

# Protein Dynamics Derived from Clusters of Crystal Structures

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**ABSTRACT** A method is presented to mathematically extract concerted structural transitions in proteins from collections of crystal structures. The "essential dynamics" procedure is used to filter out small-amplitude fluctuations from such a set of structures; the remaining large conformational changes describe motions such as those important for the uptake/release of substrate/ligand and in catalytic reactions. The method is applied to sets of x-ray structures for a number of proteins, and the results are compared with the results from essential dynamics as applied to molecular dynamics simulations of those proteins. A significant degree of similarity is found, thereby providing a direct experimental basis for the application of such simulations to the description of large concerted motions in proteins.

## INTRODUCTION

A relationship between protein function and flexibility/dynamic characteristics was postulated before the first protein structure was even elucidated (Pauling, 1948). Using NMR and other forms of spectroscopy, it is possible to obtain some information on the motions of proteins (Nicholson et al., 1995; Hage et al., 1996). However, there is no experimental method for following the motion of every atom in a protein as a function of time, although with the advent of time-resolved crystallography a step has been made in this direction (Moffat, 1989; Bolduc et al., 1995; Genick et al., 1996). In most cases, computer simulation methods are used to obtain rough impressions of the motions that are possible in a given structure. Although these simulation methods have come of age (Berendsen, 1996), there are still a few key problems: 1) computer-generated data are treated with suspicion by many scientists, because they are not hard experimental observations; 2) simulations have the tendency to be restricted to small areas of the full configurational space of the protein (Clarage et al., 1995; Balsera et al., 1996); 3) interpretations of simulations are prone to subjectivity, as it is always possible to observe something that fits a particular hypothesis; and 4) large amounts of computer time are needed to properly simulate even small proteins (i.e., <25 kDa); the larger proteins are, at present, virtually impossible to simulate.

The recently introduced essential dynamics (ED) method (Amadei et al., 1993) (similar to principal components/multivariate analysis; Diamond, 1974; Garcia, 1992; Clarage et al., 1995) is able to extract the large (biologically significant) concerted motions from a molecular dynamics (MD) simulation. All relevant conformational states can be

described by only a few degrees of freedom. These essential degrees of freedom allow us to focus on the motions important for protein function, facilitating targeted mutagenesis studies aimed at affecting protein dynamics (van Aalten et al., 1996c), more direct comparison with experimental results (van Aalten et al., 1995, 1996b), and a full exploration of the relevant protein configurational space (Amadei et al., 1996; de Groot et al., 1996a, b). However, concerted motions revealed by this approach are calculated from MD simulations, and as such require experimental verification.

In the past few years, the Protein Data Bank (PDB) protein structure database (Bernstein et al., 1977) has been expanded rapidly by the addition of crystal structures of new proteins, and of different crystal structures of proteins already in the database. Proteins are crystallized in mutated forms, with different ligands, or under different conditions, all leading to slight conformational changes, restricted by the mechanical framework defined by the protein structure (and by the crystal packing environment; Phillips, 1990). Here we show that it is possible to combine the structural variations in thermally accessible conformations in a crystal environment into a formal description of large concerted movements of atoms by using the essential dynamics method. The results reveal a pattern of mobility similar to that derived from MD simulations started from a single crystal structure.

## METHODS

Essential dynamics (similar to the single value decomposition method; Garcia, 1992; Romo et al., 1995) is based on the diagonalization of the covariance matrix, built from atomic fluctuations relative to their average positions:

$$C_{ij} = \langle (x_i - \langle x_i \rangle)(x_j - \langle x_j \rangle) \rangle \quad (1)$$

where  $x$  are the  $x$ ,  $y$ ,  $z$  coordinates of the atoms,  $\langle x \rangle$  are the average positions of the coordinates, and the average is calculated over all structures, after they have been superimposed on a reference structure to remove overall transla-

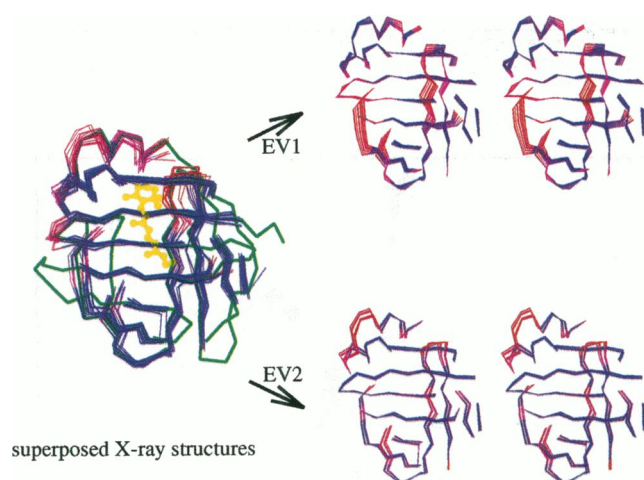
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**FIGURE 1** The results of the essential dynamics procedure. Superposition of all crystal structures in the FABP set (*left*), colored by average normalized B-factors (low B-factor = blue; high B-factor = red), and stereo pictures representing the projection of the same structures onto eigenvector 1 (*upper right*) and 2 (*lower right*) calculated by CRY-ED, colored by mobility as described by the eigenvector (i.e., regions with low mobility are colored blue; highly mobile regions are colored red).

**TABLE 2** List of eigenvalues resulting from the application of ED on the crystal structures in the test sets

| Family | EV1   | EV2   | EV3    | EV5    | EV10   | EV15    |
|--------|-------|-------|--------|--------|--------|---------|
| FABP   | 0.28  | 0.14  | 0.088  | 0.034  | 0.0036 | 0.00098 |
| PL     | 0.17  | 0.099 | 0.022  | 0.011  | 0.0036 | 0.0015  |
| MYO    | 0.035 | 0.013 | 0.0098 | 0.0068 | 0.0017 | 0.00065 |
| RAS    | 0.39  | 0.20  | 0.12   | 0.046  | 0.0074 | 0.0021  |
| LYS    | 0.032 | 0.026 | 0.019  | 0.0076 | 0.0035 | 0.0021  |
| BARN   | 0.018 | 0.013 | 0.0067 | 0.0038 | 0.0012 | 0.00056 |
| HIV    | 0.11  | 0.082 | 0.026  | 0.0099 | 0.0046 | 0.0029  |
| AAT    | 1.8   | 0.77  | 0.047  | 0.018  | 0.0073 | 0.0045  |

Eigenvalue 1 (EV1 in the table) corresponds to the largest concerted motion of atoms (described by eigenvector 1). Eigenvalues are in nm<sup>2</sup>.

vectors, we filtered out the small uncorrelated structural variations. The structural fluctuations seem to correlate with the average B-factors determined from the crystal structures. The first two eigenvectors (i.e., the eigenvectors with the largest eigenvalues, thus the most dominant concerted fluctuations of atoms) describe two distinct movements. In eigenvector 1, one of the two helices at the entrance to the binding pocket moves away from the rest of the protein. In the second eigenvector a correlated displacement of the D and E strands is observed. The displacements along the two eigenvectors together seem to create an opening toward the ligand binding pocket. Similar motions were observed in two molecular dynamics simulations, where the ligand was seen to move toward this opening (van Aalten et al., 1996b; Zanotti et al., 1994).

Comparable results were obtained for the other proteins in the test set. Application of CRY-ED provided a formal description of large concerted structural rearrangements, captured in a few eigenvectors. Such structural rearrange-

ments, which were observed to be centered around the substrate or ligand-binding site, are likely to be linked to the functional properties of the proteins. Internal motions have been demonstrated for PL by fluorescence studies (Kuipers et al., 1991), involving regions containing engineered tryptophans, which are also highly mobile in the CRY-ED analysis. CRY-ED was able to detect rigid body motions of secondary structure elements, centered around the heme-binding pocket; concerted motions have also been found experimentally in myoglobin (Srajer et al., 1996; Richard et al., 1992). Fluctuations of two loops covering the nucleotide-binding site in RAS, detected by NMR (Kraulis et al., 1994), were also found by CRY-ED. Similarly, NMR experiments predicted flexible regions for LYS (Buck et al., 1995) and BARN (Meiering et al., 1993), which are reproduced by the CRY-ED experiments. For HIV, concerted motions of the well-characterized flaps and other regions close to the substrate binding site were observed, in agreement with previously published NMR (Nicholson et al., 1995) and simulation data (Collins et al., 1995). AAT, a protein too big for long simulations on present-day computers, shows large hinge-bending of domains around the substrate-binding site, as previously observed (Moser et al., 1994).

Unfortunately, for most proteins whose structures have been solved, too few structures are available to obtain the essential eigenvectors directly by the ED approach described above. However, MD simulations analyzed with ED (MD-ED) can be used to obtain a rough approximation of these vectors. Here we quantitatively compare the ED eigenvectors revealed by the analysis of crystallographic data (CRY-ED) with those obtained from MD (MD-ED).

Fig. 2 A demonstrates the method of comparison: MD-ED eigenvectors are projected onto the first three CRY-ED eigenvectors by calculating the cumulative square inner product. If these two sets of eigenvectors are very different, a nearly straight line will be obtained. The figure shows that the largest part of the overlap is concentrated in the first few MD-ED eigenvectors, which together form the "essential subspace" (Amadei et al., 1993). Thus the large concerted motions derived from CRY-ED are similar to those found from MD-ED. This is further quantified by inner products representing the overlap of the first few MD-ED eigenvectors (5% of the total number) of the protein with the first three CRY-ED eigenvectors, listed in Table 3. It appears that on average, the first three CRY-ED eigenvectors are contained for ~50% (overlap of 0.5) in the first 5% of MD-ED eigenvectors. There are four reasons why this overlap is not 100%: 1) Even a 1-ns simulation does not provide a complete sampling of the essential subspace (Clarage et al., 1995; Balsara et al., 1996). 2) An MD simulation represents the protein in solvent without any contacts with neighboring proteins; such contacts may play a role in a protein crystal (Zhang et al., 1995). 3) The covariance matrix is built from an ensemble of thousands of structures in the case of MD-ED, whereas for CRY-ED only a few tens of crystal structures are used. 4) Structural

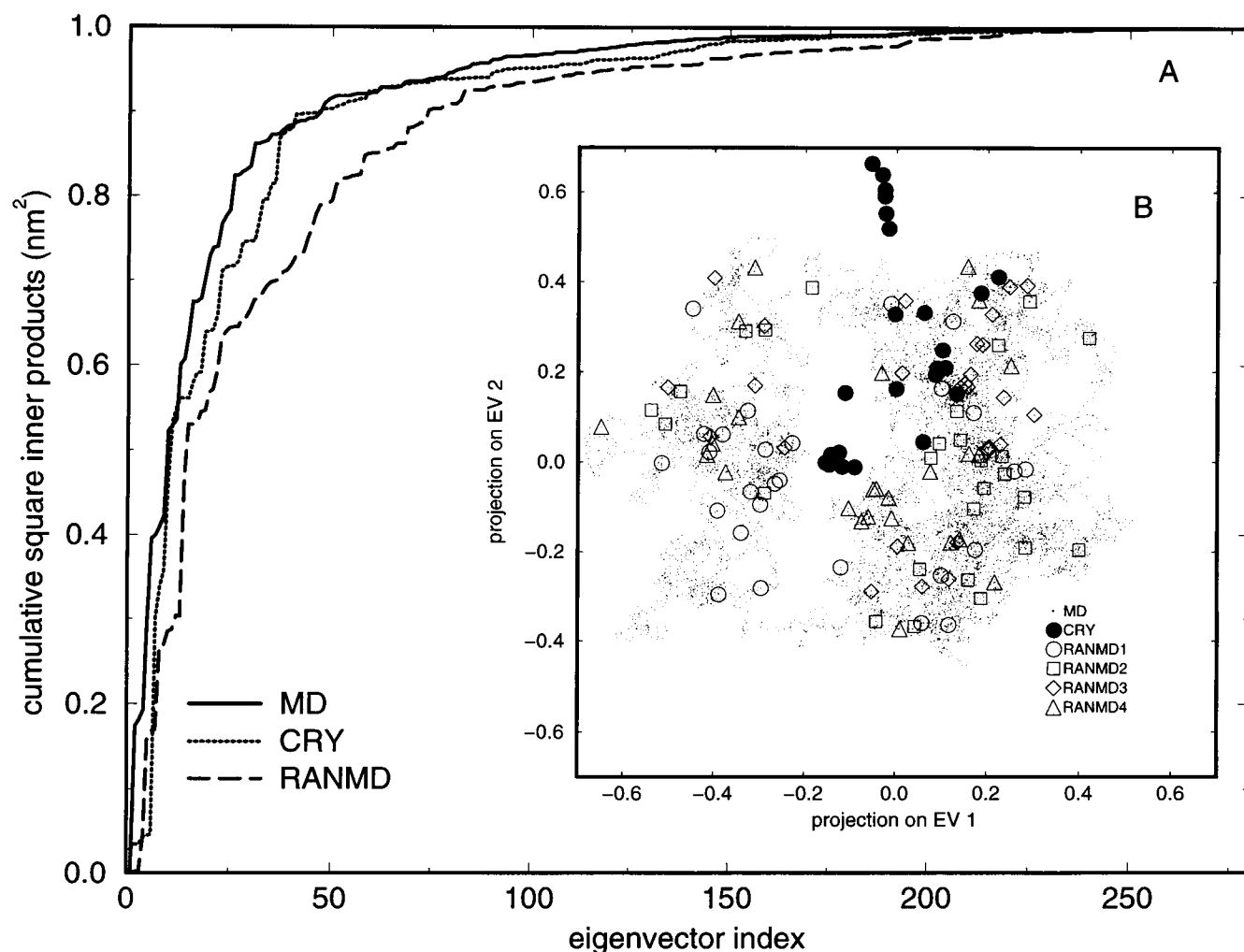


FIGURE 2 Projection of eigenvectors and trajectories. (A) Cumulative inner products from the projection of the MD-ED eigenvectors onto the first three CRY-ED eigenvectors, for FABP. EV1, EV2, EV3 are CRY-ED eigenvectors 1, 2, and 3, respectively. (B) Projection of the MD structures (MD), the crystal structures (CRY), and the four "random" (see Table 1) sets of MD structures (RANMD1-4) of FABP onto the plane defined by MD-ED eigenvectors 1 and 2.

variation in the ensemble of crystal structures is rather limited compared to that in the MD ensemble. The importance of the last two effects was investigated by randomly selecting structures (the same number and as much as possible the same spread as for the crystal structures) from the MD simulation (see Table 3). ED was then performed on these structures, and the resulting eigenvectors were compared to those calculated from the full MD simulation (Table 3). It appears that reducing the structural variability and the number of structures used reduces the overlap considerably, to  $\sim 70\%$ . Thus considering the possible additional effect of crystal contacts, and the MD sampling problem, 50% indicates a significant similarity.

Fig. 2 B shows the projection of the three sets of structures (normal MD, crystal structures, and the randomly selected MD structures, for FABP) onto the first two MD-ED eigenvectors. The crystal structures show a considerable spread in projection onto these eigenvectors. This

implies that the structural repertoire of the experimental set is not restricted to a limited number of conformations.

## DISCUSSION

Summarizing, it is possible to derive a formal description of concerted structural fluctuations of atoms in a protein from just a few tens of crystal structures. The "essential" motions found appear to have a significant similarity to those obtained from MD simulations of the same proteins, thus validating the results coming from such simulation techniques. Interestingly, the amount of overlap between the CRY-ED and MD-ED eigenvectors does not seem to depend on the cause of structural variation in the cluster of crystal structures. Both RAS and BARN contain mainly mutant crystal structures, whereas structural variation in FABP, for instance, is mainly caused by sequence diversity.

**TABLE 3** Quantitative analysis of similarities between the eigenvectors derived from MD and CRY-ED

|      | RMS deviation (Å) |       |              | #dim | #CRY | MD on CRY |      |      | MD on RANMD |             |             |
|------|-------------------|-------|--------------|------|------|-----------|------|------|-------------|-------------|-------------|
|      | (MD)              | (CRY) | $\sigma$ CRY |      |      | ev1       | ev2  | ev3  | ev1         | ev2         | ev3         |
| FABP | 0.68              | 0.82  | 0.18         | 14   | 27   | 0.61      | 0.56 | 0.47 | 0.93 (0.01) | 0.91 (0.02) | 0.89 (0.03) |
| PL   | 0.86              | 0.63  | 0.09         | 14   | 20   | 0.41      | 0.41 | 0.26 | 0.68 (0.19) | 0.58 (0.06) | 0.41 (0.03) |
| MYO  | 0.87              | 0.23  | 0.11         | 22   | 28   | 0.59      | 0.46 | 0.39 | 0.47 (0.04) | 0.38 (0.12) | 0.29 (0.10) |
| RAS  | 1.59              | 0.67  | 0.34         | 24   | 19   | 0.46      | 0.49 | 0.41 | 0.86 (0.02) | 0.78 (0.09) | 0.57 (0.20) |
| LYS  | 0.85              | 0.33  | 0.12         | 19   | 41   | 0.49      | 0.36 | 0.43 | 0.61 (0.06) | 0.58 (0.07) | 0.43 (0.07) |
| BARN | 0.93              | 0.26  | 0.07         | 14   | 63   | 0.61      | 0.69 | 0.33 | 0.42 (0.12) | 0.34 (0.10) | 0.25 (0.05) |
| Mean | 0.96              | 0.49  | 0.15         | 18   | 33   | 0.53      | 0.49 | 0.38 | 0.66 (0.07) | 0.60 (0.08) | 0.47 (0.08) |

For each protein in the test set, the following are listed: average root mean square deviation (RMSD) with respect to the average structure for the MD and crystal structures;  $\sigma$ CRY, standard deviation in this RMSD for the crystal structures; #dim, number of eigenvectors (5% of the total) taken to represent the essential subspace of the MD simulation; #CRY, = number of crystal structures in the test set; MD on CRY, cumulative squared inner products between the first #dim eigenvectors of the MD simulation and eigenvectors 1, 2, and 3 (ev1, ev2, ev3, respectively) from CRY-ED; MD on RANMD, cumulative squared inner products between the first #dim eigenvectors of the MD simulation and eigenvectors 1, 2, and 3 (ev1, ev2, ev3, respectively) derived from a set of randomly selected structures from the MD simulation. These structures were chosen by using a reference frame from the MD simulation and selecting MD frames such that the spread in the RMSDs was the same as for the set of crystal structures. This procedure was repeated four times, with different MD structures as reference (equally spread out over the simulation) to increase statistics. The standard deviation in the overlap between the eigenvectors from these four RANMD sets and the MD simulation is listed between brackets. The last line in the table (Mean) lists the means of the columns.

Collections of crystal structures of the same protein have been used before in many ways to investigate biologically important structural changes. The Diamond plot (Diamond, 1974) shows variation in a set of structures along the main axes of structural displacement, in a way similar to that presented here. In general, there have been many reports of comparison of a few crystal structures by conventional structural superposition (e.g., Sondek et al., 1994; Moser et al., 1994; Zhang et al., 1995) and domain-searching algorithms (Nichols et al., 1995). The hinge-bending motion in a mutant bacteriophage T4 lysozyme (Faber and Matthews, 1990) is a well-known example of this kind. Furthermore, crystal structures have been sorted visually to yield a movie of conformational change depicting a reaction cycle (Vornrhein et al., 1995). The new approach presented here has many advantages: it is able to extract the large concerted conformational changes, thus eliminating small irrelevant structural changes; it provides a mathematical model for protein conformational changes, which enables us to describe biologically relevant conformational states by specifying only a few variables (the displacement along the essential eigenvectors). This opens up new directions in targeted site-directed mutagenesis (e.g., van Aalten et al., 1996c), or even in automatic docking and folding algorithms, which can now be based on eigenvectors derived from experimental rather than simulated structures.

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