Luxury accommodations: the expanding role of structural plasticity in protein–protein interactions

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The recognition of multiple ligands at a single molecular surface is essential to many biological processes. Conformational flexibility has emerged as a compelling strategy for association at such convergent binding sites. Studies over the past few years have brought about a greater understanding of the role that protein plasticity might play in protein–protein interactions.

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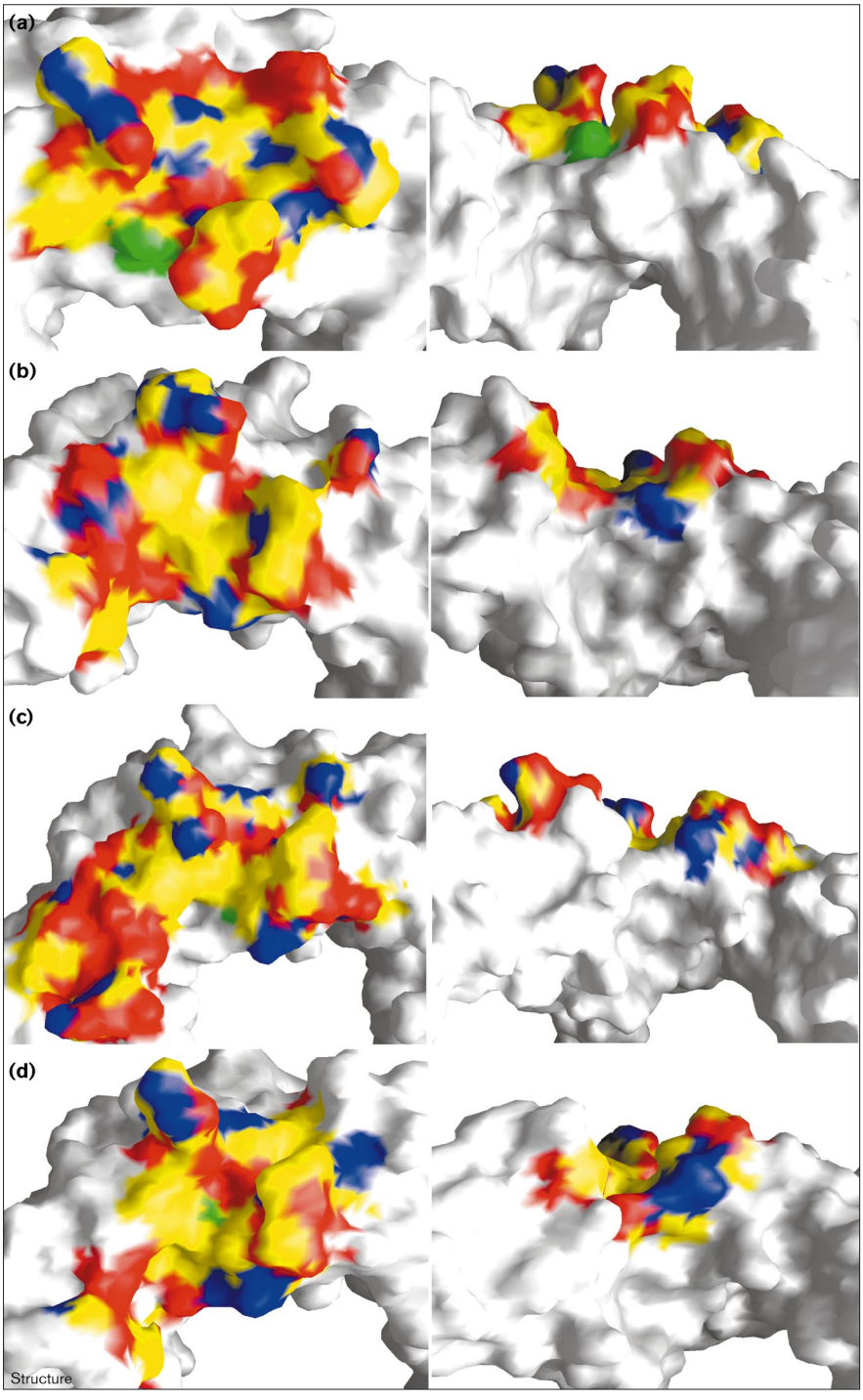
There are now many known examples of proteins that have the ability to associate with multiple ligands using essentially the same set of surface residues. Although we are just beginning to understand the properties that make these consensus binding sites unique, the role of conformational changes induced upon binding at the protein interface has emerged as a factor of key importance. Recent structural studies have shown that plasticity has at least two fundamental roles in protein–protein interactions. First, it enables protein interfaces to accommodate mutations as they co-evolve. Second, it allows a single molecular surface to interact with numerous, structurally distinct binding partners.

Associations between proteins are essential to the regulation of most cellular processes. Although the properties that govern these interactions are not understood fully, the rapidly expanding database of protein complex structures has revealed some factors that appear to be generally important for binding [1–3]. Most protein complexes share a relatively large and planar buried surface area composed of closely packed atoms, similar to those found in the protein core. This shape complementarity is usually accompanied by a high degree of charge complementarity, although water-mediated polar interactions are often substituted for direct hydrogen bonds. Recent studies [1,4,5] show that the hydrophobic effect, widely accepted as the driving force for protein folding [6], also has a significant role in protein–protein associations, even though the hydrophobic nature of residues at interaction surfaces is intermediately dispersed between those in the protein core and those at non-interacting surfaces. Small, but significant conformational changes are generally seen between non-associated and associated proteins and this flexibility appears to increase with the amount of buried surface area in the protein complex [2]. Although it is clear that proteins capable of binding multiple ligands at a single site share many of the molecular properties seen in other protein–protein interactions, they appear to follow a very different set of rules when it comes to conformational flexibility.

The Fc fragment of immunoglobulin G binds to perhaps one of the most diverse repertoires of ligands discovered to date, including protein A [7], protein G [8], rheumatoid factor [9] and the neonatal Fc receptor [10]. Remarkably, all four proteins bind to a common site located between the CH_2 and CH_3 domains of the Fc fragment. To determine whether this common site was selected on the basis of biological function or because of its intrinsic binding properties, Wells and colleagues [11] used phage display to isolate peptides that bound the Fc fragment, without any selection for biological function. The technique of phage display has revolutionized the study of macromolecular interactions by reducing the timescale of the co-evolution of protein interfaces to an experimental level. Surprisingly, following several rounds of selection, the library became dominated by a single peptide that appeared to bind to the Fc consensus site with high affinity. By determining the X-ray crystal structure of this 13-residue peptide in complex with the Fc fragment the authors found that the peptide, which is structurally unrelated to any of the physiological Fc ligands, buries nearly as much surface area (650 $Å^2$) as does the 15-fold larger rheumatoid factor (740 $Å^2$) and binds only twofold weaker than does protein A. In an effort to determine distinguishing features of this highly convergent binding site, Wells and colleagues [11] carried out patch analysis over the entire surface of the CH_2 and CH_3 domains of the Fc fragment from five available Fc complex crystal structures. From this analysis it was clear that the highly favorable consensus binding site was part of a larger region of highly accessible and hydrophobic potential binding surfaces that extend across the top of the CH_2 and CH_3 domains. To explain why the consensus site is so favorable towards binding diverse ligands, the authors propose that greater conformational flexibility might distinguish this site from other similarly exposed, nonpolar sites on the Fc. Indeed, there are significant and distinct structural rearrangements in this region induced by each of the Fc-binding partners (Figure 1). Much of this conformational change is mediated by two neighboring methionine residues that rearrange either to form a pocket to accommodate surface residues of the peptide and protein G or to form a much

Figure 1



Molecular surfaces of the Fc fragment at the consensus interface region when bound by **(a)** protein A, **(b)** protein G **(c)** rheumatoid factor and **(d)** Fe-III, the peptide produced by phage display that binds specifically to the Fc fragment consensus binding site. The molecular footprint of each ligand, as determined using CNS [40] with a probe radius of 1.4 Å, is depicted in color: buried carbon atoms are yellow, oxygen atoms red, nitrogen atoms blue and sulfur atoms green. The non-interacting surface residues are uncolored. Side-by-side representations of the differentially liganded Fc fragment molecules are shown rotated 90° about the horizontal axis of the figure. The ligands have been removed for clarity. This figure was prepared using the program GRASP [41].

flatter surface for rheumatoid factor and protein A binding. Several other sidechains in the consensus binding site adopt different rotamer positions to facilitate the binding of the distinct ligands. It is likely that further plasticity of the binding site is conferred by its position on the hinge region of Fc between the CH_2 and CH_3 domains.

The inherent flexibility of such hinge domains of many receptor families is a major factor in conferring greater adaptability to binding surfaces. The family of cytokines and their receptors [12] provide several examples of the fundamental role that structural plasticity plays in cellular signaling by enabling the accommodation of mutations

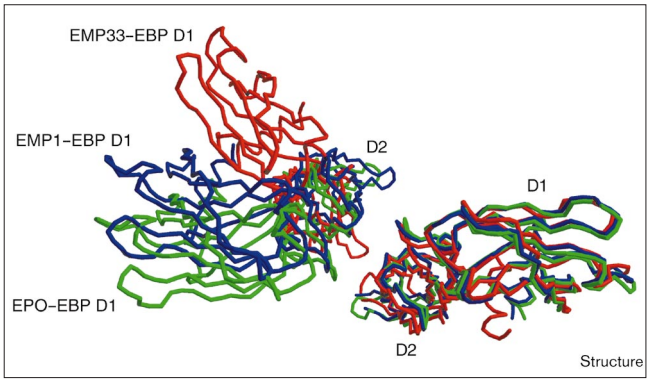
and the binding of many structurally distinct ligands. A single molecule of human growth hormone (hGH) binds to two hGH receptors to activate signaling [13], and the crystal structure of the complex revealed that the asymmetrical hGH binds to essentially the same residues on each of the two receptors, even though there is no structural similarity between the two binding surfaces of the growth hormone [14]. The interface between hGH and its high-affinity receptor site has been drastically remodeled by selecting a hGH pentamutant by phage display for binding to its high-affinity receptor site [15]. The crystal structure of the complex between the resulting pentamutant hGH and a low-affinity mutant hGH receptor revealed that the five hGH mutations central to the interface were able to rescue binding in the complex by inducing significant global conformational changes: mainchain and sidechain atoms 15 Å from the center of the binding interface were observed to move by up to 3 Å relative to neighboring residues in the wild-type structure.

Crystal structures of the erythropoietin (EPO) binding protein (EBP) in its native unliganded form [16], bound to EPO [17], to an agonist peptide [18], and to an antagonist peptide [19] have revealed that dimerization of EPO receptor molecules on the cell surface is a necessary but insufficient event to activate this hormone receptor system. In binding to the same site on the EPO receptor, which itself dimerizes in unliganded form, these structurally distinct agonist and antagonist molecules regulate the activation mechanism of the receptor by orienting the two receptor molecules in various positions relative to one another (Figure 2). The resulting juxtaposition of the cytosolic JAK-2 domains of the receptor, in turn, controls their self-phosphorylation activation mechanism.

Yet another example of structural plasticity in the cytokine family was revealed by the crystal structure of a complex between interferon-γ and its high-affinity receptor [20]. A flexible loop in the unbound form of interferon-γ undergoes radical conformational changes upon receptor binding, including the formation of a 3_{10} helix.

Because of the inherently greater morphological diversity of foreign antigens relative to the immune system recognition molecules, antibodies and T-cell receptors (TCRs), the structural flexibility of the recognition domains in these molecules has been proposed to be an essential component of immune recognition. The structures of these molecules seem to present ideal scaffolds for antigen-recognition-based structural plasticity owing to the conformational arrangement of six complementarity determining region (CDR) loops extending from the variable light (V_L) and heavy (V_H) chains of antibodies, and from the variable α and β domains of the TCR (Vα and Vβ). Indeed, a wide range of conformational changes induced upon ligand binding have been reported for both of these classes of molecules.

Figure 2

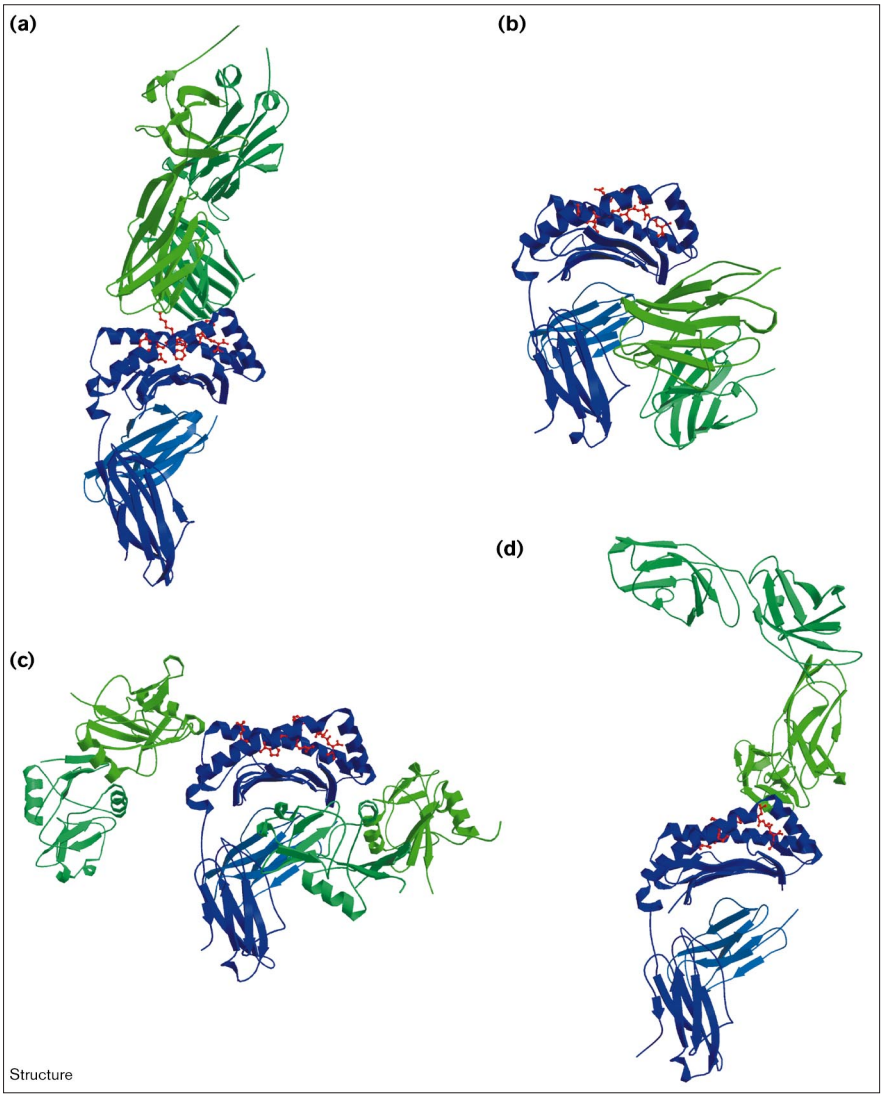


Relative juxtapositions of the erythropoietin (EPO) binding protein (EBP) dimer pairs when bound by the natural agonist EPO (green), a weak peptide agonist EMP1 (blue) and an antagonist peptide EMP33 (red). Domain 1 (D1) β-strand residues of one EBP molecule of each complex were superimposed by least-squares fitting in XTALVIEW [42]. The D1 domains of the second EBP molecule of each EBP dimer pair, when bound by EMP1 and EMP33 are situated approximately 15° and 60°, respectively, away from the axis along the two EBP D1 domains of the EPO–EBP complex. The ligands have been removed for clarity. This figure was prepared using the program MOLSCRIPT [43].

Several examples of an 'induced-fit' mechanism have been described for antibody recognition of small molecules, including peptides [21,22] and nucleotides [23], whereby substantial rearrangements of the CDR loops and relative rotations of the V_L and V_H domains were observed. Interestingly, large rearrangements of CDR loops have not been observed in antibody complexes with protein antigens [24,25]. Thus, the induced-fit mechanism of antigen binding may be pertinent only to small-molecule–antibody interactions, such as for peptides, nucleotides and haptens, necessitated by excessive antigen envelopment. A possible role of structural plasticity in antibody maturation was revealed by the crystal structures of a germline antibody in both its hapten-bound and free forms [26], in which the substantial conformational changes incurred upon complex formation were structurally similar to the mature antibody.

Functional TCR molecules must have dual specificity. In the thymus, T cells are selected for maturation on the basis of weak interactions between the TCR and self-peptides presented by major histocompatibility complex molecules (pMHC). Surviving T cells then survey pMHC complexes on cellular surfaces in the periphery. Encounters with self and foreign pMHC complexes can lead to a wide range of agonist and antagonist T-cell signaling mechanisms, dependent on the affinity of the pMHC–TCR interaction [27]. Plasticity in the CDR loops of TCR molecules provides one explanation as to how T cells can perform their immunoregulatory duties by recognizing different molecular surfaces from those on which they were originally selected. The

Figure 3



Major histocompatibility class I molecules bind numerous ligands at distinct binding surfaces, including **(a)** the T-cell receptor (TCR), **(b)** the CD8αα homodimer, **(c)** the lectin-like natural killer (NK) cell receptor Ly49A, and **(d)** the NK inhibitory receptor KIR2DL2. The MHC heavy chain and β-2-microglobulin are shown in blue, the antigenic peptide is in red, and the ligands TCR, CD8αα, Ly49A and KIR2DL2 are in green. This figure was prepared using the program MOLSCRIPT [43].

structure of a complex between self pMHC and its specific TCR [28] has provided some insights into the role of structural plasticity in the development and activation of T cells. Notably, there is very poor shape complimentarity at the pMHC–TCR interface. By comparison to structures of the individual unliganded molecules, numerous conformational changes induced upon binding pMHC were observed in the CDR loops of the TCR, similar to the antibody rearrangements induced by small molecules. The largest of these conformational changes occurs in the CDR3 loop of the TCR α chain in which a type II′ β turn in the unliganded structure undergoes a movement of 6 Å in order to accommodate the pMHC α2 helix. These types of conformational changes induced by pMHC in the CDR loops of TCRs might be important for differentiating between superagonist and weak agonist signaling [29]. Recent thermodynamic analyses of pMHC–TCR complexes [30,31] have indicated that these interactions are highly temperature dependent, characteristic of induced fit mechanisms, and implicate TCR flexibility as an essential component of specific binding to pMHC ligands.

Recently developed nuclear magnetic resonance (NMR) techniques are able to distinguish intramolecular motions over a wide range of timescales on a per residue basis [32,33]. A number of studies that take advantage of these techniques have revealed significant overlap in residues that have a high degree of structural flexibility and those that are important in protein–protein interactions; this is especially true for residues that bind multiple ligands [34,35].

Despite the finding of common binding sites on many biologically important molecules, it would be incorrect to believe that proteins, in general, are competent to bind

ligands at only very restricted locations on their surface. Several striking counter-examples to this binding strategy exist, including MHC class I molecules, which interact with TCRs, natural killer (NK) cell receptors, and the accessory molecule CD8. As shown in Figure 3, these MHC class I ligands all bind to very distinct surfaces of the pMHC complex. TCR molecules bind across the top of the MHC α helices and interact with the antigenic peptide [28]. The accessory molecule CD8 binds as a CD8αα homodimer to a surface comprised of residues from the HLA-A2 α2 and α3 domains (HLA, human leukocyte antigen) as well as residues from β-2-microglobulin [36]. Two recently solved complexes of MHC class I molecules bound by NK cell receptors also revealed very different modes of interaction. The lectin-like NK cell receptor Ly49A binds to two sites on the MHC molecule H-2D^d [37], one which partially overlaps the CD8-binding site and another at a conserved glycosylation site of the α2 domain of the MHC molecule. The NK inhibitory receptor KIR2DL2 binds in a peptide-dependent manner along the top of the HLA-Cw3 molecule displaying an importin α2 peptide [38]. In contrast to the consensus site on the Fc fragment, each of these MHC class I binding surfaces is relatively hydrophilic. Particularly in the Ly49A (both interaction sites) and KIR2DL2 complexes, the interfaces are dominated by polar interactions, including numerous salt bridges. Another counter-example to the scenario of a single receptor surface binding multiple ligands is seen in the case of hen egg white lysozyme. Crystal structures of hen egg white lysozyme in complex with a number of antilysozyme monoclonal antibodies have revealed that numerous distinct molecular surfaces can function as epitopes [39].

Thus, although one must exercise considerable caution in making generalizations about the structural characteristics of ligand-binding sites on proteins, the need for expanding the database of protein structures in both their complexed and uncomplexed forms is evident. This information, coupled with techniques for co-evolving protein surfaces and more specifically defining the energetically important residues in the binding interface and their individual atomic movements, will provide a much clearer understanding of the importance of structural plasticity in protein–protein interactions in the near future.

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