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Fold or not to fold upon binding — does it really matter?

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Protein interactions are usually determined by well-defined contact patterns. In this scenario, structuring of the interface is a prerequisite, which takes place prior or coupled to binding. Recent data, however, indicate plasticity of the templated folding pathway as well as considerable variations: polymorphism or dynamics in the bound-state. Conformational fluctuations in both cases are modulated by non-native, transient contacts, which complement suboptimal binding motifs to improve affinity. Here I discuss both templated folding and fuzzy binding mechanisms and propose a uniform scheme.

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Introduction

Protein structure is central to function. Three-dimensional arrangements of amino acid residues generate specific microenvironments, with a plethora of biological activities. Folding however, leads to marginally stable conformations, which can be realized by many iso-energetic states [1]. Conformational ensembles are easy to modulate via population shifts by posttranslational modifications, alternative splicing or a network of interacting partners. What are the mechanisms and structural requisites of molecular recognition? On the one hand, steric complementarity results in a well-defined set of specific contacts and usually provide a binary (i.e. yes or no) response to cellular signals. On the other hand, malleable interfaces may either increase complementarity via induced fit or offer alternative interactions upon variable conditions and more complex cellular responses.

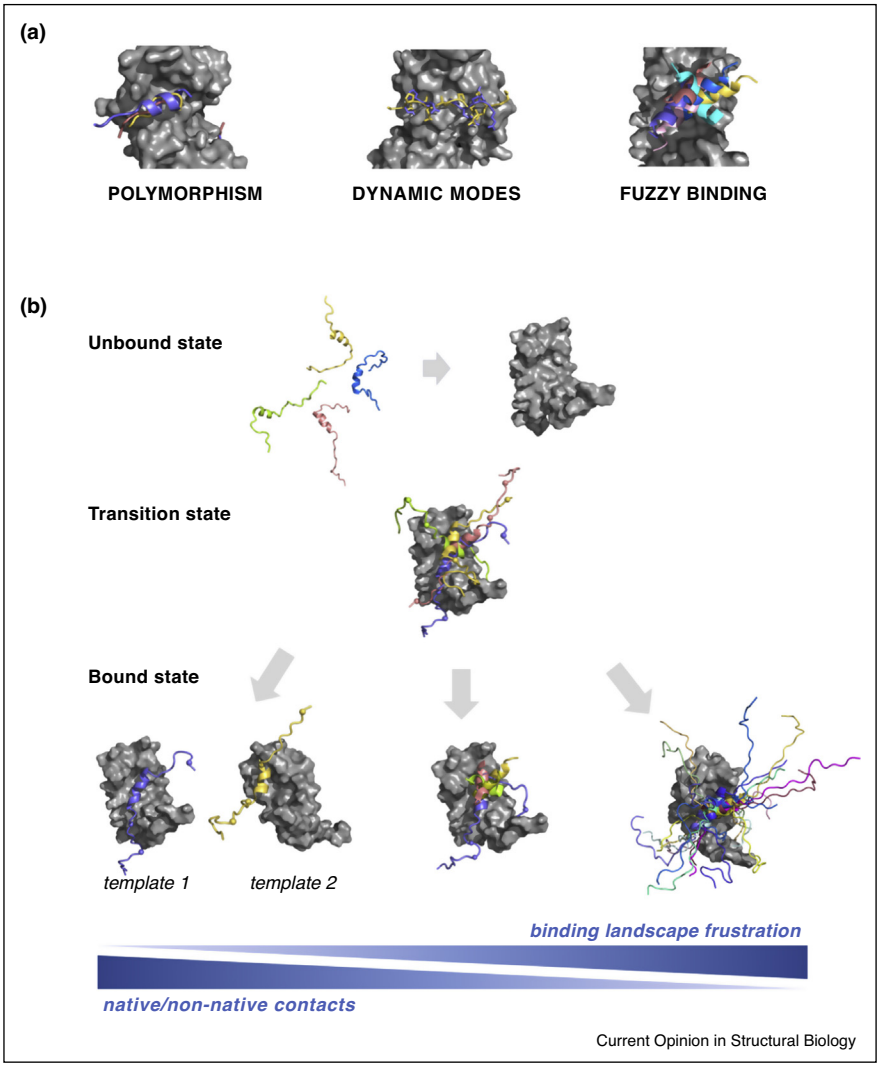
From this viewpoint, interactions of proteins with excessive flexibility are especially interesting to investigate [2]. Solution techniques [3] and advances in single molecule methods [4] provide detailed experimental characterization of highly dynamic sequences, the native state of which is represented by an ensemble of variable secondary and tertiary structure elements. What defines the recognition sites in these systems and to what extent the binding partner shapes the structure? Both conformational selection and induced fit mechanisms may contribute to partner selection [5,6], which result in a folded, complementary interface. Indeed, most studies focus on the structural elements, which are observed in the complex. Within this framework, the folded part of the protein is thought to impart specificity on the interaction.

The 'folding-coupled to binding' scheme, however, is a complicated issue [7,8]. First, it is accompanied by a considerable entropy loss upon limiting the number of conformers in the bound state. Second, most experiments use truncated model systems (e.g. without the fluctuating or sticky parts) with activities differing from the native state. Third, depending on the resolution, well-defined structures may represent averages over a range of conformers with considerable variations in critical interactions [9•]. Indeed, different pathways for partner-driven (templated) folding mechanisms have been revealed, indicating additional layers of the complexity [10•].

In parallel, significant variations in conformations and alternative interaction patterns in specific protein assemblies have been recently observed [11–13] (Figure 1**(a)**), especially in large, complex model systems [14,15]. Such heterogeneity in the bound form implies multiplicity of functionally relevant states, also referred to as fuzziness [16]. Conformational exchange in the complex could present a bottleneck for structural characterization, as the spectrum may overlap with that of the isolated state [17]. Nevertheless, conformational ensembles of a few hundred complexes have been analyzed in detail and were coupled to functional data [18]. Although intriguing, conformational multiplicity or dynamics in the bound state do not compromise specific recognition [19,20], rather offer a toolbox for regulation under varying contexts [21].

In the light of recent data, what does really matter upon protein interactions: fold [22] or not to fold upon binding [23]? In this review, I address the problem of how folded binding elements cooperate with more transient, non-native interactions, and vice versa how non-native contacts shape folding of dynamic interaction sites. I propose that under stochastic cellular conditions, a holistic binding model should operate, where the two scenarios are not distinct.

Figure 1



(a) Heterogeneity in protein complexes. (from left to right) Ribosomal protein S6 kinase alpha 1 adopts different secondary structures upon binding to S100β (PDB: 5csf, 5csi, 5csj [34]). Polymorphism is observed at the canonical interaction motif, corresponding to autoinhibited and released states to regulate ERK phosphorylation. MAPK kinase MKK4 explores multiple recognition modes upon binding to p38α [9•]. Here the docking site is relatively rigid, while the kinase specificity sequence exhibits a fast conformational exchange on the ns timescale. GCN4 activation domain interacts with the shallow binding groove of Med15 in multiple orientations, which interconvert on the μs-ms timescale [19]. Fuzziness of the system is required for optimal transcriptional activity.
(b) Binding mechanism for dynamic protein ensembles. The unbound state ensemble interacts with the target in multiple fashion. Heterogeneity of the transition state (TS) is regulated via non-native interactions (spheres) outside the binding motif. Dynamics of the final, bound complex depends on the frustration of the landscape. In case the interacting motif is close to optimal, the template-bound conformation is selected/induced from the TS ensemble. Increased frustration of the binding energy landscape is compensated by multiple, iso-energetic recognition modes. In case of suboptimal binding motif(s), non-native contacts dominate even in the bound state leading to a fast conformational exchange. This is coupled to variations in interaction patterns. (*graphics was prepared based on coordinates PDB:2lpb*).

Residual structures and polymorphism

Transiently formed, regular secondary structures were observed in highly flexible proteins, which were also sampled in the bound complex [24,25]. In analogy to the framework model in protein folding [26], these pre-formed structural elements were proposed to facilitate the formation of well-defined interfaces [27]. The propensity of structured motifs may indeed improve binding affinity [28], but more intricate relationships can also be revealed [29]. For example, helix-disrupting mutations have a negligible impact on the affinity of c-Myb:KIX binding [30]. Furthermore, folding of transient elements induced by posttranslational modifications may as well impair binding [31]. Initial contacts can also be established in the absence of a folded structure [32], so the final conformation may not be represented in the unbound ensemble [33].

Dynamic, transiently structured binding elements can adopt alternative conformations with the same, specific partner in the final, bound form [34] (Figure 1**(a)**). Static or dynamic polymorphism seems to be common in higher-order protein structures [15,35] (e.g. different prion-strains [36] are generated by interactions of β-strands in alternative registers). Different binding modes [37] — often overlooked in binary/ternary protein complexes — may initiate different pathways under different circumstances [34,38].

Plasticity of the templated folding pathway

Dynamic protein sequences are remarkable in making specific contacts with versatile partners [39]. How residual elements are tailored for different targets? Kinetic studies on templated folding pathways consistently indicate conformational heterogeneity in the transition state [29,40••,41]. Structures in the ensemble may exhibit alternative contact topologies, potentially leading to functional plasticity [40••,42,43] (Figure 1**(b)**). Interestingly, the degree of conformational heterogeneity is controlled by residues outside the binding site [40••], often referred to as non-native contacts. Here this terminology is used not only for the transition state, but also for the bound form. Non-native interactions likely reduce the encounter times between the flexible protein and its partner [44], providing a major difference in kinetics as compared to globular proteins [45]. Along these lines, majority of native hydrophobic contacts appear after the rate-limiting step of forming the HIF1α CAD: CBP TAZ1 complex [46•].

Non-native, transient interactions

Although even highly flexible sequences can achieve picomolar affinities, the binding surface may not be fully optimized [47••]. Frustration of the binding energy landscape can be minimized by non-native interactions, which could be transiently sampled in the bound state [11]. These contacts can improve affinity in parallel with increasing conformational heterogeneity [11] (see above), demonstrating that the two effects are not exclusive. Owing to their transient nature, non-native interactions might be beyond the level of detection [48,49••]. The presence of these transient contacts is often witnessed by affinity-modulating mutations outside the physically interacting segments [50] or even by ionic strength effects [51]. Transient contacts with the binding elements can tune the entropy of association [52], compete for the target site [53], or simply increase local concentration nearby the partner [54].

Dynamics in the bound state

Energetic frustration of the binding energy surface may also stem from impaired correlated conformational fluctuations [55,56••]. These long-range motions may be compensated by decreased order in the bound state, as observed for osteopontin [57], α-synuclein [56••,58] or Hepatitis C virus interactions [43]. In this strategy, the different conformers interconvert between thermodynamically equal, suboptimal sites [59•] (Figure 1**(b)**).

The functional role of the bound state plasticity however, often remains to be elucidated. In general, structural properties of the dynamic ensemble can be perturbed by minor effects or posttranslational modifications [60,61]. Population shifts in 'sensor' regions for example, may initiate functional [62] or pathological [48] polymerization. The tolerance to sample multiple orientations in the bound state keeps the interface architecture simple (e.g. using a few hydrophobic contacts and a shallow binding cleft [19,63]), which could be explored by several binding partners. This empowers a fuzzy 'free for all' mechanism, where activation domains (AD) could simultaneously screen all AD binding regions via weak, low-specificity contacts [49••] (Figure 1**(b)**). Such an efficient, combinatorial mechanism might be common in gene regulatory circuits.

Multivalency and interaction ambiguity

Recent structural and computational studies evidence fast kinetics [64,65] and high affinity [66,67] by multiple, minimalistic, suboptimal motifs. How these could be achieved? First, these associations do not require a major conformational transition between the unbound and bound forms, as both states are highly dynamic. Second, the redundant binding motifs could be combined into many iso-energetic configurations [59•], given the plasticity of the interconnecting segments. Third, contacts via multiple specific sites can increase the encounter frequency, similarly to non-native interactions ([11,40••] see above). Taken together, the multitude of topologies appearing in the bound complex increases the efficiency of target-search as compared to individual motifs.

Oligomerization via weak binding sites can thus optimize affinity and fidelity of the recognition, which is exploited as a 'quality control for signaling' [68]. For example, this can rationalize why tandem AD binding sites result in activated transcription [69]. Indeed, self-assembly of activation domains generates 'standalone' cellular compartments, similarly to proteins with multiple RNA-binding motifs [70]. These membraneless organelles are constructed via dynamic recognition modes, and their building blocks interconvert amongst many different bound conformations [71,72].

Entropy-driven specificity?

Given the multitude of conformations in the transition or bound state and the suboptimal interactions with the target site, specificity might be an issue. Surprisingly, multiple recognition modes of high-affinity, synthetic GCN4 variants correlate to specificity for Med15 [19]. Similarly, complexes of small molecules with $p27^{Kip1}$ [73], Tau [74], c-Myc [75••] exhibit significant heterogeneity,

including variations in the binding site. Conformational fluctuations, appearing because of the frustrated binding landscape, could be controlled by non-native interactions [40••]. Consistently with these observations, entropy may be specifically tailored for selected partners [76]. Indeed, a marked difference between the distributions of an inhibitor (10058-F4) and urea upon diffuse binding to c-Myc has been observed [75••]. Despite its loosely defined binding, this compound has been shown to efficiently block heterodimerization with Max, as well as decrease c-Myc aggregation. Although the generality of the 'entropic' binding has yet to be explored [76], this pioneering idea could be exploited in targeting highly dynamic sequences.

Towards a consensus model

Recent structural and kinetic data evidence conformational fluctuations in both templated folding [40••,47••] and fuzzy interaction mechanisms [56••] in different stages along the binding trajectory (Figure 1**(b)**). Fluctuations either in the transition or the final bound state are frequently controlled by partner interactions outside the binding site. Consequently, native interactions are often mediated by suboptimal motifs, which are optimized by the transient (partial) non-native contacts. As a further layer of complexity, multiple suboptimal motifs may similarly cooperate leading to a combinatorial mechanism [77•]. Thus, partner-modulated variations in conformations and contact patterns appear to be general considering the full binding trajectory of the fully functional sequence. This is a key to define a uniform binding model.

The holistic pathway should be composed of both native and non-native interactions defined in a complete model (i.e. not truncated with full activity). The relative contributions of these two contact types in different stages along the pathway determine the spatial and temporal plasticity of the recognition process, and eventually defines the interaction mechanism. Therefore, the functionally relevant variations in conformations and contacts ('fuzziness') along the binding trajectory should be characterized. This serves as a basis for regulation either in the transition state [40••] or in the final complex [57].

Future directions

Only a handful high-resolution data is available on archetypal fuzzy complexes [9•,64,66]. Detailed experimental characterization of more systems would be required to understand the driving forces of these dynamic recognition modes. Importantly, functionally complete models (e.g. with K_d equal to that of the full sequence) must be investigated to eliminate truncation artefacts. Further technical advances will be needed to overcome limitations by low-affinity and transient interactions, or motif redundancy. Further computational studies will shed light on the interplay between non-native and native contacts, and how these — often transient interactions — shape the global characteristics of the association [11,40••]. Synergy between redundant motifs also remains to be explored.

In conclusion, a unified binding model must not discretize the bound protein chain to be 'folded' or 'not folded'. Instead, the model should define the stable or critical components of the interaction and their variations owing to conformational heterogeneities in the system along the full binding trajectory. Alternative — even transient — structures must be interpreted in the light of functional data, to relate the different patterns to their biological outcomes. Albeit difficult to envisage, fuzziness is intrinsic to the holistic model, detailed characterization of which provides exciting perspectives for protein engineering and drug design.

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Combining single-molecule FRET, kinetic studies and molecular dynamics simulations, different binding models are analyzed for Nup153 and Nup214. Nup153 exhibits ultrafast kinetics by archetypal fuzzy binding mechanism (see also ref 64). Nup214 however, expands upon interactions with exportin CRM1, termed as coupled reconfiguration binding. Both scenarios are robust to glycosylation. Structural prerequisites and biological consequences of the two models are discussed.