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Conformational changes associated with protein–protein interactions

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Motions related to protein–protein binding events can be surveyed from the perspective of the Database of Macromolecular Movements. There are a number of alternative 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.

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Introduction

The Database of Macromolecular Movements [1,2] (http://molmovdb.org/) currently stores descriptions of over 250 distinct molecular motions, most of which are based on solved structures [3]. Although these conformational changes can occur upon environmental change, such as varying pH and temperature levels, the majority of the 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.

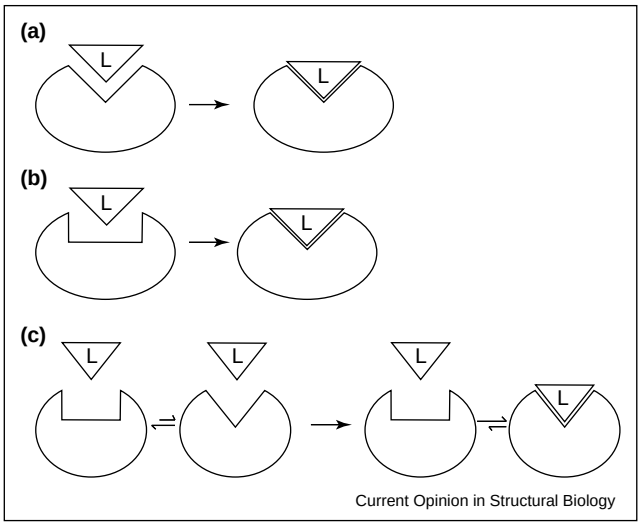
Several different models have attempted to explain protein binding mechanisms (Figure 1). The 'lock and key' concept, originally introduced by Emil Fischer in 1894, assumes that one protein has a cavity or indentation that another protein perfectly fits into. These two proteins can interact with almost no change in conformation. However, this model cannot account for proteins that can bind various substrates with different shapes. The 'induced-fit' model, introduced by Daniel Koshland in 1958 [4], 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 exhibits 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 to one area of the 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 of the unbound protein — 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 [11]. The Koshland-Nemethy-Filmer (KNF) model [12] discusses how individual subunits of oligomeric proteins will switch states in response to ligand binding. Consequently, some proteins 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 of cooperative regulation, termed the dynamic population shift model, has been 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.

During the past few years, several important studies have presented the structures of complexes that exhibit conformational rearrangements upon binding and provided further insight into the function of these proteins. Above, we have described several models of binding mechanisms and we will now 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 because there is no experimental evidence of a pre-existing equilibrium of multiple conformations. We will also present studies that support the pre-existing equilibrium model and, finally, we will

Figure 1



Models of protein binding mechanisms. **(a)** Lock and key model. **(b)** Induced-fit model. **(c)** Pre-existing equilibrium model. L, ligand.

describe occurrences of dynamic population shift in allosteric regulation mechanisms. Table 1 provides a more comprehensive overview 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 through a weak stereospecific interaction whereby the lower domain of myosin contacts 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 by a rotation of 21°, so that the cardiomyopathy loop comes in contact with the actin surface, thus doubling the total interaction surface area (Figure 2b). This creates a displacement movement of 6–9 Å in the switch 1 element, which contains the nucleotide-binding pocket. These studies therefore indicate that strong binding to actin opens up the

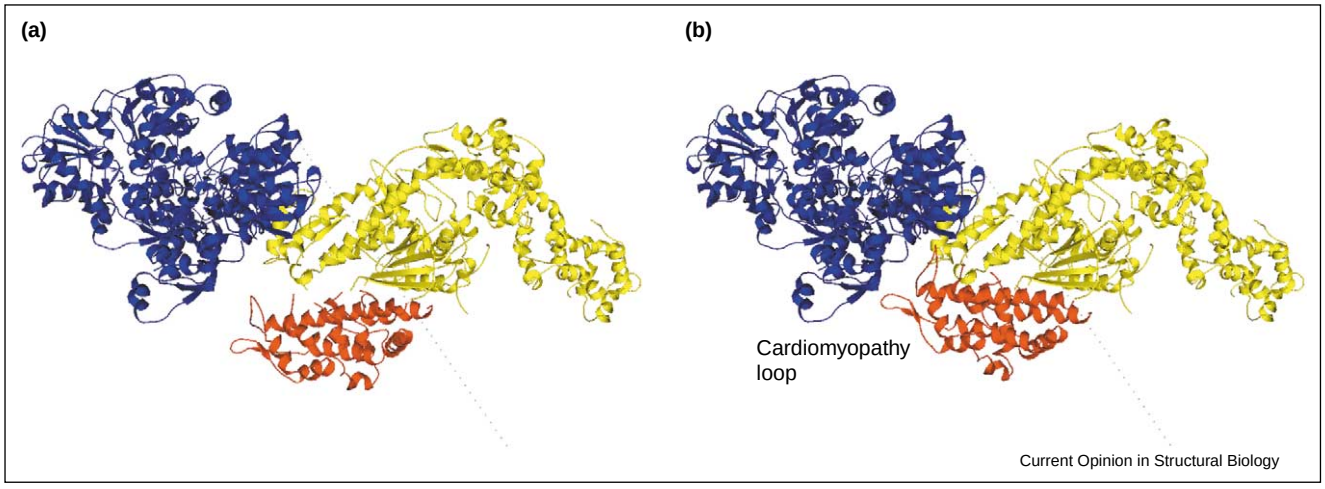
Table 1

Protein complexes with observed conformational changes.

Protein	Binding ligand	Conformation 1 (PDB code)	Conformation 2 (PDB code)	Maximum Cα displacement (Å)	Maximum θ change	Classification[b]	Discussed
Myosin	Actin	NA	NA	9[a]	21[a]	Induced	Yes
Antibody Spe7	TrxShear3	NA	NA	6.7[a]	90[a]	Equilibrium	Yes
GpIb-IX-V	Thrombin	1P8V	1OOK	37[a]	90[a]	Equilibrium	Yes
$G\alpha_i$ subunit	RGS14	1BOF	1KJY	10.2	177	Induced	Yes
FliS	FliC	1ORJ	1ORY	29.3	168.8	Induced	Yes
NtrC	P	1DC7	1DC8	12	177	Equilibrium	Yes
FcαR1	Fc	1OVZ	1OWO	11	180	Induced	
Importin β	SREPB-2	1GCJ	1UKL	19[a]	22[a]	Induced	
Btub	Colicin E3	1NQF	1UJW	13.4	179	Induced	
Leukemia inhibitory factor	GP130	1LKI	1PVH	7	169	Induced	
Lir-1	Hla-A2	1G0X	1P7Q	11	177	Induced	
GPCR kinase 2	Gβ1γ2	1BAK	1OMW	17	171	Induced	
F_1-ATPase	IF_1	1BMF	1OHH	4.5[a]	8.7[a]	Induced	
Her2	Herceptin Fab	1N8Y	1N8Z	13.7	170	Induced	
Erythropoietin	Erythropoietin receptor	1BUY	1EER	15.4	149.5	Induced	
GroEL	GroES	1AON	1OEL	48.5	169.6	Allosteric	
Calmodulin	Calmodulin-binding domain of skeletal light chain myosin kinase	4CLN	2BBM	179	61	Allosteric	
Proto-oncogene Cbl	ZAP-70 kinase	2CBL	1B47	7	58	Induced	
CDK2	Cyclin A	1FIN	1HCK	20	176	Induced	
Mms2	Ubc13	1J74	1J7D	20.7	164	Induced	
PPARγ	Steroid receptor coactivator-1	1PRG	1FM6	13	169	Equilibrium	
TRAF6	RANK	1LB4	1LB5	16.6	133	Either	
XRCC4	DNA-ligase IV	1FU1	1IK9	14	119	Induced	
RAN	RAN-binding protein 2	1BYU	1RRP	53.4	179	Induced	
CheY	CheA	3CHY	1EAY	3.8	23	Induced	

[a]Data taken from the literature. [b]The classifications are assumed based on the experimental data available. NA, not available.

Figure 2



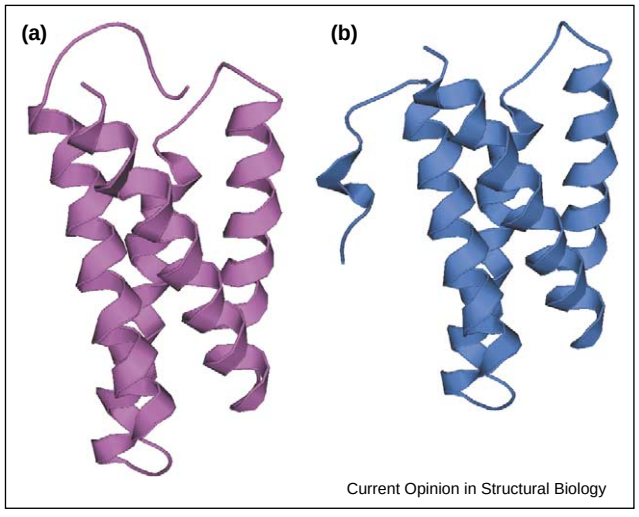
Overview of myosin (yellow) and its upper 50 kDa domain (red) docked to actin (blue) using **(a)** a rigid-body X-ray model of myosin in its open cleft, weak binding form and **(b)** fit with an independent upper 50 kDa domain that illustrates its closed, strong binding form. Figure produced with PyMOL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

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] to aid the correct assembly of the bacterial flagellum and to 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.

The recent crystal structure of a G-protein α subunit bound to the GoLoco motif found in regulatory proteins highlights important residues that control the specificity of the GoLoco–Gα interaction 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]. GoLoco 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 the adenylyl cyclase inhibitory Gα subunit (α_{i1}•GDP). Interaction with the R14GL peptide (residues 496–530 of rat RGS14 containing the GoLoco region) is shown to alter the conformation of switches I–III relative to Gβγ-bound α_{i1}•GDP•Mg^{2+}. In particular, the deviation in switch II (where Arg208 moves ~6 Å) 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 Ala114 is displaced 11 Å away from the Ras-like domain.

Figure 3



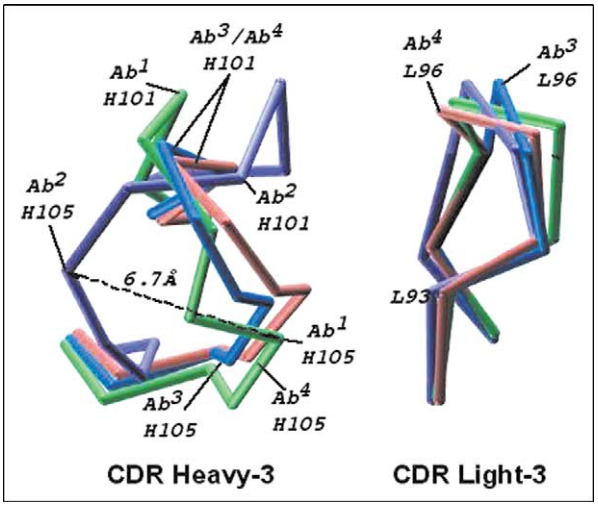
Structural conformations of **(a)** uncomplexed FliS and **(b)** FliS bound to FliC.

Pre-existing equilibrium

There are 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 (Ab^1) has a flat, regular binding site, which is reminiscent of antibodies that bind proteins or peptides. The alternative isomer (Ab^2) contains a deeper, funnel-shaped pocket, typical of antibodies that bind haptens (small molecules that become antigenic when bound to proteins). The recombinant protein antigen TrxShear3 binds to Ab^1, but does not bind to Ab^2, and haptens do not bind to Ab^1. These conformations result from large backbone alterations of the H3 and L3 loops, with Cα atoms deviating by up to 6.7 Å (Figure 4). The H3 loop flips between the Ab^1 and Ab^2 isomer, displaying different sidechain rotamers. This study highlights the potential role of conformational diversity in cross-reactivity, which can lead to autoimmune disease and allergy [33].

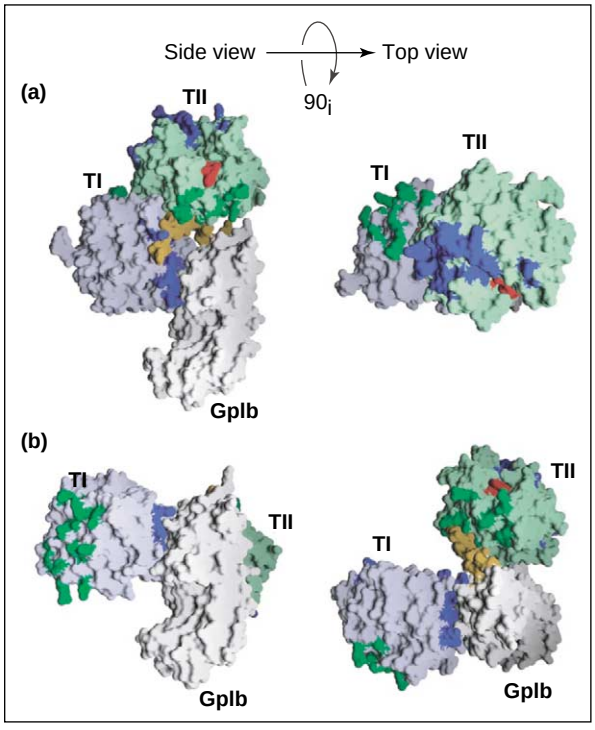
Two crystal structures recently determined by Celikel *et al.* [34••] and Dumas *et al.* [35••] (Figure 5) illustrate the existence of an ensemble of possible conformations for bound proteins. The authors describe the thrombin–GpIbα interaction, but obtain very different crystal forms. Both these papers show two thrombins bound to each glycoprotein GpIbα fragment — one thrombin bound through exosite I and the other bound through exosite II. Although the first thrombin is bound to approximately the same region of GpIbα in both structures, the structures display completely different contacts and are rotated approximately 180° about an axis perpendicular to the interface. Additionally, structures of the second thrombin interface show the flexible anionic segment of GpIbα rotated 90°, resulting in a 37 Å displacement of Tyr279, 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 a better functional understanding of the thrombin–GpIbα interaction.

Figure 4



Mainchain configurations of the IgE antibody Spe7: free isomers Ab^1 (green) and Ab^2 (purple), hapten-bound isomer Ab^3 (blue) and TrxShear3-bound isomer Ab^4 (pink). Figure provided by Dan Tawfik.

Figure 5

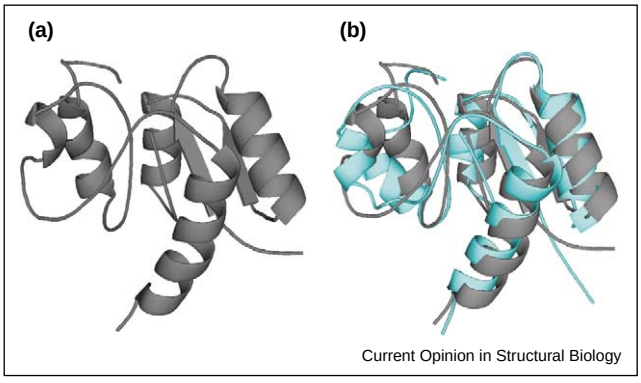


The GpIbα–thrombin interface. Surface representations of the **(a)** Celikel *et al.* [34••] and **(b)** Dumas *et al.* [35••] crystal structures. The GpIbα N-terminal fragment (gray) is shown with its anionic segment (orange). The part of thrombin that binds to GpIbα through exosite I (TI, dark blue) is shown in pale blue and the part that binds through exosite II (TII, dark green) is shown in pale green. Figure reprinted with permission from JE Sadler [45]. Copyright 2003 American Association for the Advancement of Science.

Allosteric regulation — the dynamic population shift model

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

Figure 6



Structural conformations of **(a)** the unphosphorylated form of NtrC, and **(b)** the phosphorylated form of NtrC (cyan) superimposed on the unphosphorylated form (gray). Figure produced with PyMOL [44].

study by Volkman *et al.* [37] that evidence was presented for allostery in a single-domain signaling protein. The study describes a population shift induced by ligand binding to 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 unbound calmodulin exists in a predominantly closed conformation, with a smaller population of more open conformations. For membrane proteins, kinetic studies performed on the allosteric transitions of *Torpedo* acetylcholine receptors showed that, without ligand, 11% of the receptors pre-exist in the activated (desensitized) conformation [39•]. In the presence of ligand, this population increases to 85%. Nevo *et al.* [40•] presented further evidence of multiple conformational states for macromolecular complexes such as the Ran–importin β1 binding interaction. This study demonstrated the existence of two distinct bound conformational states when importin β1 is associated with Ran that is loaded with a nonhydrolyzable GTP analog (GppNHp).

Conclusions

As more experimental work is performed to characterize the dynamics of binding interactions, it is becoming increasingly 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 be the case for 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, such as NtrC, may require a certain number of activated conformers 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 functional diversity without depending on the evolution of sequence diversity, which can greatly facilitate the potential for rapidly evolving new functions and structures [43•].

Supplementary material

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

Acknowledgements

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