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Improvements in the analysis of domain motions in proteins from conformational change: DynDom version 1.50

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Abstract

DynDom is a program that analyses conformational change in proteins for dynamic domains, hinge axes, and hinge-bending regions. Here, a number of improvements and additions are reported which have been implemented in the new version 1.50. The most significant improvement is in the determination of the hinge-bending residues. A new routine also compares quantities relating to the main-chain dihedrals of bending residues with the hinge-bending motion. This version of the program can now be run from the DynDom website at: http://www.sys.uea.ac.uk/dyndom.

Keywords: Domain motions in proteins; Proteins conformational change; DynDom; Hinge bending

1. Introduction

DynDom is a program that analyzes conformational change in proteins for dynamic domains, hinge axes, and hinge-bending regions. It is primarily used by X-ray crystallographers when they have more than one conformation of a protein for analysis of its domain motion. It is also used to analyze results from molecular dynamics simulations [1]. The first release of DynDom (version 1.02) was made available in 1998 from the DynDom website, now at http://www.sys.uea.ac.uk/dyndom, and from the Collaborative Computing Project Number 4 (CCP4) [2] website at http://www.ccp4.ac.uk/main.html. The purpose of this report is to describe some improvements and additions in the new version 1.50.

2. The basic methodology

Details of the basic methodology are given in the original DynDom article [3], but a brief description is given here. Given two conformations of a protein, the program will analyze the conformational change in terms of dynamics domains, hinge axes, and hinge-bending regions. The procedure is performed in three consecutive stages. First the dynamic domains are found, second, the inter-domain screw axes are determined, and finally the inter-domain bending regions. The basis of the methodology is that the dynamic domains can be distinguished by their differing rotational properties. After the two conformations are superposed, the rotation vector is calculated from the rigid-body movement between the two conformations of each main-chain fragment (generated by use of a sliding window). The components of each vector are treated as co-ordinates of a point in a rotation space. Therefore, main-chain segments belonging to domains with different rotations should form separated clusters of points in the rotation space. The *K*-means clustering algorithm is used to determine these clusters, which form the basis of the domain decomposition. The next stage is the determination of the inter-domain screw axis between each domain pair. To do this one domain is fixed in space, and the motion of the other domain, the rotating domain, is described by a screw motion about an unique axis. In the final stage, hinge-bending residues are determined. These residues are situated at a transition between the rotational properties of two dynamics domains.

This first release of DynDom version 1.02 was used to analyze 24 proteins, for which at least two X-ray conformers were known, for their domain motions [4]. The nature of the inter-domain bending regions was of particular interest in that study. In some of the cases, it was clear that bending regions comprised a complex set of rotations, and the

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original method resulted in some residues that were clearly involved in the inter-domain bending, being missed. Below a new method, implemented in version 1.50, of determining the bending regions is described as are a number of other improvements and additions.

3. Improvements and additions in DynDom version 1.50

3.1. Determination of bending residues

Each ball in the top of Fig. 1(a and b) is at a point in the rotation space representing the rotation of a main-chain segment in canine lymphoma immunoglobulin (the conformational change is between chains B and D in the PDB file: 1IGT [5]). The central residue of each segment can, therefore, be associated with a point in the rotation space and, therefore, a cluster. When these clusters are mapped onto the protein structure, boundaries between the domains are found. The residues at the boundaries are automatically assigned as bending residues. The new algorithm determines whether neighboring residues that belong to the same domain as a boundary residue, lay outside the main distribution of points of the corresponding cluster. If they do, they are also assigned as bending residues. To make precise what is meant by "outside the main distribution of points" the clusters are modelled as 3D normal distributions. Associated with each normal distribution are ellipsoids of constant probability density that can be associated with confidence levels. In other words, an ellipsoid can be chosen such that the probability of finding a point outside that ellipsoid is small enough for one to consider it unlikely to occur by chance. Each ellipsoid is associated with a value Q:

$$Q = (\boldsymbol{X} - \boldsymbol{\mu})^{\mathrm{t}} \boldsymbol{\Sigma}^{-1} (\boldsymbol{X} - \boldsymbol{\mu}) \quad (1)$$

where $\boldsymbol{X}$ is the position vector of any point on the ellipsoid corresponding to Q, $\boldsymbol{\mu}$ is the position vector of the average point of the distribution, and $\boldsymbol{\Sigma}$ is the variance–co-variance matrix of the distribution. The superscript "t" denotes the transpose. Q follows the χ^2-distribution, and is dependent on the number of degrees of freedom, which is 3 in this case. The value of Q can be chosen such that a certain proportion, P, of the points lay outside the corresponding ellipsoid. In 1D, Q is equal to Z^2, where Z is the standardized normal variate in the two-tailed test for a given value of P. The 24 proteins used in a previous study [4] were used to determine a P-value that gives reasonable results. The value chosen was 0.2. In many proteins this produced little difference over the old method in regions assigned as bending, but improved results significantly in some. Fig. 1(a) shows the result using version 1.02 and Fig. 1(b) using version 1.50 for canine lymphoma immunoglobulin. It is clear that there are more rotation points colored green in Fig. 1(b). These are indeed separated from the two main clusters and it is apparent that the old method has not worked well in this case. This is even more apparent in the bottom of Fig. 1, where only part of the flexible linker region is assigned as bending using the old method, whereas, using the new method the whole of this region is assigned as a bending region. This improvement was seen in other proteins with a single flexible linker between domains.

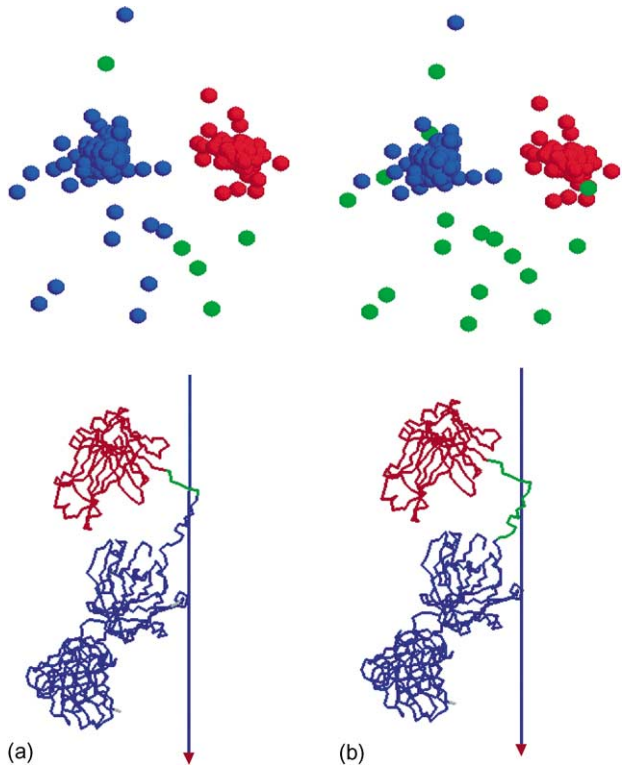

Fig. 1. Top, rotation points from the conformational change seen in canine lymphoma immunoglobulin between chains B and D from PDB file: 1IGT [5]. One domain is colored blue, the other red, the bending residues green. In (a) from DynDom version 1.02, very few residues are assigned as bending, in (b) using DynDom version 1.50, far more are assigned as bending. Bottom, canine lymphoma immunoglobulin chain B from PDB file: 1IGT [5] colored as in top. In (a) from DynDom version 1.02, only a small portion of the clear linker region is assigned as bending, in (b) from DynDom version 1.50, the whole of the linker region is assigned as bending. Bottom of figure also shows in blue chain A.

3.2. Dihedral angle analysis

Sometimes individual dihedral angles can be responsible for a considerable proportion of the rotation between the fixed and rotating domain. It would be helpful, therefore, to compare individual dihedral angle rotations with the inter-domain rotation. DynDom now does this for bending residues. The change in the angle between the rigid tetrahedra, formed by N, C^{α}, C^{β}, and C atoms at residues i and $i + 1$, is largely dependent on $\Delta\psi_i + \Delta\phi_{i+1}$. In other words, if this quantity is small then there will be very little relative rotation of these tetrahedra and their attached side chains. Even if $\Delta\psi_i$ and $\Delta\phi_{i+1}$ are large, the fact that their

sum is small means it is the intervening peptide plane that undergoes a rotation [6], leaving the flanking regions relatively unperturbed. This means that we should be assessing changes in both these dihedral angles, when comparing them to inter-domain rotations. This latest version of DynDom calculates $\Delta\psi_i$ and $\Delta\phi_{i+1}$ for bending residue pairs. It also calculates the scalar product between the ψ-dihedral axis of residue i, and the ϕ-dihedral axis of residue and $i+1$, with the unit vector in the direction of the inter-domain screw axis, as a measure of how parallel these axes are. It does this for both the conformations. If the rotation vector at tetrahedron i is denoted $\boldsymbol{\theta}_i$ (this corresponds to the rotation of the tetrahedron of residue i relative to the fixed domain), the unit vector in the direction of the inter-domain screw axis, $\boldsymbol{n}$ and ξ, the inter-domain rotation, then $((\boldsymbol{\theta}_i \cdot \boldsymbol{n})/\xi) \times 100$ will give a percentage measure of the extent to which residue i is rotating together with the rotating domain.[1] Another interesting quantity is the difference of this quantity between residues i and $i+1$, which measures the progress in reaching the rotation of the whole rotating domain in going from residue i to $i+1$. Both these quantities are output to a file dedicated to dihedral angle analysis. The classic hinge-bending motion in lactoferrin [7] represented by the conformational change between the structures in PDB files 1LFG and 1LFH gives an example of the use of this analysis. The $\Delta\psi$ of Thr90 is 43° and $\Delta\phi$ of His91 is 27° with both their axes making an angle of about 20° to the inter-domain screw axis in both the conformations. What is more the inter-domain screw axis passes very close to these axes, being <2 Å from the C^{α}s of these residues in both the conformations. The inter-domain rotation angle is 55°, where Thr90 is rotating at −36.5%, and His91 at 95.5%, of the total rotation of the rotating domain. These results indicate the great extent to which the two dihedral axes between Thr90 and His91 are involved in the inter-domain motion.

3.3. Other improvements

There are improvements in visualization including the ability to view the two conformations simultaneously with RasMol [8], where the first conformation is colored to highlight the domains and hinge-bending residues, but the other conformation is left uncolored. Another feature is the ability to see each residue colored according to the rotation of the segment it is centered on. This can be seen by selecting "temperature" from the "color" menu in RasMol. There are also some improvements in the underlying algorithm that make it more robust. For more details please see the complete paper.

[1] In this case, the fixed domain is the one in which the bending region originates and the rotating domain is the one in which the bending region ends.

4. The DynDom website

DynDom version 1.50 is available to run server-side at the DynDom website at http://www.sys.uea.ac.uk/dyndom, where PDB files can be selected or files uploaded. A feature of the server, not available in the downloadable version of the program, is a pre-processor that determines residue equivalencies for superposition from a pairwise sequence alignment. Users are advised to select chain pairs that have high sequence identity. Results from a successful run are displayed at the website and an image of the protein can be seen from a direction that looks down the hinge axis. JavaScript enables one to see the domain movement from this vantage point, by swapping the two images of the protein, one from each conformation, upon a "mouseover" or "mouseoff" movement. A RasMol script file is also provided to download for viewing locally. The source code of DynDom program version 1.50 itself is available from the DynDom website and from the Collaborative Computing Project Number 4 (CCP4) [2] website at: http://www.ccp4.ac.uk/main.html.

For more details regarding this program, the readers are referred to the complete text and the DynDom website.

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