Conformational Changes Associated with Protein-Protein Interactions

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"Teaser": A discussion of current articles revealing conformational changes upon protein-protein binding shows there is increasing experimental data supporting the hypothesis that unbound proteins exist in multiple conformations.

Summary

Motions related to protein–protein binding events can be surveyed from the perspective of the Database of Macromolecular Movements. There are a number of alternate conceptual models that describe these events, particularly induced fit and pre-existing equilibrium. There is evidence for both alternatives from recent studies of conformational change. However, there is increasing support for the pre-existing equilibrium model, whereby proteins are found to simultaneously exist in populations of diverse conformations.

Introduction

The Database of Macromolecular Movements [1,2] (http://molmovdb.org/) currently stores over 250 distinct molecular motions, most of which are based on solved structures [3]. While these conformational changes can occur upon environmental changes such as varying pH and temperature levels, the majority of large conformational movements in the database take place upon the event of binding interactions between proteins, ligands, sugars, lipids, and/or small molecules. Here we focus on protein-protein interactions and the diverse conformational changes that are observed upon protein binding.

Several different models have attempted to explain protein binding mechanisms (Figure 1). The "lock and key" concept, originally introduced by Emil Fischer in 1894, assumes that one protein has a cavity or indentation that another protein perfectly fits into. These two proteins can interact with almost no change in conformations. However, this model cannot account for proteins that can bind various substrates that have a different shape from the ligand. The "induced fit" model [4] introduced by Daniel Koshland in 1958, tries to account for this by assuming that there is a certain amount of plasticity in the active site to accommodate its ligand, analogous to a hand and a glove. The ligand would induce a conformational change at the binding site, shifting it toward an active state.

The pre-existing equilibrium hypothesis [5] is based on protein folding theories of the funnel energy landscape[6-9]. The native state of the protein contains an ensemble of conformations at its binding site. The ligand will bind selectively to an active conformation, thereby biasing the equilibrium toward the binding conformation.

For proteins that exhibit allosteric behavior, the binding of a ligand in one area of a protein can affect the conformation of the protein at a distant region away from the binding site. The Monod-Wyman-Changeux (MWC) model [10] describes a two-state switch. The model assumes an equilibrium between the two conformational states for the protein in the unbound state – tense (T) and relaxed (R). When the ligand binds, the equilibrium is shifted toward the relaxed, or high affinity state. This model explains positive cooperativity but doesn't address the issue of negative cooperativity as well [11]. The Koshland-Nemethy-Filmer (KNF) model [12] discusses how individual subunits of oligomeric proteins will switch states in response to ligand binding. Subsequently some molecules can exist with some subunits in the weak binding state and others in the strong binding state. This model can adequately describe both positive and negative cooperativity. A more recent model for cooperative regulation, termed the dynamic population shift model, was proposed [13]. The dynamic population shift model assumes

that proteins exist in a population of conformations. Upon ligand binding, the probability distribution of the ensemble of native states will be redistributed, changing the stability of certain residues throughout the protein molecule and propagating a conformational change at specific residues.

In the past few years, several important studies have presented structures of complexes that have exhibited conformational rearrangements upon binding and provided further insight into the function of these proteins. Above, we described several models of binding mechanisms and here we will discuss how specific examples relate to these models. First, we will discuss some induced fit interactions. These systems are assumed to be induced fit binding mechanisms since there is no experimental evidence for a pre-existing equilibrium of multiple conformations. We will also present studies that support the pre-existing equilibrium model and, finally, we will describe occurrences of dynamic population shift in allosteric regulation mechanisms. Table 1 provides a more comprehensive viewpoint of motions and their corresponding models.

Induced Fit

An electron cryo-microscopy study illustrates that the closing of myosin's actin-binding cleft is structurally coupled to the opening of the nucleotide binding pocket [14]. Initiation of binding occurs in a weak stereospecific interaction where the myosin lower domain contacts with the actin filament in an open conformation (Figure 2A). As this interaction progresses toward strong binding, the cleft in the myosin domain closes as has been previously suggested [15]. The upper myosin domain swings around at a rotation of 21 degrees so the cardiomyopathy loop comes in contact with the actin surface, thus doubling the total interaction surface (Figure 2B). This creates a displacement movement of 6-9 A in the switch 1 element, which contains the nucleotide-binding pocket. These studies therefore indicate that strong binding to actin opens up the nucleotide-binding pocket. This and other studies suggest that strong binding of the myosin cross-bridge to actin probably closes the actin-binding cleft [16,17].

A structure of the FliS-FliC complex demonstrates the binding mechanism of the bacterial export chaperone and its role in type III secretion [18]. FliC monomers polymerize to form the tail filament of the bacterial flagellum [19]. Export chaperone FliS binds specifically to FliC [20,21] in order to aid the correct assembly of the bacterial flagellum and avoid premature interactions with other structural components of the flagellum [22,23]. The structure of FliS is an antiparallel four-helix bundle with a quasi-helical cap formed by 16 N-terminal residues [18]. Upon binding FliC, the N-terminal cap of FliS is displaced and re-orients to form a short helix on one side of the helical bundle (Figure 3) while a helical segment of FliC (residues 499-505) moves into the position that was formerly occupied by the FliS N-terminal cap. This suggests that the N-terminal cap of FliS works as a "molecular stopper" to block the hydrophobic binding site when FliS is not bound to FliC.

A recent crystal structure of a Gα subunit bound to the GoLoco motif found in regulatory proteins highlights important residues that control the specificity of the GoLoco-Gα interactions and suggests mechanisms for preventing the binding of Gβγ [24]. Regulatory proteins with the 19-amino-acid GoLoco motif [25,26] can bind to Gα subunits and maintain G-protein subunit disassociation [24,27-30]. Go-Loco motif proteins interact specifically with GDP-bound $G_{i/o}$ Gα subunits, preventing both GDP

release [24,27-29] and Gβγ reassembly [29,30]. Kimple *et al* [24] determined the crystal structure of the RGS14 GoLoco region bound to an adenylyl cyclase-inhibitory Gα subunit (α_{i1}·GDP). Interactions with the R14GL peptide (residues 496-530 of rat RGS14 containing the GoLoco region) is shown to alter the conformations of switch I-III relative to Gβγ-bound α_{i1}·GDP·Mg^{2+}. In particular, the deviation in switch II (where Arg 208 moves about 6 A) could hinder Gβγ binding to GoLoco-complexed α_{i1}·GDP. The largest change occurs in the αB-αC loop of the $G\alpha_{i1}$ helical domain where Ala 114 is displaced by 11 A away from the Ras-like domain.

Pre-Existing Equilibrium

There is some experimental data that can discriminate between induced fit and pre-existing equilibrium models. For hemoglobin, numerous convincing experiments support the MWC model and rule out the induced-fit model [31]. Recently, there have been a number of studies reported that support the pre-existing equilibrium hypothesis. A breakthrough study reported by James *et al.* [32] presents crystal structures of a monoclonal IgE antibody, SPE7, that exists in two very different conformations each binding structurally distinct antigens. The predominant unbound isomer (Ab1) has a flat, regular binding site, which is reminiscent of antibodies that bind proteins or peptides. The alternative isomer contains a deeper, funnel-shaped pocket, typical for binding haptens (small molecules that become antigenic when bound to proteins). The recombinant protein antigen, TrxShear3 that binds to Ab^1 does not bind to Ab^2, and haptens do not bind to Ab^1. These conformations involve large backbone alterations of the H3 and L3 loops with Cαs deviating up to 6.7 A (Figure 4). The H3 loop flips between the Ab1 and Ab2 isomer, displaying different side-chain rotamers. This study highlights the potential role of conformational diversity in cross-reactivity which can lead to auto-immune disease and allergy [33].

Two recent crystal structures by Celikel *et al* [34] and Dumas *et al* [35] (Figure 5) illustrate the existence of an ensemble of possible conformations for bound proteins. The authors describe the thrombin-GpIbα interaction, but obtain very different crystal forms. Both these papers show two thrombins bound to every glycoprotein GpIbα fragment – one thrombin bound through exosite I and the other bound through exosite II. While the first thrombin is bound to approximately the same region of GpIbα in both structures, these structures display completely different contacts and are approximately rotated 180^o about an axis perpendicular to the interface. Additionally, the structures of the second thrombin interface show the flexible anionic segment of GpIbα rotated 90^o, resulting in a 37 A displacement for Tyr^{279}, a sulfated tyrosine located on the anionic segment and shown to be necessary for optimal thrombin binding [36]. These two structures of thrombin-GpIbα binding illustrate the very different conformations that GpIbα can have while bound to the same protein (Figure 5). Further studies may be able to elucidate the predominant structural interface and lead to better functional understanding of the thrombin-GpIbα interaction.

Allosteric Regulation - Dynamic Population Shift Model

Although allosteric regulation is well accepted for multidomain proteins, it is not as commonly thought of in single domain proteins. However, it wasn't until a recent

NMR study by Volkman *et al.* [37] presented evidence for allostery in a single domain signaling protein. The paper describes a population shift induced by ligand binding in the phosphorylation-regulated bacterial response regulator NtrC. This work characterized the motions of NtrC in the unphosphorylated and phosphorylated states (Figure 6). For unphosphorylated NtrC, both the active and inactive conformations are evident. However, upon phosphorylation, the protein is activated and the equilibrium is shifted toward the active conformation. Similarly, a previous study had reported multiple conformational states for apo-calmodulin, illustrating a conformational exchange process [38]. It was shown that the unbound calmodulin exists between a predominantly closed conformation with a smaller population of more open conformations. In membrane proteins, kinetic studies performed on the allosteric transitions of *Torpedo* acetylcholine receptors showed that without the presence of ligand, 11% of the receptors pre-exist in the activated (desensitized) conformation [39]. In the presence of the ligand, this population increases to 85%. Nevo *et al*. [40] presented further evidence of multiple conformational states in macromolecular complexes such as the Ran-importin β1 (impβ) binding interaction. This study demonstrated the existence of two distinct bound conformational states when impβ is associated with Ran that is loaded with a nonhydrolyzable GTP analog, GppNHp.

Discussion and Conclusions

As more experimental work is performed to characterize the dynamics of binding interactions, it is becoming more evident that proteins can exist in an ensemble of conformational states. If this hypothesis is true, then unbound proteins should have a population of activated conformers and exhibit some activity. This seems to occur in proteins such as the single-domain response regulator CheY which shows a low level of activity in its unphosphorylated state [41]. However, other proteins do not exhibit a basal level of activity in their unbound state. It is possible that these proteins, like NtrC, may require a certain amount of activated conformers in order to demonstrate activity [42] and show a sharp signal response.

The results reported here show that unbound proteins can exist in different conformational states. Flexibility within regions of a protein allows it to adopt new conformations and, in turn, bind structurally distinct ligands. This ability of proteins to adopt multiple structures allows for functional diversity without depending on the evolution of sequence diversity, which can greatly facilitate the potential for rapidly evolving new functions and structures [43].

Supplemental Material

Most of the structures discussed for which 3D data is available are listed online at http://molmovdb.org/cosb. These listings include additional images and animations.

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FIGURES

Figure 1
Models of protein binding mechansisms. (A) Lock and Key Model. (B) Induced Fit Model. (C) Pre-Existing Equilibrium Model.

Figure 2
Overview of myosin (yellow) and its upper 50 kDa domain (red) docked to actin (blue) using a (A) rigid-body X-ray model of myosin in its open cleft, weak binding form and (B) fit with an independent upper 50 K domain that illustrates its closed, strong binding form. This figure was produced with PyMOL [44].

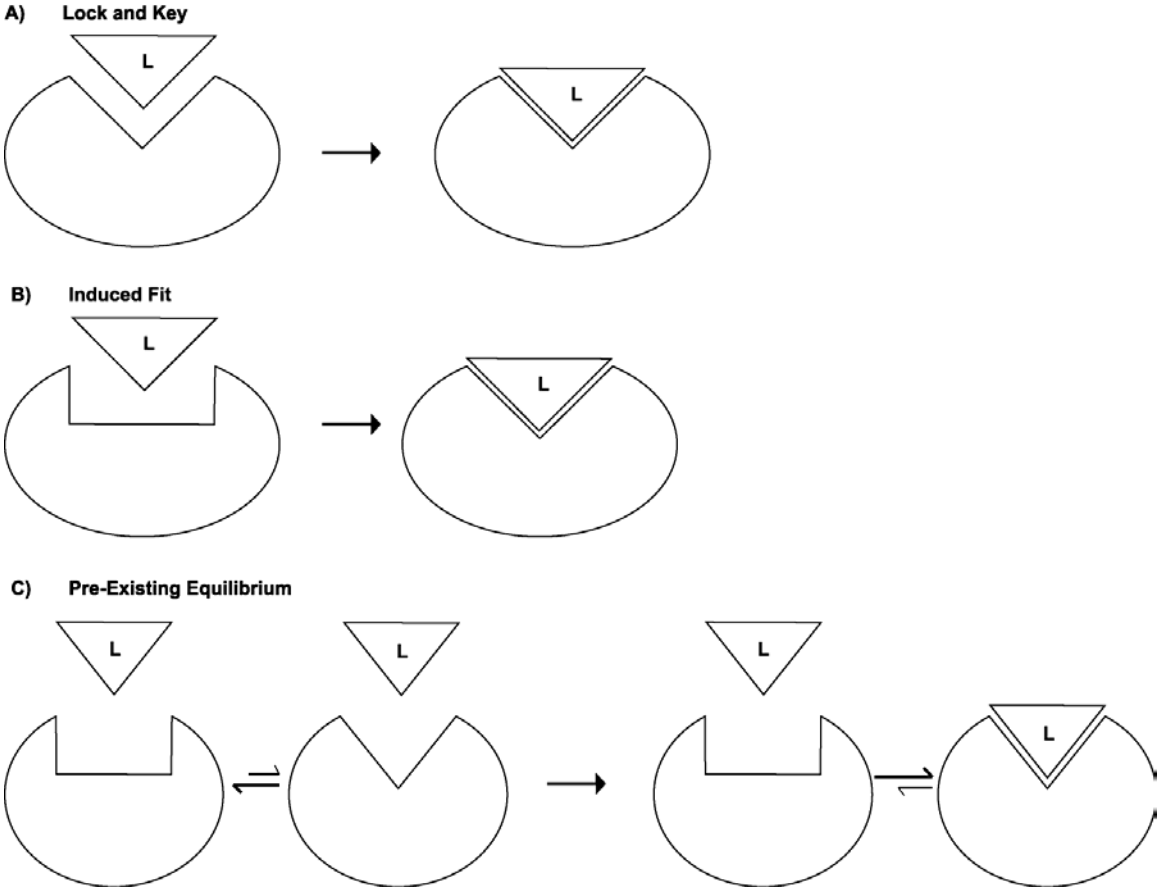
Figure 3
Structural conformations of (A) uncomplexed FliS and (B) FliS when bound to FliC.

Figure 4
Main chain configurations of IgE antibody Spe7 – free isomers Ab^1 (green) and Ab^2 (purple), hapten-bound isomer Ab^3 (blue), and Trx-Shear3-bound isomer Ab^4 (ochre). This figure was provided by Dan Tawfik.

Figure 5
The GpIbα-thrombin interface. Surface representations of Celikel (top) and Dumas (bottom) crystal structures. The GpIbα amino-terminal fragment (gray) is shown with its anionic segment (orange). The part of thrombin binding to GpIbα through exosite I(TI, dark blue) is shown in pale blue, and the part binding through exosite II (TII, dark green) is shown in pale green. This figure was reprinted with permission from JE Sadler, Science, Vol 301 (2003) [45]. Copyright 2003 American Association for the Advancement of Science.

Figure 6
Structural conformations of the (A) Unphosphorylated form of NtrC and the (B) Phosphorylated form of NtrC (cyan) superimposed on the unphosphorylated form (gray). This figure was produced with PyMOL [44].

Figure 1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C) Pre-Existing Equilibrium
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Figure 2A

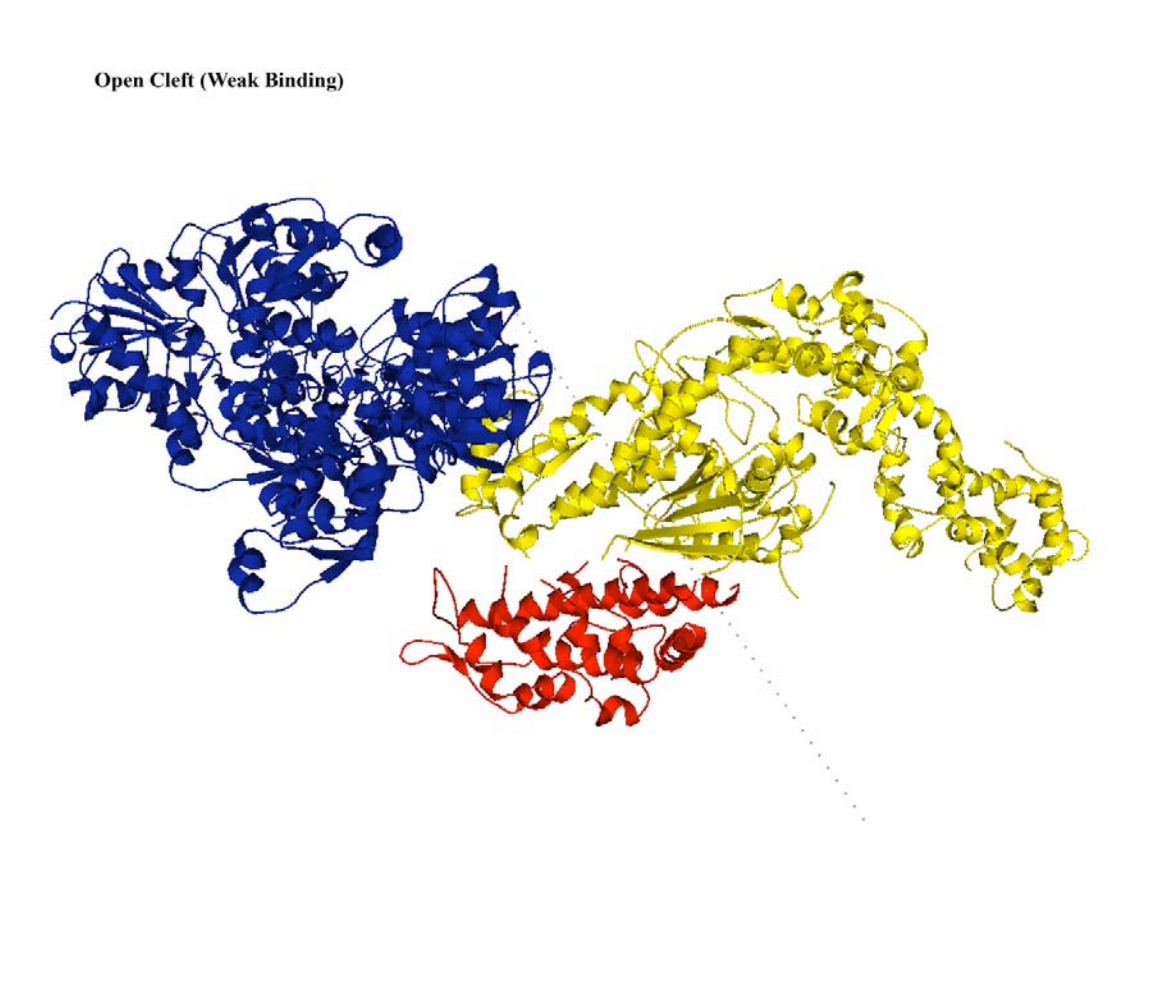


Figure 2B

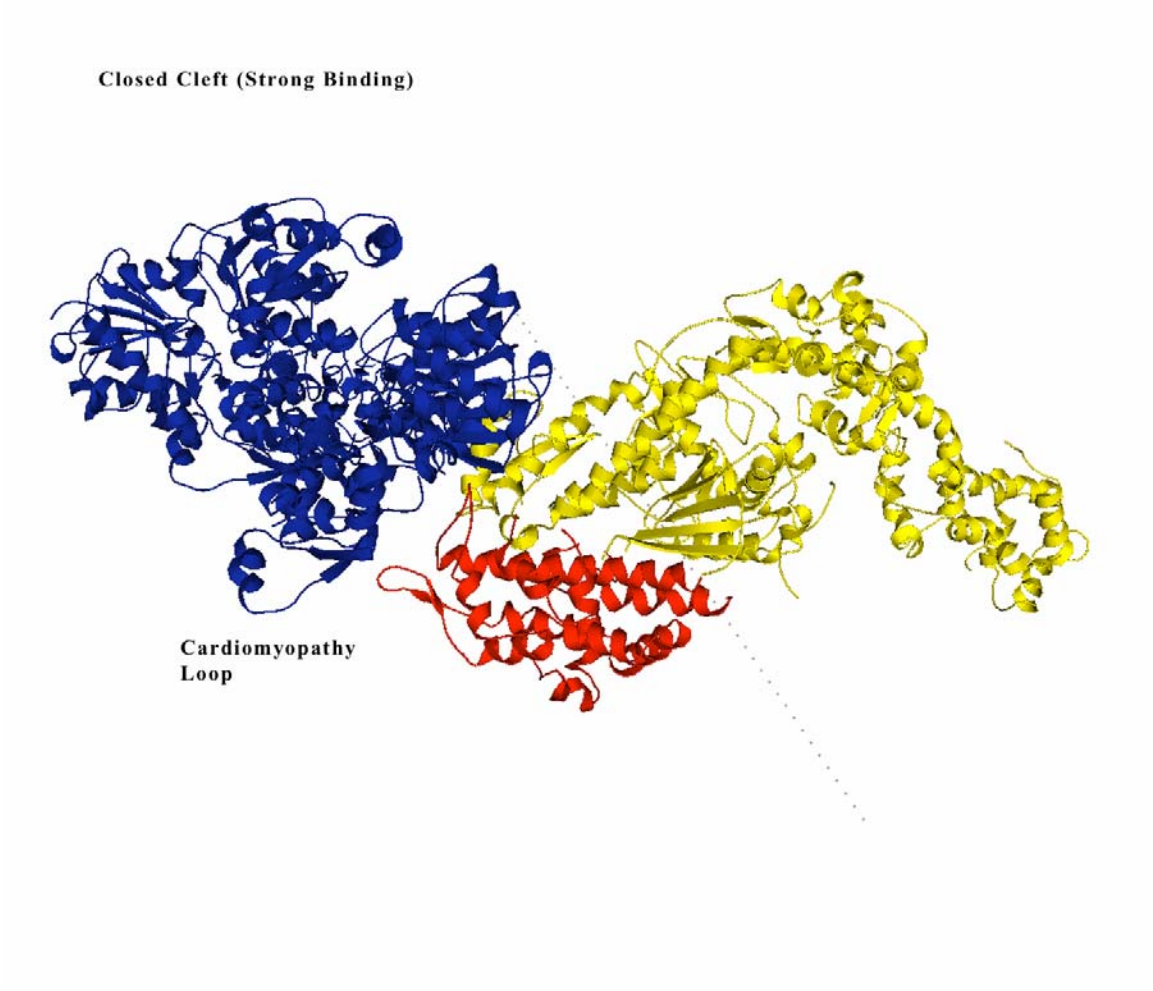
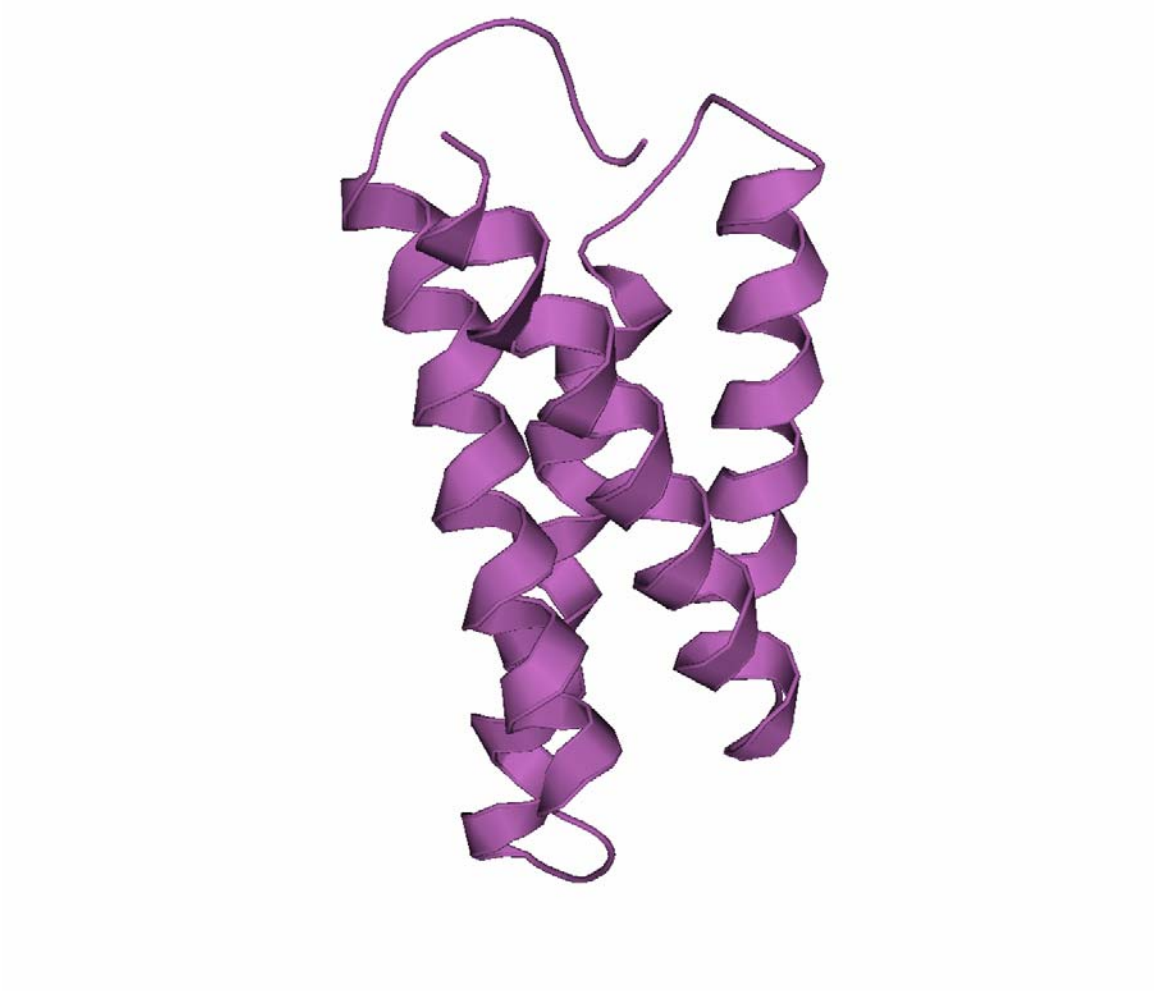


Figure 3A



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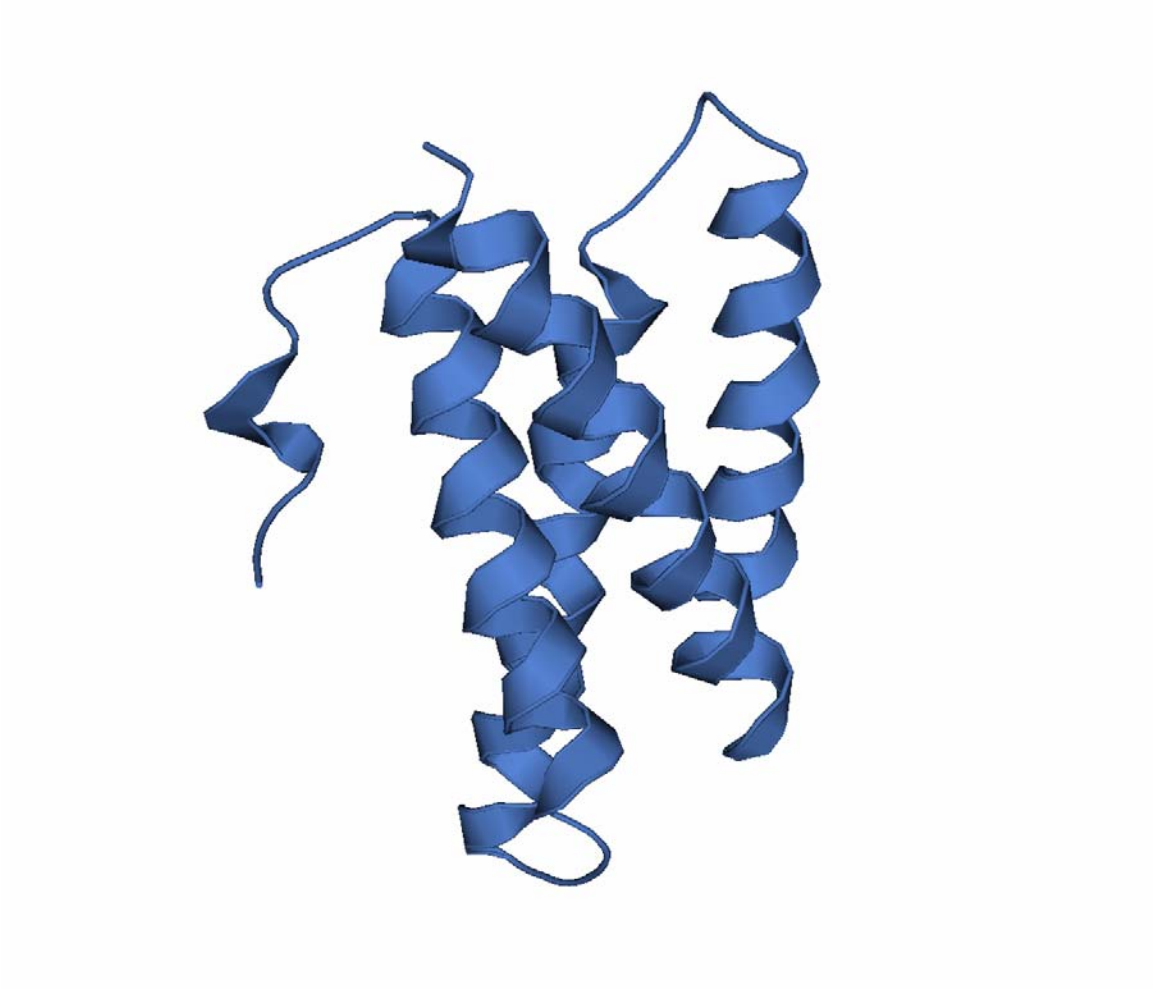

Figure 4

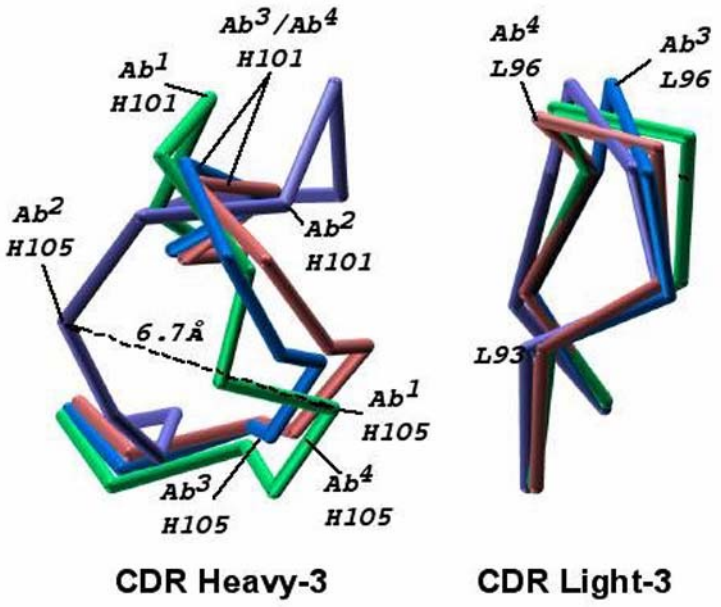
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Figure 5

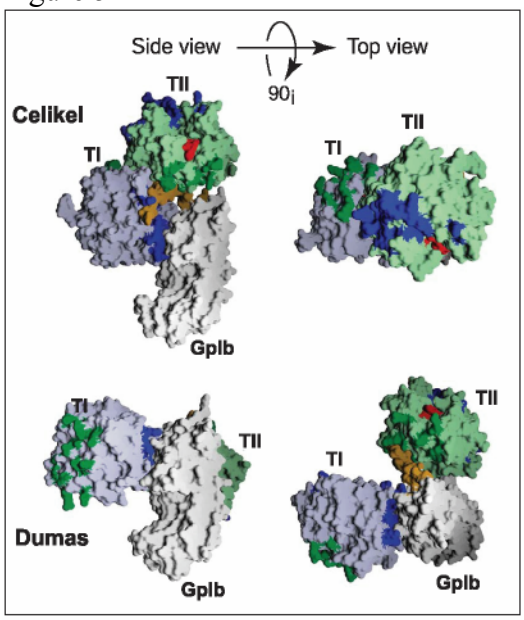
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Figure 6A

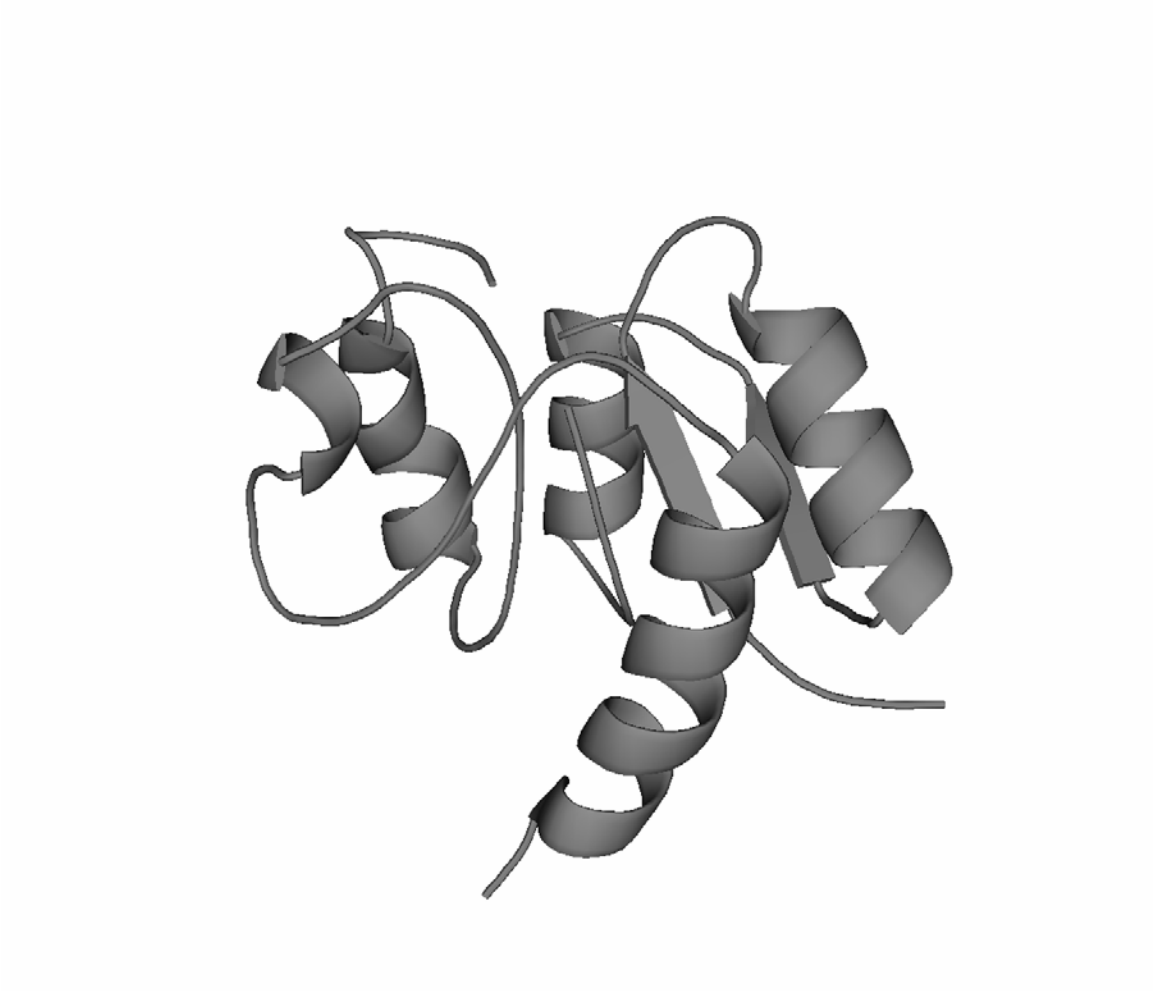

Figure 6B