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The protein dance:
partner selection and adjustment

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Opinion

Review

Cover: Binding of proteins involves highly complex events as described by the extended conformational selection model put forth by Csermely et al. on pages 539–546 of this issue. The mutual conformational selection and adjustment resemble a protein dance. Induced fit can be viewed as a subtype of this dance, whose contribution is affected by the bond types stabilizing the interaction and the differences between the interacting protein dancers. Protein segments whose dynamics are distinct from the rest of the protein ('independent dynamic segments') can govern conformational transitions and allosteric propagation through which the movements of the protein dancers take place. Design by Attila Kovacs.

Opinion

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Induced fit, conformational selection and independent dynamic segments: an extended view of binding events

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Single molecule and NMR measurements of protein dynamics increasingly uncover the complexity of binding scenarios. Here, we describe an extended conformational selection model that embraces a repertoire of selection and adjustment processes. Induced fit can be viewed as a subset of this repertoire, whose contribution is affected by the bond types stabilizing the interaction and the differences between the interacting partners. We argue that protein segments whose dynamics are distinct from the rest of the protein ('discrete breathers') can govern conformational transitions and allosteric propagation that accompany binding processes and, as such, might be more sensitive to mutational events. Additionally, we highlight the dynamic complexity of binding scenarios as they relate to events such as aggregation and signalling, and the crowded cellular environment.

The induced fit and the original conformational selection models

The prevailing view of binding mechanisms has evolved from the early 'lock-and-key' hypothesis [1] to the now popular 'induced fit' model [2]. According to the induced fit scenario, the interaction between a protein and a rigid binding partner induces a conformational change in the protein. However, several systems follow the 'conformational selection' (or, with a different nomenclature, population selection, fluctuation fit, selected fit) paradigm, where among the conformations of the dynamically fluctuating protein the ligand selects the one that is compatible with binding, and shifts the conformational ensemble towards this state. Selective binding to a single conformation in the ensemble was suggested by Straub as early as 1964 [3]; initial experimental evidence for the hypothesis came from Zavodszky et al. [4] in 1966. Over 25 years later, in a landmark paper, Frauenfelder, Sligar and Wolynes [5] described the energy landscape of proteins, which in 1999 led to the generalized concept of 'conformational selection and population shift' [6,7]. The observed widespread occurrence of such conformational selection phenomena and their importance in functional scenarios is increasingly supported by X-ray and cryo-electron microscope images, kinetics studies, extensive single molecule fluorescence and, in particular, NMR data showing a repertoire of conformational states of unliganded proteins reflecting *in vivo* occurrences, including conformations corresponding to the bound form [8–10].

The duality of the induced fit and the original conformational selection models resembles the model pair of the Koshland–Nemethy–Filmer (KNF, [11]) and the Monod–Wyman–Changeux (MWC) models [12] that explain allosteric interactions. Both models describe the allosteric effect as a binding event at one site that induces a conformational change affecting the activity at another site. However, the KNF model views the conformational change as a consequence of allosteric binding, whereas the MWC model describes the conformational change as an allosteric ligand-induced shift of the equilibrium of two pre-existing conformational states. Recent data suggest that in several cases (e.g. adenylate kinase and catabolite activator protein) allostery can be mediated by transmitted changes in protein motions [13,14]. In this dynamic sense, allosteric regulation involves a population shift; that is, a change in the population of conformations preferring the state whose binding site shape is complementary to the incoming partner. In many cases, the overall average conformation does not change, and the allosteric effect can be seen only by the increased (or decreased) dynamics of the protein segments [15,16].

It is well understood that the distinction between the induced fit and the original conformational selection models is not absolute; indeed, an increasing number of cases show that conformational selection is often followed by conformational adjustment [17,18]. The growing volume and precision of data relating to protein dynamics now enables a general discussion of binding events involving small ligands such as substrates, antigens or drugs, proteins and DNA and relating these to allosteric effects [6,8,19]. Extending the list of binding partners, Gorfe *et al.* [20] showed that conformational selection might play an important role in the insertion of proteins into membrane. Here, we use the above generality of binding scenarios to extend the original models, and to show that induced fit can be perceived as an extremity of this extended conformational selection model. We propose that independent dynamic segments, i.e. protein segments with dynamics distinct from the rest of the protein, could be key contributors to binding processes. Finally, we discuss intrinsically disordered proteins,

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extreme temperatures, aggregation, chaperones and the crowded cellular environment from the point of view of the extended conformational selection model, and show how this mechanism contributes to cellular signalling.

The extended conformational selection model

Recent data on protein dynamics make the discrimination between the induced fit and the original conformational selection models less rigid. An increasing number of examples in which conformational selection is followed by conformational adjustment [17,18] provide support for the extension of the original conformational selection model [6,7] (Figure 1). The extended conformational selection model describes the general scenario, where both selection- and adjustment-type steps follow each other. Recent data suggest that the conformational selection model holds also for RNA [21,22], which suggests that the extended conformational selection model could describe the binding mechanism of RNA molecules.

The ensemble of the conformational states available for mutual selection and adjustment is positioned at the low-energy region of the folding funnel. Decades ago, this conformational ensemble was considered as the single conformational state of the 'native' protein. As binding

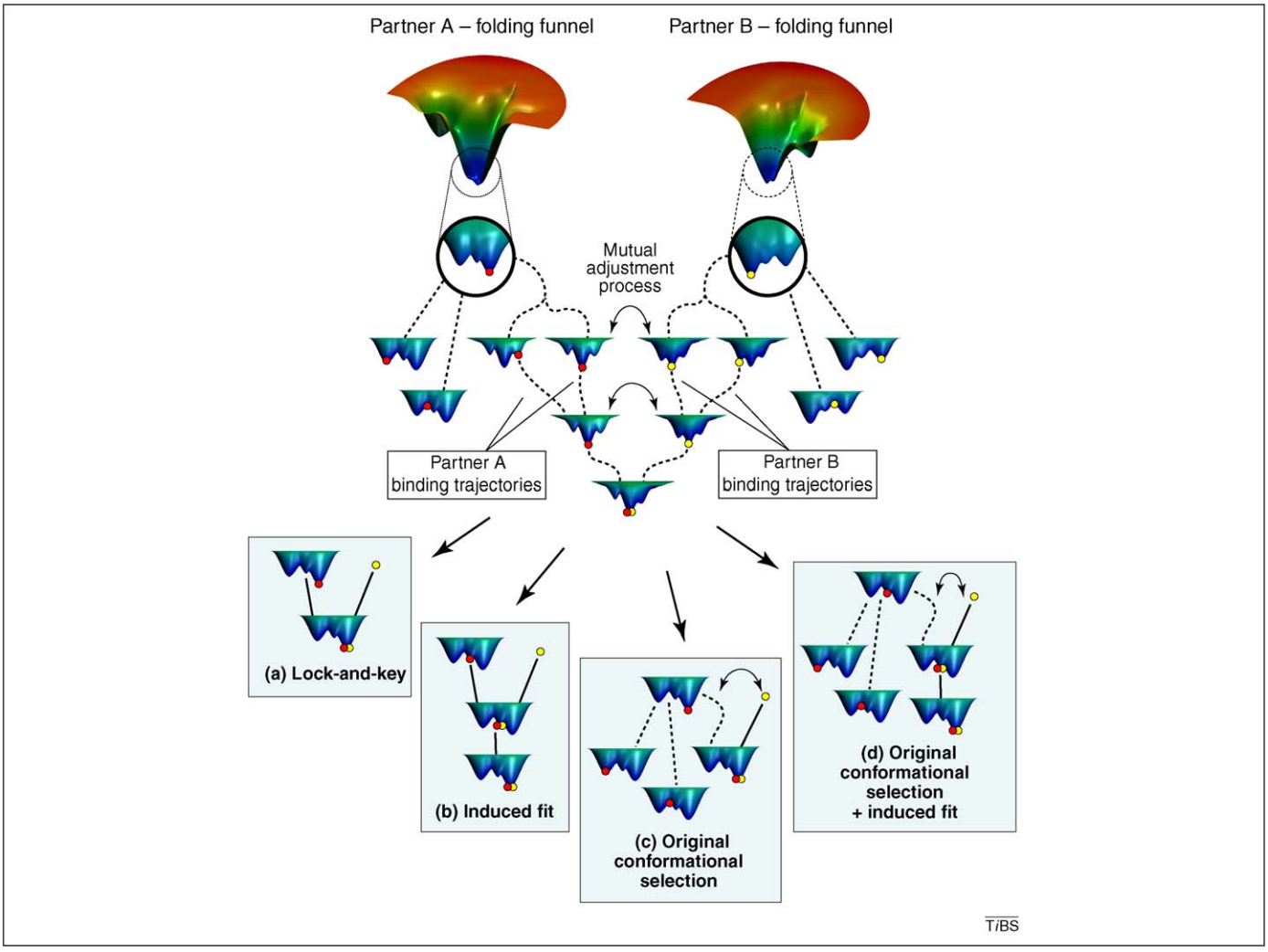

Figure 1. The extended conformational selection model. The native state at the bottom of the two illustrative energy landscapes (folding funnels) of the two binding partners usually can be described as an assembly of conformers, which are visualized as secondary minima at the magnified bottom sections of the energy landscape in the circles at the upper part of the figure. The top segment of the figure contains our proposed extended conformational selection model. For simplicity, we restrict our treatment to the encounter of two partners, because the probability of simultaneous binding of more than two partners is extremely small. The actual conformation of partner A and B is described as their position on the energy landscape marked by red and yellow dots, respectively. The double-headed arrows mark conformational selection. As the two partners approach each other, the probability of occurrence of their conformers changes and so does the shape of their energy landscape. The actual sequence of these mutual conformational selection and preceding or subsequent conformational adjustment steps forms the 'binding trajectory' (marked with lines) covered by partners A and B. For the sake of simplicity, we have restricted the number of binding trajectories to two, which is several magnitudes higher in real situations. This general scenario is obviously much less complex; if the conformational ensemble of one of the partners is negligible compared to that of the other partner, e.g. in the case of binding of small substrates or allosteric modifiers to a protein as shown in the four bottom panels. The lower part of the figure contains four scenarios of the extended conformational selection model, where binding trajectories are less complex. **(a)** The classical lock and key model, where both partners are either rigid or have exactly matching binding surfaces. **(b)** The classical induced fit model, where partner B first binds to the single available conformation of partner A, and then induces a conformational change of partner A. **(c)** The original conformational selection model, where partner B binds one of the several fluctuating conformations of partner A and no further conformational rearrangement occurs. **(d)** Partner B first binds one of the several fluctuating conformations of partner A, and induces a subsequent conformational rearrangement of partner B. As a discriminating feature of all scenarios in the figure from the extended conformational selection model shown at the top centre, in all panels partner B has a single conformational status instead of a conformational ensemble and, for the sake of simplicity, its energy landscape is not shown. This approximation works well if partner B is small and/or rigid, like a small molecule or DNA. As a further simplification, the conformational landscape of partner A is not changed by partner B in **(a)** through **(d)**.

proceeds, the partners' conformations change (accompanied by the changing position of the participating proteins on the energy landscape; Figure 1) and the mutual encounter changes the shape of the energy landscape of both partners. As the two partners approach each other, electrostatic and water-mediated hydrogen-bonding signals emerge, and they increasingly change the partners' environment, thereby altering the energy landscape [23,24]. Partner proteins can follow different sequences of conformational selection and adjustment steps (which we call binding trajectories [13,24]). Such alternative pathways converging to a common end-state are typical also of the behaviour of other complex systems, such as gene expression of differentiating cells [25]. The encounter of the two binding partners involves a large number of conditional steps, where the next step of the encounter by partner A depends on a preceding conformational change by partner B and vice versa. We have previously compared this process to wooing, terming it an 'interdependent protein dance' [24]. Such a mutually conditional step-wise selection and encounter process can be described as a series of games ([23,24], Box 1).

Box 1. The application of game theory models in the description of protein-binding mechanisms

In protein binding, the conformation of one partner serves as an environment (a set of preconditions) for the other partner. This scenario is typical of a game where the strategy (the repertoire of possible responses) of a partner depends on the last step of the other partner. The application of game theoretical models to protein binding was first proposed by Kovacs et al. [23], who suggested that binding events accompanied by parallel folding and unfolding, or unilateral folding (fly-casting) might correspond to well-defined games.

Protein binding might correspond to a hawk–dove game, where rigid proteins are hawks and flexible proteins are doves [69]. In this game, if a hawk meets a hawk, a fight starts, both gain food but both are injured, and the cost of injury has to be deducted from the price of food at both sides symmetrically. If a dove meets a dove, the available food is shared equally. If a hawk meets a dove, the hawk will have the food and the dove gets nothing. We propose to set the payoff of the hawk–dove game as the decrease of free energy. By this game definition, a rigid protein (a hawk) might indeed be a winner compared to a flexible protein (a dove), because the enthalpy gain of binding is not accompanied by an entropy cost for the rigid protein, but the more flexible protein loses several degrees of freedom during the binding event. If two rigid proteins (hawks) meet, no binding occurs, thus none of the partners wins anything. If two flexible proteins (doves) meet, the free energy-gain is shared. Induced fit corresponds to a hawk–dove encounter, whereas conformational selection corresponds to either a dove–dove or a hawk–dove game in which the 'protein-hawk' is selecting the appropriate conformation of the 'protein-dove'.

We note also that the *strictu senso* induced-fit model resembles the ultimatum game. In this game the first player (the rigid protein) proposes how to divide the sum between the two players and the second player (the flexible protein) can either accept or reject this proposal. If the second player rejects the proposal, neither player receives anything (i.e. no binding and no free energy decrease occurs). If the second player accepts the proposal, the money (the free energy decrease) is split according to the proposal. It is worth mentioning that Chettaoui et al. [70] described a 'games network theory' to model multiple binding cascades as a network of different games and players. They applied this model to describe the signalling cascade of the plasminogen activator system containing seven binding partners.

Our increasing knowledge of the details of the binding process might allow us to distinguish between the local and global versions of the original conformational selection and induced fit models [26]. As an example of this diversity, side chains in the ubiquitin-binding site tend to follow induced fit-type behaviour, whereas conformational selection type changes are more typical within the rest of the protein [18]. This distinction emphasizes the possibility that a unique categorization of the mechanism of the entire protein might not hold for all of its parts, thereby lending further support to our proposal of the extended conformational selection model described above (Figure 1).

Special cases of the extended conformational selection model

The lock-and-key (Figure 1a), the induced fit (Figure 1b), the original conformational selection (Figure 1c) and the conformational selection plus adjustment models (Figure 1d) are all special cases of the extended conformational selection model. Both partners in the lock-and-key model have an exactly complementary binding surface (Figure 1a). Conformational selection-type binding scenarios (Figure 1c and d) shift towards the induced fit mechanism (Figure 1b): (i) if the interactions helping the mutual encounter are strong and long-range, like ionic interactions, or directed, like hydrogen bond interactions [27]; (ii) if the partner's concentration is high [28,29]; and (iii) if there is a large difference in size or cooperativity [30]. These latter cases can be rationalized in terms of rigidity; indeed, a small ligand is often more rigid than its large protein partner, which displays greater flexibility. Although $\sim$ 90% of cellular protein–protein interactions are homomeric [30], thus precluding any of the above three scenarios leading to an induced fit, dimerization could involve different conformers of the same protein [31]. The duality of the induced fit (Figure 1b) and the original conformational selection models (Figure 1c) can be rationalized in other complex systems (Box 2).

Mechanisms of conformational dynamics during the binding process

The mechanism of the dynamic changes that occur during protein binding has been the subject of intense interest. The main question in these studies can be summarized as follows: is the binding site the only key player, or are a few selected regions in the protein involved? Over the past few years, an increasing number of key contributors to conformational changes have been identified. We discuss a few elementary steps of binding scenarios below, highlighting the importance of independent dynamic segments.

In the first step, transient encounter complexes are formed. These complexes, which were identified originally by paramagnetic relaxation enhancement experiments, are mostly stabilized by electrostatic forces, have a small, planar contact area in the range of only a few Å^2, and cover a rather large segment (e.g. 15%) of the total surface area around the binding site [32,33]. 'Anchor residues' can play an important role in the next step. Anchor residues are in conformations similar to their final arrangement in the bound complex, and have a large surface area ($\sim$100 Å^2). Conformational selection of one to three anchor residues

Box 2. The duality of the induced fit and the original conformational selection models in various complex systems

The induced fit and the original conformational selection models can be rationalized in complex systems beyond just macromolecules (Table I). As the first example, the dipole–dipole interaction of two molecules can be viewed as an induced fit or conformational selection-type interaction in the case of the induced dipoles (Debye forces) and fluctuating transient dipoles (London forces), respectively. Here, the differences in the original rigidity (an original, rigid dipole *versus* a molecule with a large polarization) help in the development of the induced fit-type Debye interactions from the London forces induced by fluctuating transient dipoles. Similarly, the pleiotropic nature of stem cells makes them ideally flexible partners to adapt to the demands of their environment, which is a clear induced fit-type interaction whereas, in general, neither of the interacting cortical neural cells can be regarded as much more flexible than the other, making the establishment of inter-neuronal contacts similar to a conformational selection process. Low-diversity ecosystems, hierarchical animal or human communities (dictatures) are all determined by a few key species, which resembles an induced fit-type interaction, whereas the interactions in a high-diversity system and in a cross-cooperating animal or human community (democracy) all resemble a conformational selection model.

Table I. 'Induced fit' and 'conformational selection' in complex systems other than macromolecules

Name of complex system member	Induced fit-type interaction	Conformational selection type interaction
Chemical molecules	Induced dipoles (Debye-forces)	Fluctuating transient dipoles (London forces)
Cells	Stem cell differentiation	Multi-directional electronic and neurochemical interactions of cortical neurons
Ecosystem species	Food consumption in low-diversity systems with a dominant herbivorous or carnivorous organism	Food consumption in high-diversity systems with non-hierarchical (e.g. omnivorous) organisms
Animals	Division of labour in hierarchical communities of ants, bees, wasps etc. having a leading figure (alpha male or female)	Complementary cooperation between fishes, lions, monkeys, etc. without a dominant member of the community
Humans	Setting common norms and goals in a dictatorship	Setting common norms and goals in a democracy

can be followed by an induced fit completion of the binding event involving several 'latch residues' that 'click in' to their final conformation and thereby stabilize the interaction [34].

Anchor and latch residues are not the only protein segments involved in the conformational changes accompanying binding. Hinges and hinge-like motions were among the first suggested to play a decisive role in binding-induced conformational transitions [35]. Other studies have revealed that crucial nodes between communities of amino acid networks play an important role in the reorganization of the protein structure during a binding event [36–39], (Figure 2). Protein segments with 'discrete breather' behaviour accumulate kinetic energy and dynamically exchange as much as 20–65% of the accumulated energy during the conformational changes [40,41]. These segments are often located close to the binding or catalytic sites. We call these one or a few amino acid long fragments independent dynamic segments to emphasize their distinct behaviour. We note that hinges, critical nodes and independent dynamic segments might overlap, because independent dynamic segments are located in stiff, hinge-type regions of proteins and both are often positioned at inter-modular boundaries [35–38,40–42]. The possible involvement of specific regions of co-evolving amino acids, called protein sectors in conformational changes has also been suggested [43]. Protein sectors, sparse networks of amino acids that span the entire protein, operate collectively and rather independently from each other. Segments of protein sectors at the interface of protein domains might constitute independent dynamic segments.

Independent dynamic segments might play a crucial role in binding. These independently vibrating protein segments can shift their energy content between each other [41]. This energy relocation might underlie the transition between various conformations in the conformational selection model and, as such, play a key role in allosteric propagation. Independent dynamic segments can also trigger induced fit-type mechanisms, as in the conformational adjustment steps. In the relative absence of independent dynamic segments (due to a high degree of rigidity or flexibility) the protein probably lacks markedly differing conformational states, and its primary binding mechanism might shift from the original conformational selection model towards a lock-and-key or induced fit-type mechanism.

The binding process requires a gross reorganization of interactions (including desolvation), which is a source of

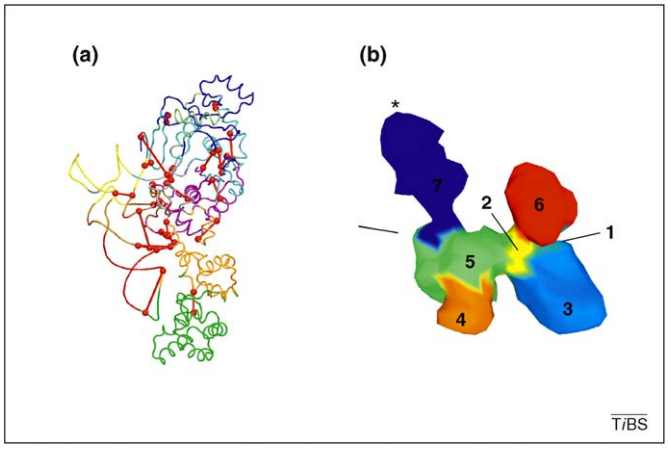

Figure 2. Inter-modular regions might act as key structural contributors of conformational changes in proteins. The two examples highlight amino acids located in the overlapping regions of residue-network modules. These inter-modular segments regulate the coordination of motion of the two modules they connect. **(a)** Key inter-modular links and their end-point residues of Glu-tRNA synthase at the centre of the majority of shortest paths connecting nodes in different communities of the residue-network are marked with red lines and spheres [39]. Residue network communities are marked with different colours. (The image is courtesy of Zaida Luthey-Shulten and John Eargle). **(b)** Inter-modular regions of clusters of the ribosome-bound termination factor RF2 are noted. Clusters of correlated motions were determined from data from the Electron Microscopy Data Bank (EMBD) using normal mode analysis and are marked with different colours and numbers [66,67]. The asterisk (*) denotes the peptidyl-transferase centre. The region between clusters 7 and 5, as well as clusters 6, 1 and 2, connect clusters 7 (blue) and 6 (red) with the rest of the RF2 structure, respectively. Clusters 7 and 6 are the most distinctly moving segments of RF2. (The image is courtesy of Mark Bathe and Do-Nyun Kim.).

frustration and conflict in need of efficient mediation [17,44–47] that can be provided by (i) key residues [44] that could be positioned at inter-modular segments and the independent dynamic segments described above [38,40,41]; (ii) transient bonds (e.g. transient, non-native hydrogen bonds) [35,44,48]; (iii) water [25,44,45]; and (iv) molecular chaperones [25]. We note that mediation is transient. Water molecules are expelled by binding-induced gradual desolvation [17,44–47] and molecular chaperones release their targets before complex formation is completed. Finally, independent dynamic segments might become trapped as binding proceeds [40,41]. By definition, before binding, independent dynamic segments have separate dynamics from the rest of the protein. Binding-induced stabilization might decrease the number and individuality of these key protein segments. In all cases, the decrease of mediation is gradual and occurs in concert with the decreasing need for conflict mediation. This self-regulated withdrawal of conflict mediators, which provides their presence for optimal help but disturbs the process at the possible minimum, is the true beauty of the complex events accompanying protein binding.

Intertwined binding and folding events: intrinsically disordered proteins, extreme temperatures, aggregation and chaperones

Binding is often coupled with protein folding and unfolding. As an example of binding-induced protein folding, binding of tetracycline increases the stability and rigidity of the neighbouring DNA-binding domains, which ensures the correct positioning of the DNA-recognition helices of the two monomers in the major groove of the DNA [19]. Binding-induced folding is particularly evident in the case of intrinsically disordered proteins (IDPs). Importantly, a purely induced fit-type binding mechanism is not characteristic even in these cases, because IDPs also have a broad fluctuating conformational ensemble, thus conformational selection can take place [49–51]. The initial binding event of IDPs was proposed to be assisted by a 'fly casting-like' search mechanism in which multiple weak binding events favour initial complex formation [52]. Subsequently, 'Velcro'-like multivalent binding steps often increase the stability of the resulting complexes [49], illustrating further the utility of the proposed extended conformational selection model (Figure 1). A recent proposal posits that strongly interacting proteins can bind IDPs efficiently [53]. In these binding events, selection of a high-complementarity variant is followed by rigidification of the IDP partner. This rigidification step can be regarded as a subsequent induced fit, where the fit is achieved not by changing the average conformation, but by changing the distribution of the conformational sub-states in the assembly.

Temperature has a profound effect on protein stability and folding: cooling pauperizes, whereas heating expands the conformational ensemble. An increase in temperature makes the binding mechanism more similar to that of IDPs; however, at high temperatures both partners can become 'unstructured'. Efficient adaptation occurs in nature: flexible loops or salt bridges within proteins help to adapt organisms to extremely low or high temperatures, respectively [14]. At higher temperatures the contribution of ion pairs and hydrogen bonds decreases, whereas that of hydrophobic bonds might increase. In comparison with other data [27], less involvement of strong, long-range and directed bonds in binding at higher temperatures could imply that the increase of temperature decreases the induced-fit components of the binding mechanism, with a parallel increase in the conformational selection.

In recent years, aggregation has been increasingly perceived as a potential 'side-effect' of protein binding. Protein interface regions were proposed to be more prone to aggregation than other surface regions [54]. Aggregation is also a manifestation of conformational selection of higher energy (less populated) monomer states leading to highly polymorphic aggregate species.

Aggregation is often prevented by molecular chaperones, which can temporarily cover aggregation-sensitive surfaces. Chaperones also facilitate the assembly of protein complexes; indeed, it was this property that led to the definition and the name of this protein family [55]. Several types of molecular chaperones partially unfold their client proteins at the expense of their parallel folding [56]. Partial unfolding might promote the assembly of protein complexes because one of the two binding partners becomes more similar to the IDPs. Chaperone-induced flexibility of one partner increases the possibility of an induced fit-type scenario, and efficiently resolves the conflict of "who unfolds first", which often arises when two equally rigid partner proteins meet.

Binding mechanisms in the crowded cellular environment

Molecular crowding of the extremely packed cellular environment generally promotes the association of molecules *via* the excluded volume effect [55,57–59]. However, a crowded environment also decreases diffusion, which decreases the chances that two proteins will actually meet. Wang *et al*. [60] recently calculated the optimal intracellular concentration of proteins taking into account the balance of the opposing effects of the excluded volume effect-induced increased association and the decreased diffusion. They found that the optimal protein concentration is about 1 mM, which is within the range of the estimates of the actual intracellular protein concentration. The slower diffusion in crowded environments has a positive effect on binding in addition to decreasing the chances of protein encounter. Once the two binding partners find each other, crowding allows a longer contact time. The increased time in the vicinity of each other makes a more thorough conformational search possible, which might increase the dominance of conformational selection-type processes in crowded environments.

Conformational selection in signal transduction

In signal transduction networks, the fine-tuning of signalling targets and the requirement of alternative pathways ensuring the robustness of the response require conformational flexibility of the binding sites. Conformational fluctuations are not restricted to the nano- to picosecond range, but occur also in the range of milliseconds to seconds (or even hours), where they probably affect signalling steps [9,61]. This conformational dynamism opens the way for a

large involvement of conformational selection in signalling-related binding events [62,63].

Recent data on the PDZ domains of the human tyrosine phosphatase 1E and calmodulin indicate that binding to a signalling protein at certain sites changes its conformational ensemble observed at another site. This induces a cascade of binding events providing both the exact targeting and amplification of the initial signal [29,62,63]. Phosphorylation and other post-translational modifications involved in signalling shift the equilibrium of the initial conformational ensemble, and promote its subsequent binding by stabilizing a rarely populated pre-existing, active conformation of the protein [26,48]. Thus, phosphorylation-induced stabilization of certain states provides yet another example where binding specificity is governed by folding-related events. A single step of conformational selection in its original sense generally helps to achieve a high degree of specificity. However, if the selection process is not followed by a conformational rearrangement, then binding affinity could remain low. High-affinity binding typically requires an additional adjustment step, which could be a general increase in protein dynamism at the binding site after specific binding has been established [64] or, conversely, a rigidification of the IDP partner [53]. Other scenarios exist: for example, the small and flexible protein ubiquitin [8] undergoes a multitude of conformational selection steps to achieve sufficiently high affinity, while rigid spots at the binding site maintain the specificity.

The dynamic fluctuations of the conformational ensemble [13,29,62] can be perceived as noise. Noise is not an enemy of efficient signalling; rather, it might help sigmoidal responses and switch-like behaviour (which can be perceived as an extremely steep sigmoidal response), which are crucial features of efficient signalling [9,61]. Increased noise might lead to the phenomenon of stochastic resonance [65], a process that occurs when noise occasionally helps a sub-threshold signal to surpass the sensitivity threshold. During signalling-related binding events, stochastic resonance might assist in populating certain relevant conformational states. Thus, noise of protein dynamics appears to be an essential component of signal amplification and function.

Concluding remarks and future perspectives

Recent data on protein dynamics has uncovered the complexity of the mutual conformational selection and adjustment process and prompted us to suggest the extension of the original conformational selection model to include the classical lock-and-key, induced fit, conformational selection mechanisms and their combinations (Figure 1). The increasing precision of molecular dynamics simulations permits increasing mechanistic detail. Current observations implicate a key role of hinge-type regions, crucial nodes at intermodular boundaries and, in particular, independent dynamic segments in the binding mechanism. Targeted mutations within these regions could modify the dynamics of the protein; that is, the distribution of the conformational ensemble and thus binding specificity. *In vivo*, such mutations can lead to disease.

With more data on protein dynamics and with the improvement of analytical tools, including network dynamics, perturbation analysis and game theory [23,24], further mechanistic details will be clarified. We believe that the identification of special protein regions governing binding mechanisms will be a major challenge in the near future; however, these advances would allow targeting of malfunctioning proteins in disease and in interface design. The analysis of the conformational dynamics of 452 of the 681 entries of the Electron Microscopy Data Bank (EMDB) [66,67] provides a novel rich source of data for the examination of protein dynamics.

Overall, instead of targeting entire proteins, specific interactions between proteins are becoming increasingly important targets for drug design [68]. Here, we propose that independent dynamic segments should be added to the allosteric drug design repertoire. We expect that the increasing knowledge of the dynamics and mechanism of binding processes we summarized here would assist in developing drugs with substantially fewer side-effects and less toxicity in the future.

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