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Building Blocks, Hinge-Bending Motions and Protein Topology

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Abstract

Here we show that the locations of molecular hinges in protein structures fall between building block elements. Building blocks are fragments of the protein chain which constitute local minima. These elements fold first. In the next step they associate through a combinatorial assembly process. While chain-linked building blocks may be expected to trial-associate first, if unstable, alternate more stable associations will take place. Hence, we would expect that molecular hinges will be at such inter-building block locations, or at the less stable, 'unassigned' regions.

On the other hand, hinge-bending motions are well known to be critical for protein function. Hence, protein folding and protein function are evolutionarily related. Further, the pathways through which proteins attain their three dimensional folds are determined by protein topology. However, at the same time the locations of the hinges, and hinge-bending motions are also an outcome of protein topology. Thus, protein folding and function appear coupled, and relate to protein topology. Here we provide some results illustrating such a relationship.

Introduction

We have recently presented a building blocks folding model (1,2). A building block is a contiguous fragment of the sequence. If cut out of the chain, the resulting peptide is likely to have the same conformation as when it forms part of the native protein fold. While this conformation might not be stable, it is nevertheless likely to have a higher population time than other conformations of this fragment. A building block constitutes a local minimum on the chain. Building blocks fold early in the folding pathway, and associate *via* a combinatorial assembly process. The building blocks assignments are according to a score, based on hydrophobicity, compactness, and isolatedness (1). Recently we have shown the consistency of the cutting with the limited proteolytic cleavage (3). This definition of a building block suggests that if we were to dissect the protein structure into its building block components, hinges between domains should largely fall between building blocks.

Here we examine the validity of such a proposition. During the folding process, each building block folds on itself. The stabilities of the building blocks vary, and hence the population times of their predominant conformations. Consequently, hinges may fall within building blocks. Nevertheless, we would expect hinges to largely fall between the building blocks, straightforwardly enabling their combinatorial assembly process.

To examine a potential relationship between the location of the building blocks and the location of the hinges, we have extracted some well documented cases where hinge bending motions have been observed. These are picked from the database of molecular motions (4). This database illustrates cases where hinge bending has been observed experimentally, when conformations of both the open and the closed

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forms of the same chains are available. Alternatively, if that is not the case, structures have been solved for mutants or for related protein chains. For these cases, we have cut the proteins into their building blocks components. Our results, while still on a limited number of cases, illustrate that as expected, hinges tend to occur either between building blocks, or in regions unassigned into any building block. Unassigned regions are those having low stabilities on their own, and not increasing the stabilities of their sequentially linked building block neighbors.

Methods

The coordinates were extracted from the protein data bank (PDB) (5). The hinging regions and hinges are as assigned in the database of molecular movements (DMM) (4). A building block is considered to be a contiguous fragment with substantial interactions between its residues. The building blocks were assigned as described in Tsai *et al.* (2). For every candidate fragment of the protein, the relative buried accessible surface area (ASA) was calculated. The fragment was considered as a building block when the obtained relative buried ASA value was larger than a threshold value (2). The relative buried ASA is the ASA of the first half-fragment buried by the second half of the fragment plus the ASA of the second half of the fragment which is buried by the first half-fragment divided by the total ASA of the fragment. For further details see Tsai *et al.* (2).

Among all combinatorially possible assemblies of building blocks at all levels of the anatomy trees, the ones selected are based on the average score of the best two fragments (3). The scores of the hydrophobic folding units (HFUs) and the building blocks are based on compactness, hydrophobicity and isolatedness (1). The anatomy trees are generated through an iterative top-down dissecting procedure, first revealing domains, then HFUs and then a set of building blocks (3).

Molecular hinges

Hinge bending motions are essential for protein function. They are critical for binding to other molecules, whether proteins, nucleic acids, or small molecule ligands. They are essential for motility. Calmodulin, T-4 lysozyme, HIV protease and adenylate kinase are well documented cases where molecular movements have been shown to be related with their respective functions. Despite their sequence variability, retroviral proteases share a common structural fold and similar modes of movements. Significant conformational changes have been observed in the HIV protease upon binding with an inhibitor. Two flexible β-strand “flaps”, forming a ceiling of the binding pocket, open about 7Å as compared to the corresponding closed conformation (6-8). These movements have been detected by NMR (9) and by fluorescence changes (10). The functional hinge associated with flap movements (11) further validates the association between the flap movements and enzyme catalysis.

Calmodulin undergoes conformational change upon binding with Ca^{2+}, which triggers its association with target proteins (12,13). X-ray scattering (14,15) suggested that the interdomain helix, connecting the *N* and the *C*-terminal domains is flexible. NMR studies further show the details of the conformational transition induced by Ca^{2+} binding (16-19). ^{15}N spin relaxation studies in unbound (20) and Ca^{2+} bound (21) states provide further evidence that the inter-domain linker in Calmodulin is inherently flexible. This allows the *N* and *C*-terminal domains to orient independently relative to each other, providing an efficient way to adopt different inter-domain orientations when binding different targets.

Adenylate kinase is a universal multi-domain enzyme, which catalyzes phosphoryl group transfer from ATP to AMP, thereby controlling the cellular energy balance. *E. coli* adenylate kinase is made up of three domains, CORE, AMPbd and LID (22,23). LID, consisting of four stranded anti-parallel β-sheet, undergoes a significant displacement relative to AMPbd upon substrate binding (24,25). This allows the proper orientation of the active site, and the resulting closed conformation.

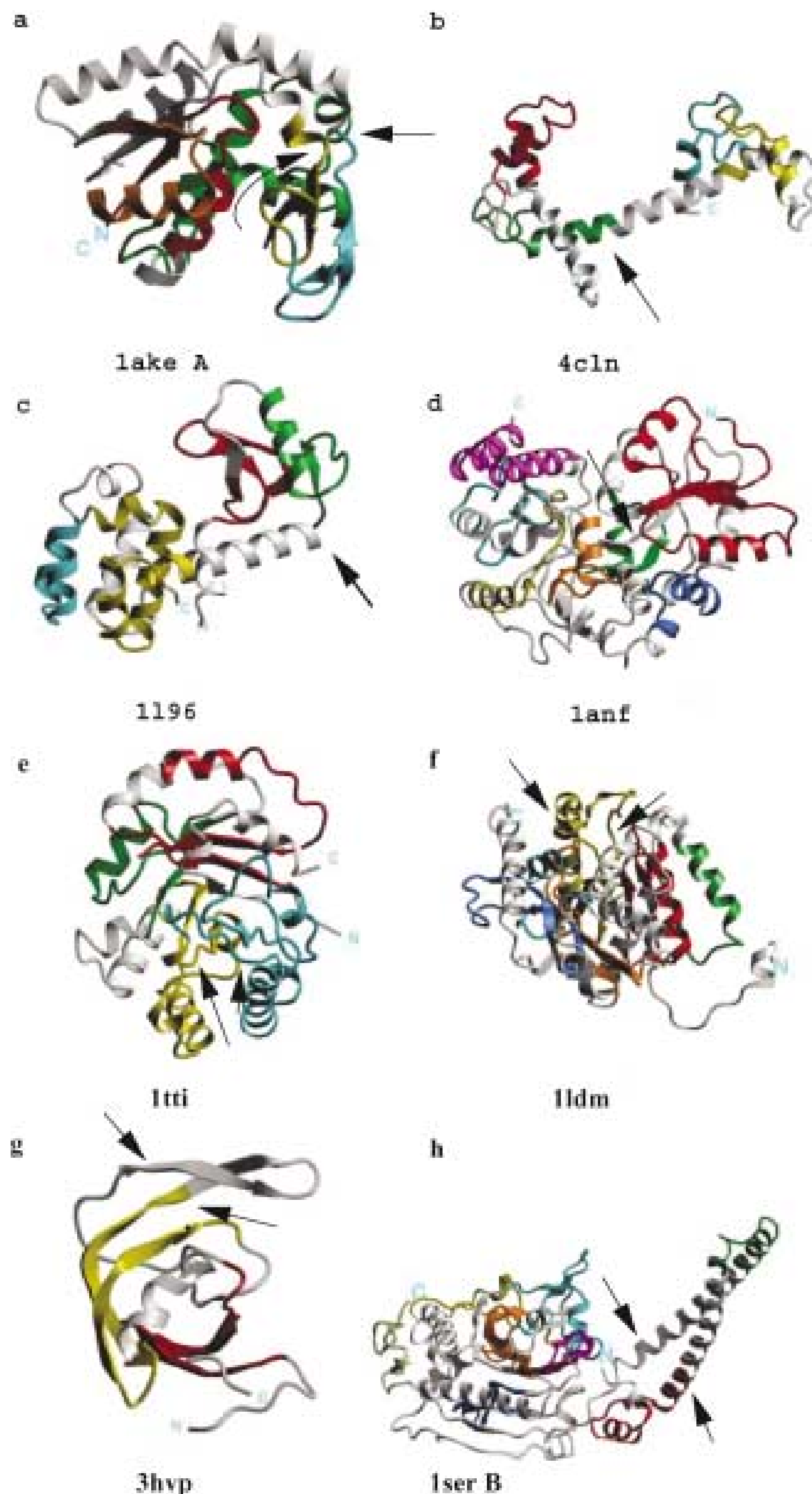


Figure 1: Building block assignments for some of the hinge-bending cases. (a-d) fall in the category of domain movement cases; (e-f) fragment movement cases. (a) Adenylate kinase (1ake); (b) Calmodulin (4cln); (c) T4 lysozyme (1l96); (d) Maltodextrin binding protein (1anf); (e) Triosephosphate isomerase (1tti); (f) Lactate dehydrogenase (1ldm); (g) HIV proteinase (3hvp); (h) Seryl-tRNA synthetase (1ser B). Each color depicts a different building block. The unassigned regions are not stable enough to form an independent building block nor do they increase the stability of their adjoining building blocks. The hinge points for the movements are marked by arrows. These points were taken from the molecular motions database (4).

T-4 lysozyme has two domains, connected by a long α-helix. The two domains move away from each other to open the active site cleft (26-28). The two domains close around the substrate *via* a hinge-bending movement. The domain movement between the closed and open conformations is supported by NMR studies (28), and by molecular dynamics simulations (29,30).

Analysis of hinges between moving domains has shown that the packing between

the moving parts is not as optimal as in the protein interior. In a classical paper, Gerstein *et al.* (31) have proposed classifying molecular motions into two types, according to the packing at their inter-domain interfaces. They labeled these as either hinge-bending, or the smaller shear motions. Packing has further enabled prediction of the modes of motions of the proteins (32,33).

We have recently analyzed the inter-domain interfaces, probing the types of interactions (34). We have observed that the number of close range electrostatic interactions, such as hydrogen bonds and salt bridges is small between the domains in the closed conformations. We have further carried out electrostatic calculations on the strength of the salt bridges at the inter-domain interfaces and found them to be roughly the same as those within the protein chains. On the other hand, comparisons of the non-polar surface areas between the domains in the closed conformations have shown that these can be extensive. For example, in glutamate dehydrogenase the non-polar surface area buried between the domains in the closed conformation is 2587 $Å^2$. In adenylate kinase the area buried between the domains in the closed conformation is substantially smaller (650 $Å^2$). Nevertheless, the energetic penalty of opening the domains is overcome. Inspection of the non-polar buried surface areas at the inter-domain interfaces in the open conformations have illustrated that in those cases where the areas in the closed conformations are large, they are as large, or even larger in the open conformations. For example, for glutamate dehydrogenase the non-polar surface area buried between the domains in the open conformation is 4677 $Å^2$. Furthermore, interestingly, at least for the cases we have examined, if the non-polar buried surface areas are large in the open conformations, the extent of the opening of the domains is smaller. Thus, the interdomain distance in the open as compared to the closed conformation in glutamate dehydrogenase is 0.6 Å. On the other hand, in adenylate kinase the observed distance is 11 Å. These smaller hinge bending motions enable retention of the larger extent of non-polar buried surface at the interdomain interface. However, the side-chain contacts are not identical in the closed and open conformations, owing to conformational rearrangements. Hence, at least in the cases examined, smaller motions were generally observed between larger domains. And, conversely, in cases where a more limited extent of non-polar buried surface areas were observed in the closed conformations, the interdomain opening was larger, with a smaller extent of non-polar buried surface areas in the open conformations too. However, as expected, the non-polar surface areas buried within the domains is larger than that buried between them, whether in the closed or in the open conformation (N. S, S. Kumar and R. N., unpublished data).

Molecular hinges, topologies, and protein folding

The building blocks fold early in the folding process. Chain-connected folded building blocks are expected to trial-associate first. If the association is unstable, the building blocks will separate, with alternate associations taking place. Trial associations yield folding intermediates, trapped in local minima wells. Depending on the depth of the well, and the barrier heights, these would dissociate to continue rolling down the folding funnels. This process implies flexible regions existing between the building blocks. On the other hand, as discussed above, many of the hinges are critical to protein function.

Folding relates to function. Regions essential for folding, are also essential for function. We have already observed a relationship between folding and function. Ma *et al.* (35) and Kumar *et al.* (36) have shown that specific building blocks, which are critical for correct protein folding, are critical for function. With respect to function, in the cases that we have analyzed these critical building blocks were observed to be part of the binding sites. With respect to protein folding, these critical building blocks are in contact with a number of other building blocks, and are inserted between sequentially-connected ones. They bury a substantial extent of non-polar surface areas at their interfaces with other building blocks. Most importantly, if pulled out of the structure, the protein “shrinks”, collapsing upon itself.

While the conformations of the individual building blocks remain practically unchanged, they form an altered association.

That protein folding may relate to functional mechanisms is also straightforwardly shown through considerations of protein topology. Topologically similar proteins have hinges at the same sites (33). Topology determines the location of the hinge. Furthermore, topology largely determines the sites of small scale movements, as shown by analysis of mutant structures (37). There, regardless of the location, and the identity of the mutations, the regions that move the most are at the same sites. Hence, the location of the hinges is relatively immune to the details of the side-chain interactions, and the residue identity. However, in addition, topology determines the folding pathways of the protein. Folding is guided by the native state, with similar folds largely following similar folding pathways, and hence similar anatomy trees and similar building blocks cuttings. This suggests that we should expect that the location of the hinges be related to the building block cuttings along the protein chain. Below we illustrate that this is largely the case.

The proteins, and their anatomy trees

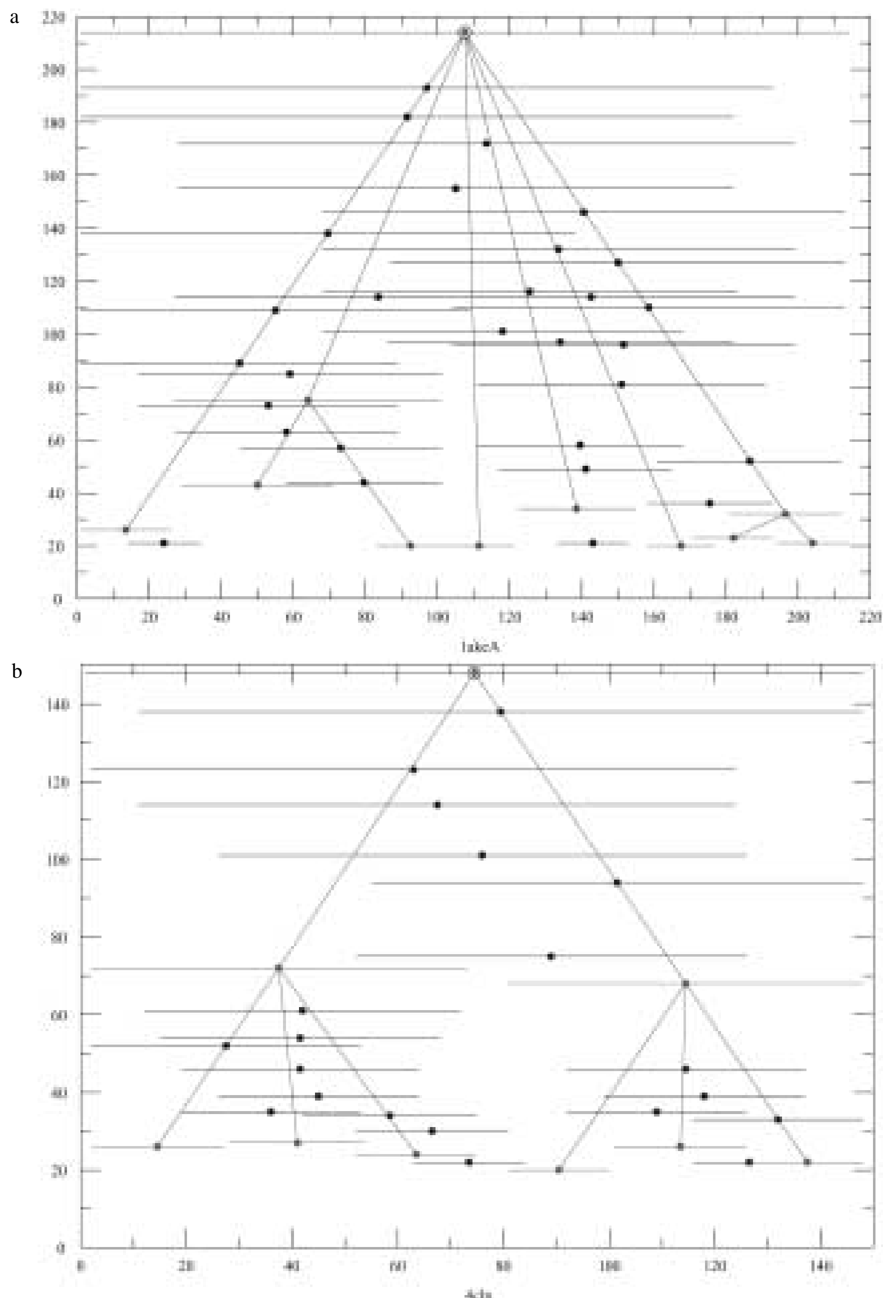
Table 1 lists some of the cases we use. It gives the protein names, PDB files, the positions of the hinging regions and the locations of the hinge points in the protein tertiary structures. It also notes whether these are 'domain' or 'fragment' motion cases. These categories were taken from the database of molecular motions.

The building blocks are cut along the protein chain using three parameters: the non-polar surface area buried within the building blocks, the compactness of the candidate building blocks, and the 'isolatedness', *i.e.*, the surface areas buried between the candidate building block and their structural neighbors which become exposed after the cutting. Some examples of the cuttings into building blocks are given in Figures 1a-f. Figures 1a-d depict cuttings of proteins illustrating domain motion (Adenylate kinase (1ake), Calmodulin (4cln), T4 lysozyme (1l96), Maltodextrin binding protein

Table I

Hinging domains and fragments: Protein sizes are shown in parentheses. [a]The region which moves with respect to the rest of the protein. The residue positions of the hinging regions are shown. The sizes of the hinging regions are shown in parentheses. [b]A and B categories show the levels of correspondence between hinges and building block assignments. The cases in category A are those where the hinging region meets any of the following criteria: (*i*) It is an independent building blocks; (*ii*) It is connected to the rest of the protein *via* an unassigned region; (*iii*) It does not share a building block with rest of the protein. Cases in category B are where the hinging region does not meet any of the above criteria, and shares a building block with a small part of the rest of the protein. Thus, the cases in category A have higher level of correspondence. However, a more extensive analysis needs to be carried out.

Protein	PDB ID	Hinging region[a]	Hinge location	Correspondence[b] (category)
Domain motions				
Adenylate kinase	1ake A (214)	118-163 (46)	Coil	A
Calmodulin	4cln (148)	*N*-72 (72)	α-helix	A
T-4 lysozyme	1l96 (164)	*N*-59 (59)	Coil	A
Maltodextrin BP	1anf (370)	*N*-109 (109)	β-strand	A
Fragment motions				
Triosephosphate isomerase	1tti (243)	166-176 (11)	Coil	B
Lactate dehydrogenase	1ldm (329)	96-110 (15)	Coil	B
HIV protease	3hvp (99)	33-62 (30)	β-strand, Coil	A
Seryl-tRNA synthetase	1ser B (421)	528-598 (71)	α-helix	A



(1anf)). Figures 1e-f provide the building blocks cutting for proteins showing fragment motions (Triosephosphate isomerase (1tti), Lactate dehydrogenase (1ldm), HIV proteinase (3hvp), Seryl-tRNA synthetase (1ser B)). For a sample of these cases we also provide the fragment maps (Figures 2a-d), *i.e.*, all the fragments constituting local minima on the protein sequence. Figures 3a-d depict the corresponding top-down anatomy trees. Figures 2a-c and 3a-c are for domain hinge bending cases (adenylate kinase, calmodulin and T-4 lysozyme). Figures 2d, 3d are for fragment hinge bending cases (HIV protease). The protein structure is iteratively cut as described in Tsai *et al.* (3). The anatomy trees (Figs. 3a-d) provide the major folding pathways of the proteins, corresponding to the red fragments and their connecting lines in Figures 2a-d. The colored building blocks in Figures 1a-f correspond to the lowest level of cuttings, *i.e.*, the red fragments at the bottom of the fragment maps in Figures 2a-d and at the node-leaves of the anatomy trees (Figures 3a-d). Figure 4 shows the building block assignments at all the levels of anatomy trees. Figures 5 a-h illustrate the hydrophobic folding units into which the building blocks assemble. Figures 5a-d are domain hinge-bending cases, corresponding to Figures 1a-d, and Figures 5e-h are the hydrophobic folding units of proteins showing fragment hinge-

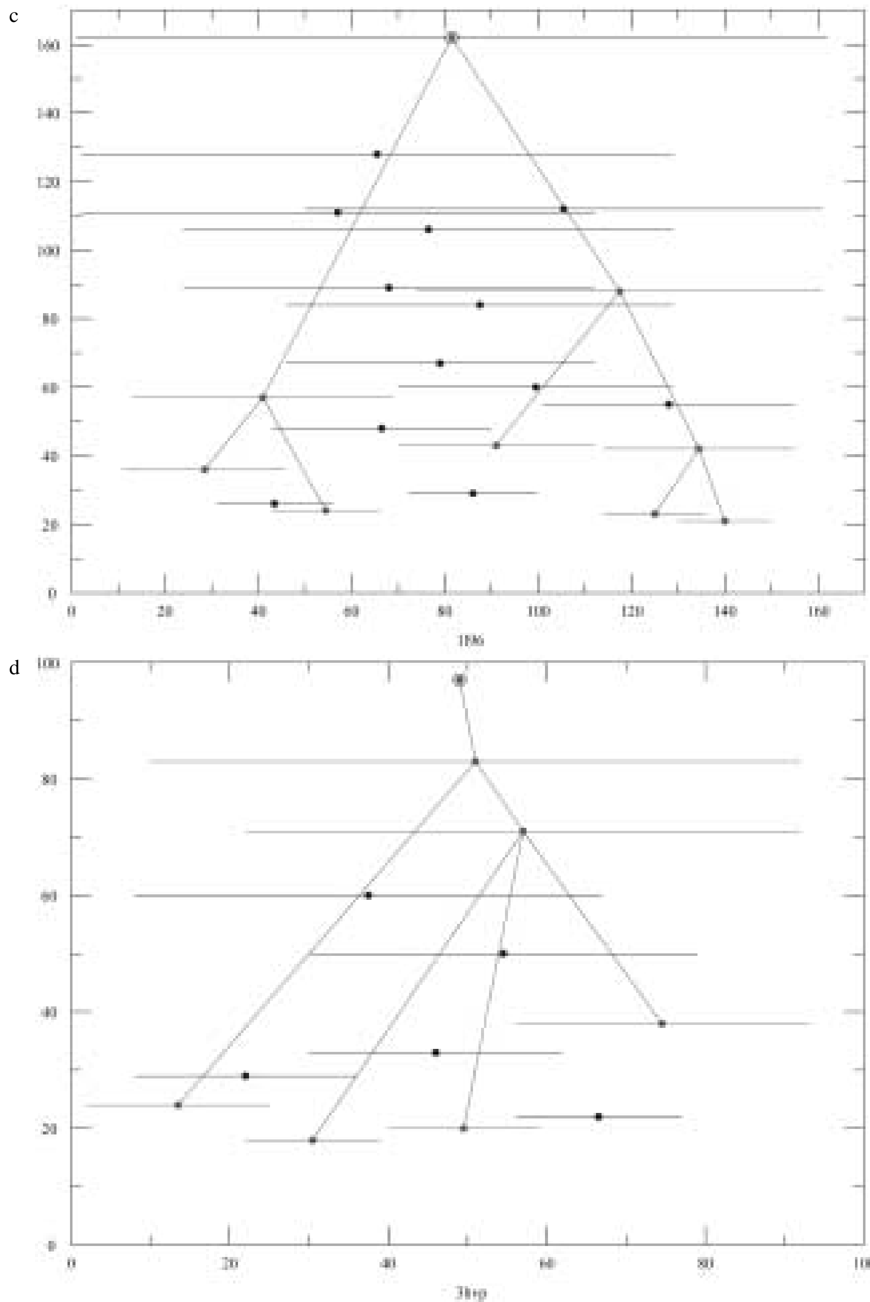


Figure 2: Fragment maps for four of the proteins we have examined, (a) Adenylate kinase (1ake), (b) Calmodulin (4cln); (c) T4 lysozyme (1l96); and (d) HIV proteinase (3hvp). The first three are domain hinge-bending cases. The last is a fragment hinge-bending case. The X-axis marks the position on the sequence. The Y-axis marks the building block size. The building block fragments are local minima on the structure. The building blocks drawn in red are the ones whose combinatorial assembly yields the major protein folding pathway. Further details are given in Tsai *et al.* (3).

bending, corresponding to the proteins shown in Figures 1e-h. Details of the cutting algorithm, the calculations of the stability function, and the detailed step by step examples and their correspondence with experiment, can be found in Tsai *et al.* (3).

The locations of the hinges

The classification of domains and fragments is taken from the molecular motions database (4). A fragment may be either a surface loop, or it may be too small to be classified as a domain. The building block assignments are considered to fit the hinge-bending locations if any of the following criteria is satisfied: (*i*) The location of the hinge-bending falls between independent building blocks; (*ii*) It is separated from the rest of the protein via an unassigned region; (*iii*) It does not share a building block with the rest of the protein. In all the domain movement cases we have studied, the building block assignments are in agreement with the movements. Some of our results are shown in Figures 1a-h. In calmodulin (4cln) (Fig. 1b) the hinge position lies in an α-helix (38), just following the green building block. Part of the α-helix is unassigned. In the case of T-4 (1l96) lysozyme the hinge position (27) lies just following the green building block (Fig. 1c). Similarly, in adenylate kinase (Fig. 1a) the hinges (39) are just prior to the yellow building block and just following the

blue building block. Figure 4 shows that building block assignments agree with the locations of hinges, at almost all levels of the anatomy tree, for both hinging fragment and hinging domain cases. In all the fragment movement cases we have examined, where the building block assignment slightly disagrees with the location of the

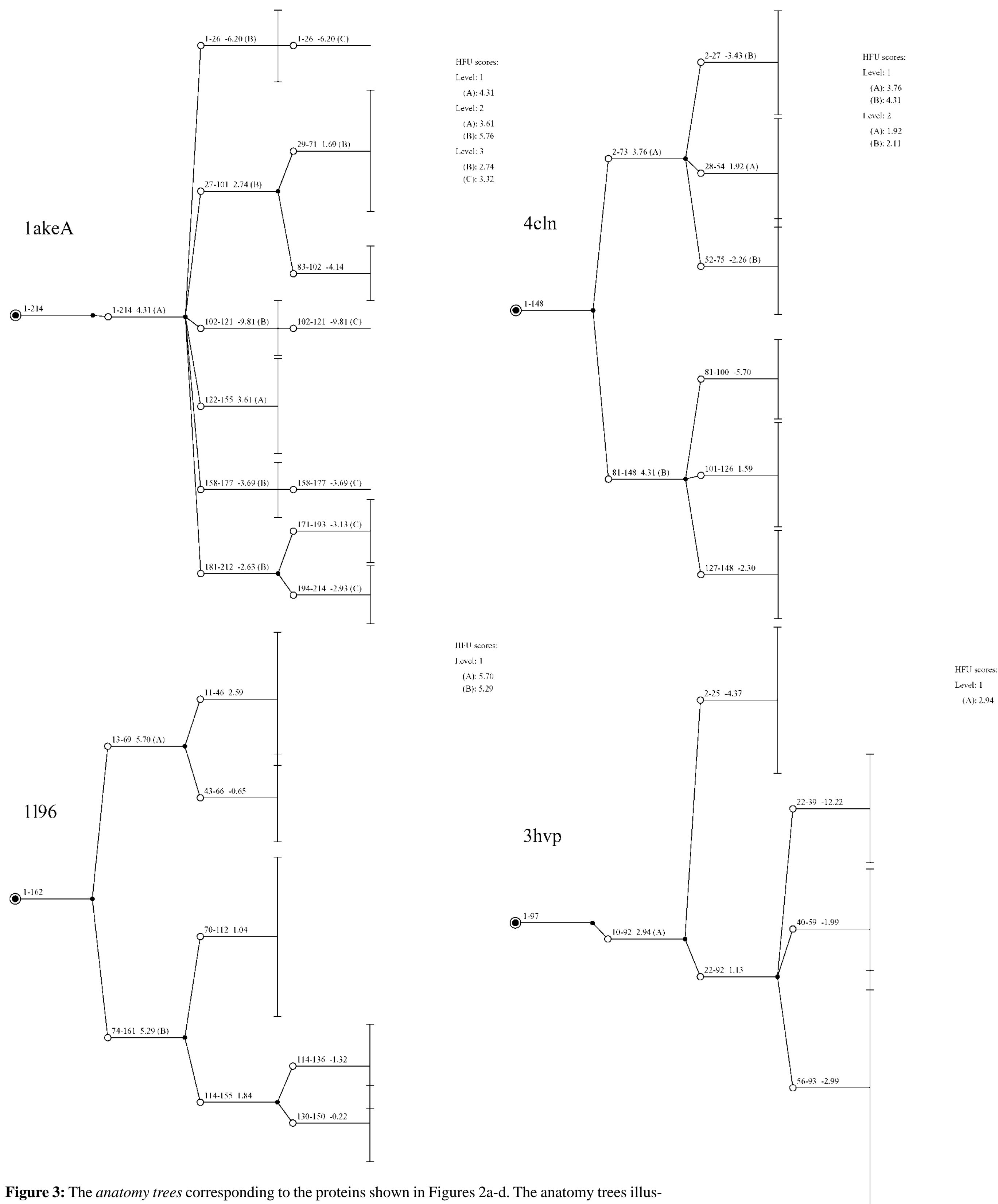


Figure 3: The *anatomy trees* corresponding to the proteins shown in Figures 2a-d. The anatomy trees illustrate the major folding pathway of the protein. These can be traced by the red building block fragments, and their red line connectors.

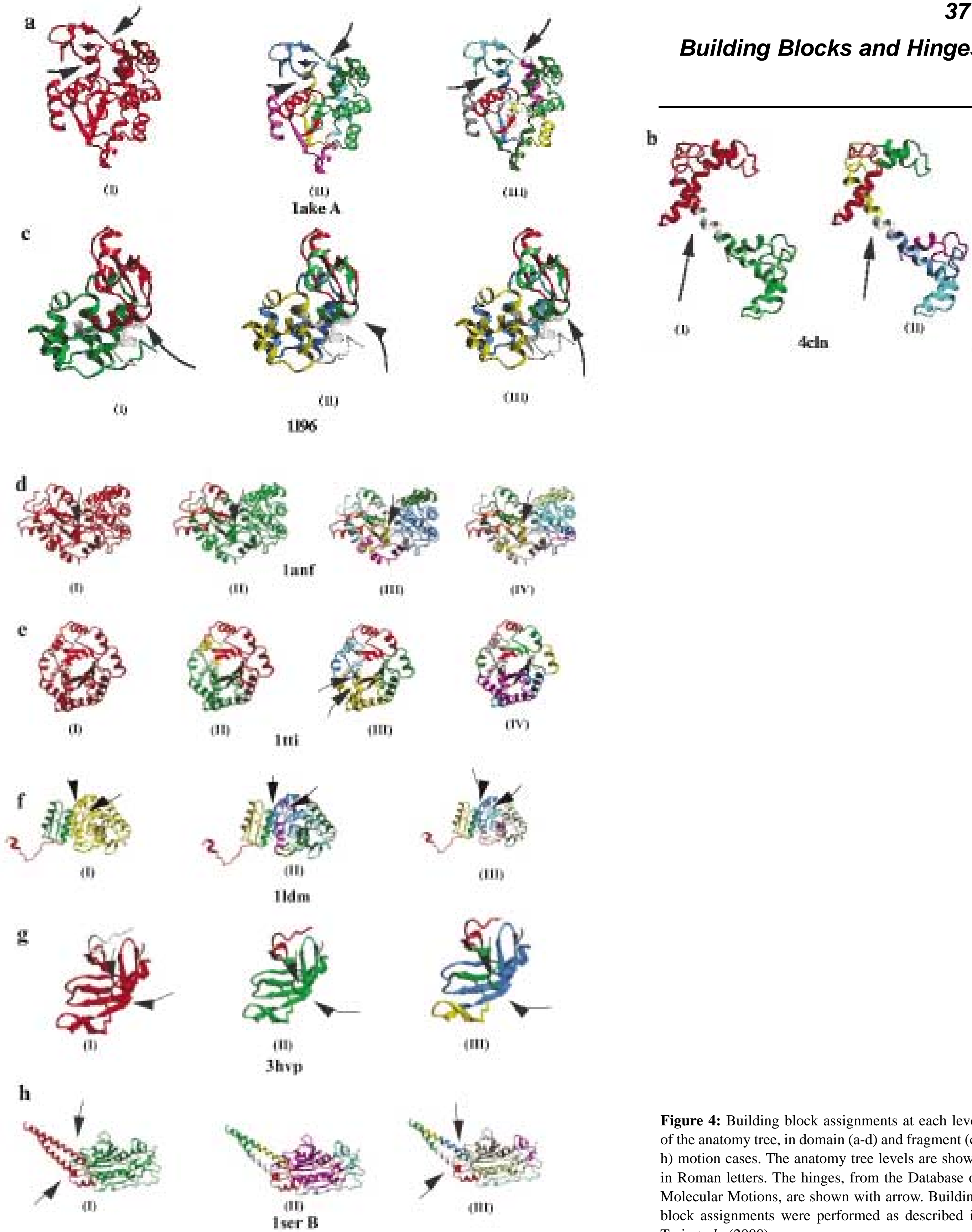


Figure 4: Building block assignments at each level of the anatomy tree, in domain (a-d) and fragment (e-h) motion cases. The anatomy tree levels are shown in Roman letters. The hinges, from the Database of Molecular Motions, are shown with arrow. Building block assignments were performed as described in Tsai *et al.,* (2000).

hinges, the fragments are of relatively small size (9 - 14 residues), and consist largely of surface loops.

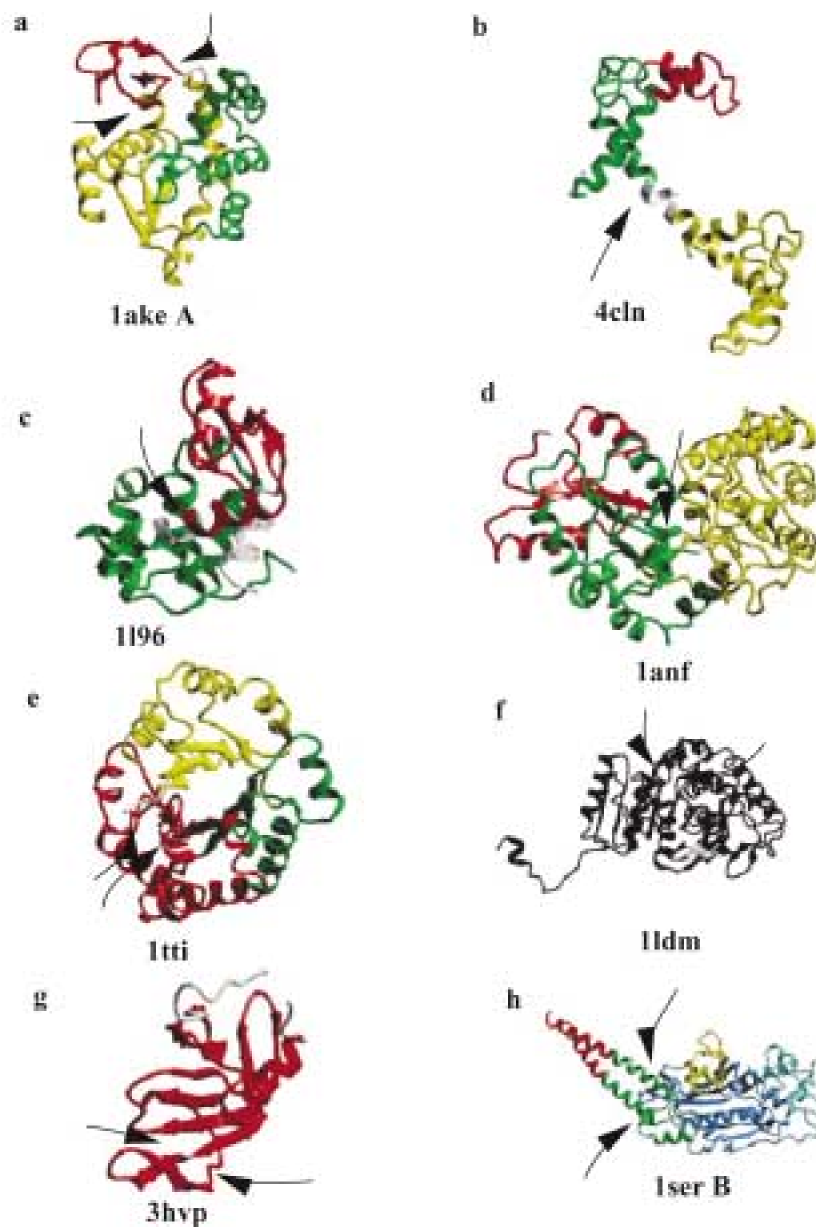


Figure 5: The hydrophobic folding units (HFUs) produced via a *combinatorial assembly* process of the building blocks. (a-d) fall in the category of domain movement cases; (e-h) fragment movement cases. (a) Adenylate kinase (1ake); (b) Calmodulin (4cln); (c) T4 lysozyme (1l96); (d) Maltodextrin binding protein (1anf); (e) Triosephosphate isomerase (1tti); (f) Lactate dehydrogenase (1ldm); (g) HIV proteinase (3hvp); (h) Seryl-tRNA synthetase (1ser B). Each color depicts a different HFU. The hinge points for the movements are marked by arrows. These points were taken from the molecular motions database (4).

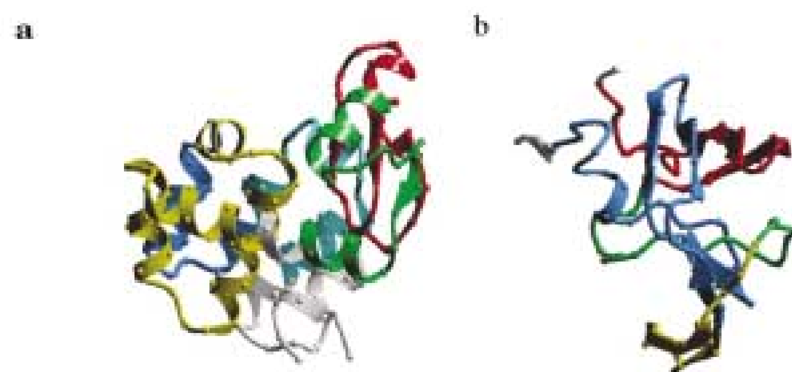


Figure 6: Building block assignments of the mutant structures, in the cases of T-4 lysozyme (a) (PDB: 1l36) and HIV protease (b) (PDB: 1gnn A). The assignments at the final level of the anatomy tree is shown here. 1l36 and 1gnn are E128a, V131a, N132a and V82d point mutants, respectively.

Figures 5a-h illustrate that there is a correspondence between the locations of the hinges and the assignments of the hydrophobic folding units (HFUs). The hydrophobic folding units are stable, independently folding units, with a strong hydrophobic core. They are the outcome of a combinatorial assembly process of the conformationally fluctuating building blocks. In all the cases of hinging domains, the HFU assignments agree with the location of hinges. Among the swivelling fragments, in the cases of triosephosphate isomerase and seryl-tRNA syn-

thetase the HFU assignments agree with the hinge locations. On the other hand, both lactate dehydrogenase and HIV protease are comprised of a single unit HFUs.

Protein topologies

Figures 6a,b present two cases of homologous proteins. These are point mutant structures of T-4 lysozyme (Fig. 6a) and HIV protease (Fig. 6b). The figures illustrate that for similar topologies, despite the dissimilarities in the details of the sequences and the side-chain contacts, the building blocks cutting and the location of the hinges are same. Additional comparisons of both building blocks cuttings across families, and of the locations of building blocks in the SCOP families, has illustrated the consistency in the recurrences of the locations of the hinges (40).

Conclusions

Here we show that for those cases we have examined, the locations of the hinges in protein molecules largely fall in regions separating building blocks. A larger, more extensive analysis needs to be carried out to confirm this observation. In the cases we have analyzed, the hinges are either at the joints of the building blocks, or in the less stable `unassigned' regions. Building blocks are fragments along the protein chain which constitute local minima. These regions fold first. Subsequently, via combinatorial assembly they associate to finally yield the native protein fold. This suggests that these regions should be more flexible to enable the trial-association process.

Molecular hinges are essential for protein function. Binding, catalysis and motility are all enabled by movements of parts of the protein with respect to each other. Rigidifying the proteins results in reduced activity. On the other hand, too much flexibility is also selected against, as shown by avoiding Gly at the hinge (41). Hence, here we illustrate that folding and function are inter-related. Fragments along the protein chains which are critical for function are also critical for folding (35,36). The more flexible regions responsible for enabling swiveling of local folded elements to interact with each other are also those where the hinges are likely to be important functionally.

Both folding and the location of the hinges are determined by the protein topology. Hence a correlation between folding and hinge-regions makes sense.

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