Full text

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 version 1.50. The most significant improvement is in the determination of the hinge-bending residues. In the previous method often only a portion of an obvious linker region was assigned as a bending region. This problem has been solved by assigning interdomain residues to a bending region if they have rotational properties that make them significantly different from the domain they belong. This improvement is demonstrated in the cases of canine lymphoma immunoglobulin, diphtheria toxin, and tomato bushy stunt virus protein, where now the entire linker regions are assigned as bending regions. In most other cases, however, the difference in the results from the previous method is small. An additional feature includes a dihedral analysis routine, in which changes in the dihedral angles of the residues in the bending region are compared to changes in the interdomain hinge-angle. In a set of 24 examples the domain motion in lactoferrin, stands out as the example of where the dihedral axes of ψ and ϕ of residues i and $i+1$, respectively, make a reasonable approximation to the interdomain screw axis. Other improvements in the algorithm and output are also described which should make this new version a more powerful tool for the analysis of domain motions in proteins. The program is available to run server side at the DynDom website at: http://www.sys.uea.ac.uk/dyndom where a preprocessor determines residue equivalencies for superposition of structures from a pairwise sequence alignment.

Keywords: Domain Motion, Hinge Axis, Hinge Bending, Lactoferrin, Citrate Synthase, Canine Lymphoma Immunoglobulin.

INTRODUCTION

Protein function is a dynamical process that necessarily involves some form of change in conformation of the protein. In many cases this conformational change will involve the motion of protein "domains" as quasi-rigid units [1-3]. In such cases it is often possible to talk in terms of hinging motions whereby one domain hinges about a conceptual hinge axis relative to another. If we are provided with two conformations of a protein, then it is useful to analyze whether this conformational change can be adequately represented by this model or not. If it can be then the benefit is twofold. First, the description has been lifted from the atomic level to a level that is on the scale of the protein itself and is therefore more easily comprehended, and second it also allows one to focus on regions that control the conformational change on this larger scale.

Given two conformations of a protein, the program DynDom[4] 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 interdomain screw axes are determined, and finally the interdomain 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 coordinates 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 interdomain screw axis between two dynamic domains. The direction and angle of rotation represented by this axis, in a sense, represents the vector joining the average points between the two clusters that correspond to the two domains. In the determination of the hinge-bending residues, the rotation of each segment from a domain is projected onto this vector and only the segments at the domain boundaries that are outside one standard deviation from the average of the domain to which they belong, are assigned as bending segments. It is the residues at the centers of these segments that are assigned as bending residues.

The 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[3]. The nature of the interdomain bending regions was of particular interest in that study. In some cases, it was clear that bending regions comprised a complex set of rotations, and the projection method described above resulted in some residues that were clearly involved in the interdomain bending, being missed. Below a new method of determining the bending regions is described. Although this constitutes the most significant improvement in version 1.50, a number of other improvements have been also been implemented. These new methods are described and the results demonstrated on selected examples.

METHODS

Determination of Hinge-Bending Residues

As already described above the determination of the hinge bending residues in version 1.02 relied on a projection method. The basic algorithm is similar in version 1.50, but the rotation vectors are no longer projected. Figure 1(a) schematically illustrates how the hinge bending residues are determined in version 1.02 and Figure 1(b) shows how they are determined in version 1.50. In 1.02 the method is basically one-dimensional, in 1.50 it is 3-dimensional. Each point represents the rotation of a segment and is associated with the central residue of that segment. Each point belongs to a particular cluster, and consequently domain. 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 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 three-dimensional 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:

$$Q=(\underline{X}-\underline{\mu})^t\ \Sigma^{-1}(\underline{X}-\underline{\mu}) \qquad (1)$$

where $\underline{X}$ is the position vector of any point on the ellipsoid corresponding to Q, $\underline{\mu}$ is the position vector of the average point of the distribution, and Σ is the variance-covariance 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 1-dimension, 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[3] are used to determine a P-value that gives reasonable results.

Dihedral Angle Analysis

DynDom chooses pairs of domains in order to calculate the interdomain screw axes and bending residues. One of the pairs is selected as the fixed domain (the domain whose two conformations are superposed), the other is the moving or rotating domain. 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 interdomain 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 that there will not be an appreciable relative

rotation of regions that flank this pair. In such cases it is the intervening peptide plane that undergoes a rotation[5], leaving the flanking regions relatively unperturbed. This means that we should be assessing changes in both these dihedral angles, when comparing them to interdomain rotations. This latest version of DynDom calculates $\Delta\psi_i$ and $\Delta\phi_{i+1}$ for bending residues *i* and *i+1*. 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 interdomain screw axis, as a measure of how parallel these axes are. It does this for both 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 interdomain screw axis, $\boldsymbol{n}$, and, ξ, the interdomain 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. 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.

Improvements in Visualization of Domain Motions

DynDom prepares its output for visualization using the molecular graphics program RasMol[6]. This version of DynDom now prepares a coordinate file in PDB format within which the two chains are put together with the coordinates of the "arrows" that are used to depict the interdomain screw axes. The coordinates of the first chain are the same as in

its original PDB file. The second chain is moved so that its fixed domain is superposed on the fixed domain of the first chain. If there is more than one fixed domain then the second chain is not written to this file. The domains and bending regions of only the first chain are colored using the RasMol script file output from the program, the second chain is colored grey or white. This visualization of both conformations allows one to see the conformational differences more clearly. If a ligand is present in the first PDB file, this can be appended to the DynDom coordinate file for visualization of the conformational change in relation to this ligand.

The rotation of each main-chain segment is calculated relative to the fixed domain, and output in the B-factor column of the DynDom coordinate file at the residue at the center of the segment. Selecting "Temperature" from the "Colours" menu allows one to visualize the degree of rotation of each residue relative to the fixed domain. Again if there is more than one fixed domain, this data is not available. The rotation points themselves can, as with version 1.02, also be inspected using RasMol.

Previously short regions were eliminated from domains if they were found not to be part of the corresponding cluster in rotation space. In this version, for reasons of aesthetics only, these regions are reunited with the domains they are embedded in if they are shorter than the minimum domain size set by the user[4].

Changes in Underlying Search Algorithm

In order to get a successful result from DynDom, two main criteria must be met. The first is that one must have at least two domains that are larger than the minimum domain size set in the command file (default: 20 residues). The second is that for each domain pair connected directly (not through another domain), their ratio of interdomain displacement to intradomain displacement must be larger than the minimum set in the command file (default: 1.0). In version 1.02 the search algorithm was perhaps unnecessarily strict. It would only perform the domain motion analysis if at some level of clustering, the clusters produced domains (a single cluster in rotation space can produce more than one domain if the residues do not form a connected region in real space) that satisfied the ratio alluded to above for *every* domain pair larger than the minimum domain size. This is no longer the case for version 1.50 where the results from any domain pair that satisfies these two criteria will be analyzed, provided that the previous level of clustering did not produce domains for which *every* domain pair satisfied the two criteria. As with version 1.2, version 1.50 also stops when it finds a cluster whose domains are *all* smaller than the minimum domain size, except in one particular circumstance. Sometimes a small flexible region, often a terminal region, will be the first cluster found. If this is the case, version 1.02 will stop without producing a result. Version 1.50 will not stop if the first cluster found is smaller than the minimum domain size, but will eliminate it from any further analysis.

RESULTS AND DISCUSSION

Bending Regions

The bending regions found by DynDom version 1.02 were found to be too short in comparison to the obvious linker regions seen in canine lymphoma immunoglobulin[7], diphtheria toxin[8,9], tomato bushy stunt virus protein (tbsvp)[10]. These three cases are distinguished by the fact that they have a single rather unstructured connecting region. Perhaps not surprisingly for an apparently very flexible linker, the rotations between the domains can be very large: 176 degrees for diphtheria toxin and 103 degrees for canine lymphoma immunoglobulin. Inspection of the rotation vectors in these linker regions revealed a complex set of rotational motions combine to produce the domain rotation. In such cases the method used in version 1.02 could miss residues that are obviously involved in the bending (see Figure 1).

In order to determine an appropriate *P*-value, the 24 proteins from the previous study were used[3]. Commonly used *P*-values are 0.1 and 0.05 but these are rather stringent and did not give expected results on proteins such as citrate synthase (see below). In the 1-dimensional case, the value Q=1.0 was chosen. In the 1-tailed test that was used there, this corresponds to a *P*-value of 0.16. Here in this 3-dimensional case, a value of 0.2 has been found to give good results. With this *P*-value about 70% of 68 bending regions differed by two or fewer residues, 90% in four or fewer residues, from the bending regions determined by the old method.

The top of figure 2 shows the rotation points for canine lymphoma immunoglobulin(from PDB file: 1IGT). Figure 2(a) shows the result using version 1.02 and Figure 2(b) using

version 1.50. It is clear that there are more rotation points colored green in Figure 2(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 Figure 2 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. The same is seen in diphtheria toxin and tbsvp (see Table I), the only proteins out of the 24 that have a single flexible linker region. It is clear that in general the new method of determining interdomain bending regions is superior to the old method. However, in many cases, and particularly for those where a more extensive interdomain region is present, results do not appear to differ significantly.

Dihedral Angle Analysis

The dihedral angles of the bending regions from the 24 proteins are compared to the interdomain screw axes by the method described in the Method section. It is clear that the simple idea of a domain hinge-axis coinciding with the dihedral axis is in most cases wrong. In most cases the domain bending is an accumulation of small changes in dihedral angle and bond angle variations that do not have any obvious relation with the interdomain screw axis. Consider the interdomain α-helix in asparate aminotransferase[11]. This helix bends about 12 degrees but its backbone hydrogen bonding structure is undisturbed by this bending[3]. In such a case, the correlated dihedral changes have a complex relationship to the overall interdomain rotation. Amongst the 24 proteins studied, lactoferrin[12] (PDB files: 1LFG, 1LFH) stands out as the classic example of a

direct relationship between the dihedral axes of ψ and ϕ of neighboring residues in the bending region, and the interdomain screw axis (see Figure 3). $\Delta\psi$ of Thr90 is 43 degrees and $\Delta\phi$ of His91 is 27 degrees with both their axes making an angle of about 20 degrees to the interdomain screw axis in both conformations. What is more the interdomain screw axis passes very close to these axes, being less than 2 A from the C^{α}'s of these residues in both conformations. The interdomain rotation angle is 55 degrees where Thr90 is rotating at –36.5%, and His91 at 95.5%, of the total rotation of the rotating domain. Lactoferrin has two interdomain bending regions, forming a double-hinged β-sheet[3]. The dihedrals of the other strand do not have such an obvious relation to the interdomain screw axis. Although not quite so impressive as lactoferrin, maltodextrin[13] and DNA polymerase β[14] also have similar hinges. The one in maltodextrin is not sited at one of the obvious interdomain linkers but at residues Asn332 and Ile333. For DNA polymerase β the residues involved are Gln90 and Asp91 as well as Gln264 and Tyr265.

Visualization of the Conformational Change in the Presence of a Ligand

In the previous version of DynDom it was not possible to view both conformations simultaneously fitted at the fixed domain. This is an important improvement in the new version. To illustrate how this might lead to new insights the case of citrate synthase is used as an example. Citrate synthase is open in unliganded but closes in the presence of its substrate oxaloacetate[15]. The closed structure has been solved with oxaloacetate and carboxymethyl coenzyme A to reveal the binding site of the oxaloacetate[16]. Figure 4 shows residues 265-285 from the closed structure (PDB:5CTS) and open structure (PDB

file: 5CSC)[17] in the presence of oxaloacetate shown in ball and stick model. Also in ball and stick model is the catalytic residue His274. Interesting to note is that the side chain of His274 hardly moves in relation to oxaloacetate. The ψ angle of His274 undergoes a 93 degree change in going from the closed to open structure which can be seen in this figure by looking at the change in the position of the carbonyl oxygen. Most of the conformational change occurs after this residue. This dihedral does not act as a hinge axis in the classical sense as it is about 12A from the interdomain screw axis, but is clearly implicated in the domain motion as the main conformational change is seen to occur after this residue. It is tempting to think that the negatively charged oxaloacetate forces this change in orientation of the carbonyl group leading to domain closure[18]. This example shows how inspection of the two conformations in the presence of ligands that cause conformational change can lead to new insights.

Coloring of Individual Residues According to their Rotation

Figure 5 shows the domain motion due to the conformational difference between subunit structures from the cis and trans rings of GroEL[19] colored at each residue according to the rotation of the segment it is the center of. The fixed domain, the central one, is colored blue and the rotating domains are visible in colors between blue and red. The domain on the left colored in warm colors rotates 90 degrees relative to the fixed domain. The domain on the right colored in lighter blues rotates 29 degrees relative to the fixed domain. Such a plot can highlight regions that are rotating more than others, as well as reveal those regions where the rotational transition takes place, i.e. the hinge-bending

regions. It should be borne in mind, however, that the residues are colored according to the magnitude of their rotation, so directional information is lost in this plot.

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 preprocessor 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. A JAVA program 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)[20] website at:

http://www.ccp4.ac.uk/main.html

CONCLUSIONS

A number of improvements in the latest version of DynDom (1.50) have been described and demonstrated using examples from a set of 24 proteins for which two or more X-ray conformations are known[3]. The most significant change is in the way the bending regions are determined. It is shown that whilst the new method has a generally good correspondence with the previous method, it does offer an obvious improvement in those cases where there is a complex rotational transition between the domains. The results are demonstrably better on canine lymphoma immunoglobulin, diphtheria toxin and tbsvp. The present version also allows one also to compare directly the rotations at backbone dihedral axes with the interdomain rotation. The domain motion of lactoferrin stands out as a clear example of the classic hinge-bending protein where the rotation on one of the two connecting regions occurs about the dihedral axes between the neighboring residues. These improvements together with the other improvements in visualization described above, and its availability at the DynDom website are certain to provide the user of this program with a better insight into the function of the proteins they are studying.

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Table I

	Bending Residues	
	Ver1.02	Ver1.50
Diphtheria toxin	383-389	379-387
Canine lymphoma immunoglobulin	245-249	232-250
Tbsv	271-273	265-276

FIGURE LEGENDS

Figure 1

Schematic illustration of how bending regions are determined. Each symbol indicates a point in the rotation space corresponding to the rotation of a residue or segment (the residue at the center of the segment represents the segment). Residues with the same color symbol belong to the same domain. A star indicates a bending residue. The curved continuous lines indicate the connectivity of the residues. In (a) residues indicated as bending are between the vertical broken lines which represent one standard deviation of the two distributions when projected onto the horizontal line. This was the method used in version 1.02. In (b), the ellipses indicate a boundary of constant probability density. This can be set such that points outside are considered to be different from the main distribution. In version 1.50 this is the method used. Note that three residues in (a) that are not assigned as bending are assigned as bending in (b).

Figure 2

Top:Rotation points from the conformational change seen in canine lymphoma immunoglobulin between chains A and C from PDB file: 1IGT[7]. 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 A from 1IGT[7] 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.

Figure 3

A close up look at the hinging region of lactoferrin (PDB: 1LFH) colored according to the conformational change between the structures in 1LFH[12] and 1LFG[12]. One domain is colored red the other blue, the bending residues green. The blue shaft indicates the hinge axis. In ball and stick model are Thr90 and His91. The axis passes within 2A of their C^{α} atoms and at about 20 degrees to the ψ axis of Thr90 and the ϕ axis of His91. Rotations about these two axes combine to produce most of the interdomain rotation along this strand.

Figure 4

Colored are residues 265-285 from the closed conformation of citrate synthase (PDB file: 5CTS)[16], in gray the same residues from the open conformation (PDB file: 5CSC)[17]. Oxaloacetate is shown in ball and stick model as is the catalytic residue His274. The carbonyl group is seen to rotate by 93 degrees causing most of the conformational difference seen beyond this residue.

Figure 5

Domain motion in GroEL. Shown is chain A from PDB file 1AON[19]. The coloring of the residues is according to their absolute rotation in the conformational change between chain A and chain H, keeping domain 137-191 and 374-408 fixed (colored predominantly dark blue). The domain colored in warm colors (residues 192-373) rotates 90 degrees relative to the fixed domain, and the domain colored in the lighter hues (residues 1-136 and 409-523) rotates 29 degrees relative to the fixed domain.

Figure1

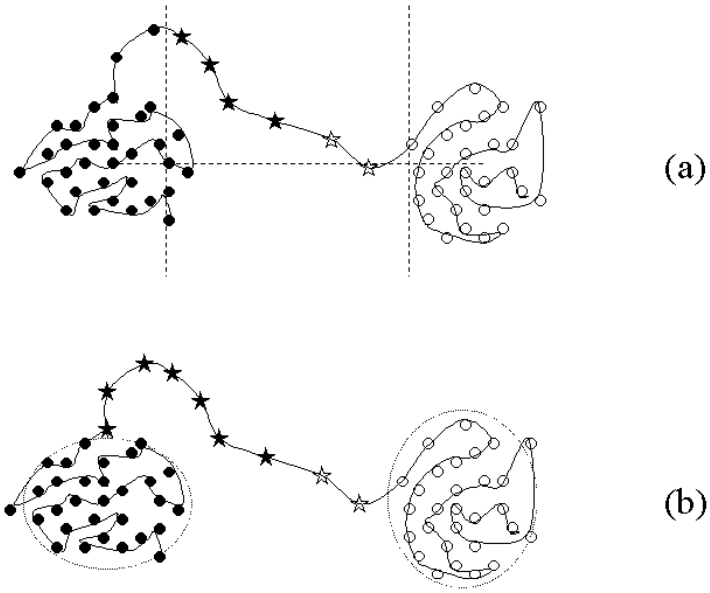
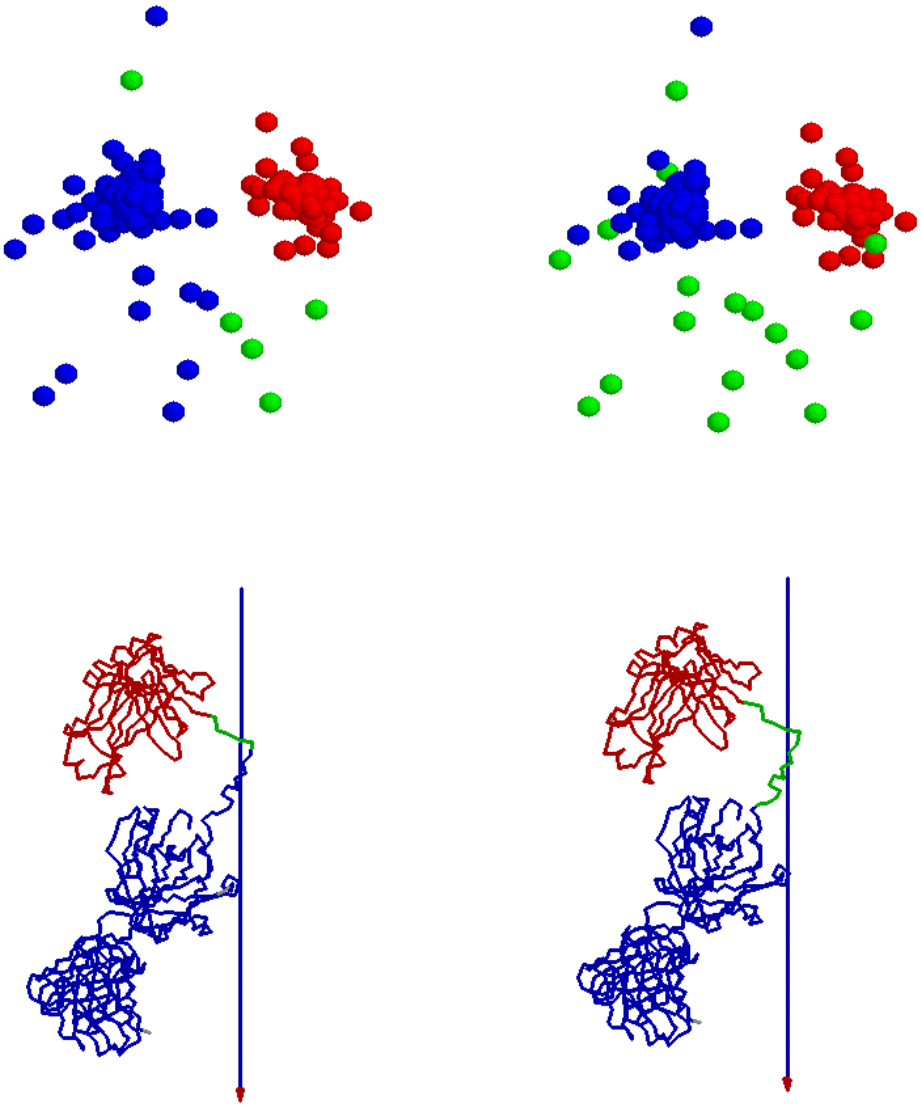


Figure2



(a) (b)

Figure 3

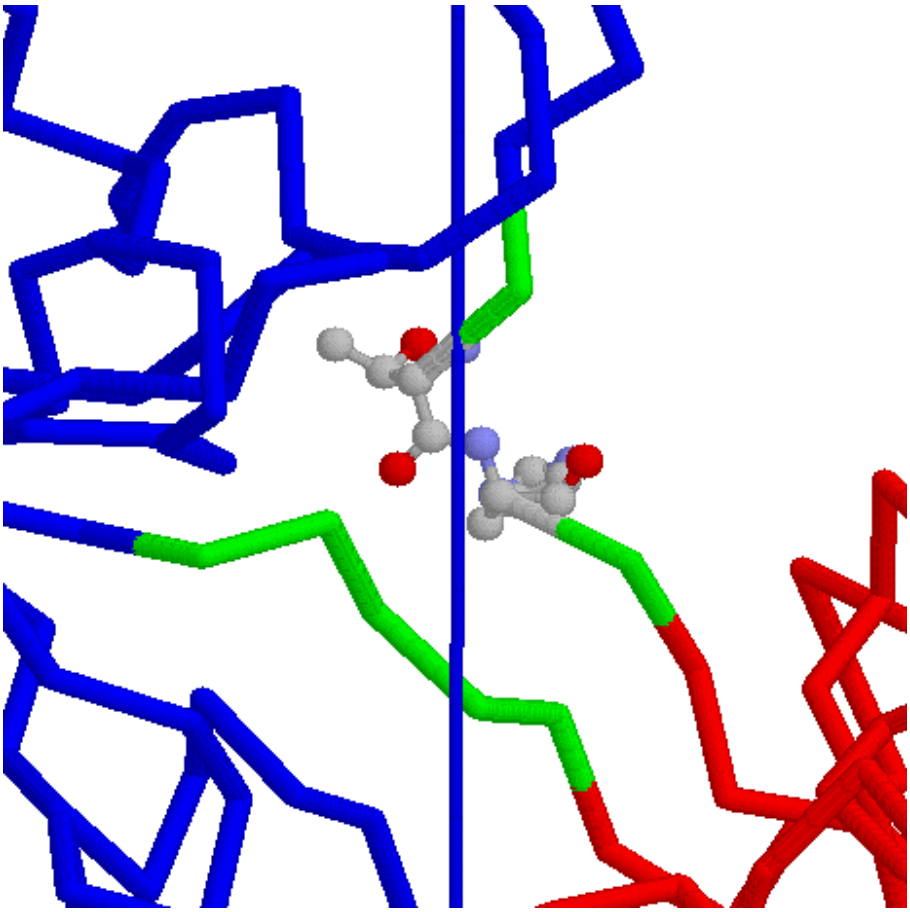


Figure 4

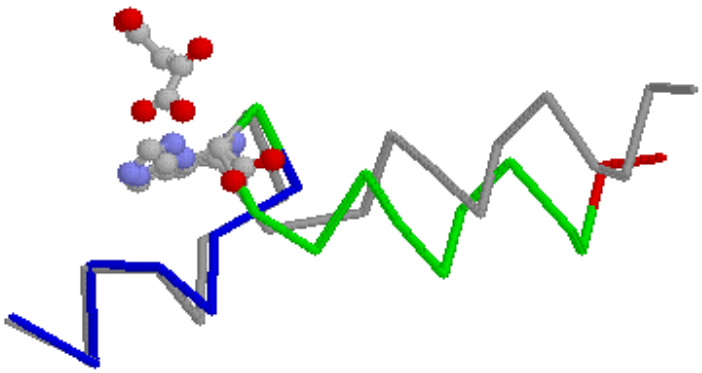


Figure 5