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Review

Multiple conformational selection and induced fit events take place in allosteric propagation

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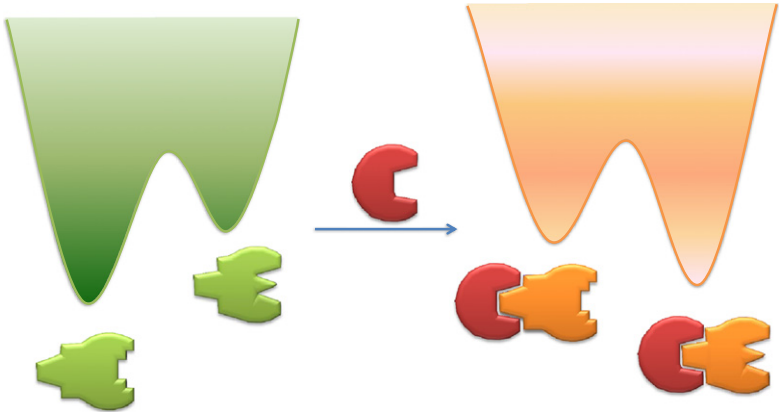
HIGHLIGHTS

- Pre-existing conformational states have been exploited by evolution.
- Conformational selection takes place at every step along the allosteric pathway.
- This view generalizes conformational selection also to other allosteric events.
- At each step in the pathway conformational selection is coupled with induced fit.
- Allostery induced by PTMs and light relates to conformational selection as well.

GRAPHICAL ABSTRACT



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ABSTRACT

The fact that we observe a single conformational selection event during binding does not necessarily mean that only a single conformational selection event takes place, even though this is the common assumption. Here we suggest that conformational selection takes place not once in a given binding/allosteric event, but at every step along the allosteric pathway. This view generalizes conformational selection and makes it applicable also to other allosteric events, such as post-translational modifications (PTMs) and photon absorption. Similar to binding, at each step along a propagation pathway, conformational selection is coupled with induced fit which optimizes the interactions. Thus, as in binding, the allosteric effects induced by PTMs and light relate not only to population shift; but to conformational selection as well. Conformational selection and population shift take place conjointly.

Contents

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1. Introduction

The mechanism of molecular recognition has attracted considerable interest over the years. Early on, in the classical description, molecules were viewed as rigid bodies. Recognition then focused on the question of *how a ligand selects a specific protein from a pool of proteins*. The proposed solution was 'lock-and-key'; that is, a perfect match of rigid complementary shapes [1]. In this view each protein has a single shape. A ligand distinguishes between the proteins through their different shapes. Subsequent realization that proteins are flexible altered and refined the question: *how a protein binds its ligand if their shapes do not match well*. This led to the hypothesis of the 'induced fit' [2]; that is, the interacting ligand induces conformational change in its partner, which optimizes their assembled complex. According to the induced fit hypothesis a protein has two states: open and closed. The ligand binds to the open state and induces a conformational change which results in the closed state. The subsequent 'conformational selection and population shift' hypothesis [3–8] argued that the induced fit hypothesis overlooked the fact that in solution, there is a large number of pre-existing states and substates of each protein. That being the case, based on simple equilibrium arguments, the state with the most complementary shape will bind, followed by a population shift toward this state, which results in redistributing the ensemble. Thus, the 'conformational selection and population shift' hypothesis turned the question around, asking *which conformation of the protein*, *out of the many in the pool*, *will bind*, and if it is a distinct conformation, how the equilibrium will be maintained so binding can continue.

Current descriptions of the binding mechanisms often do not clearly distinguish among the questions addressed by the 'lock-and-key' versus the 'induced fit' and 'conformational selection' mechanisms. The lock-and-key mechanism addressed the question of *which* protein – out of the many in the cell – will bind; the induced fit mechanism and the conformational selection mechanisms posed the question of *how* will a specific target protein bind. Eventually, the conformational selection comes back to a solution which at first sight resembles the lock-and-key mechanism in that it also invokes selection by a good-matching shape; however, the selection is of a conformer out of the many different conformers in the ensemble, rather than of a protein out of the many different proteins. Thus, the key difference between the lock-and-key and conformational selection mechanisms is that conformational selection induces a change in the equilibrium of the states, which is forced to re-equilibrate, unlike the lock-and-key. This re-equilibration is the origin of the population shift which cannot be present in the lock-and-key mechanism, where the ensemble consists of different molecules. Proteins can flip between states; however, one protein cannot be converted to another. The classical induced fit mechanism is also unrelated to the equilibrium, since it is assumed that the transition from the open state to the closed state is induced by the ligand. Fig. 1 distinguishes between the three mechanisms of lock-and-key (Fig. 1A), induced fit (Fig. 1B) and conformational selection (Fig. 1C). These mechanistic descriptions explain why induced fit can extend conformational selection [9,10]: starting with some well-bound state via conformational selection, induced fit can optimize it [10–12].

Conformational selection as it relates to allostery is now broadly accepted by the community as the major binding mechanism in diverse mechanisms in the cell [9,13–61]. However, in the literature, conformational selection and population shift are often viewed as a single event taking place upon ligand binding. This is not the case. Allosteric propagation across a structure [62–64] can be viewed as entailing multiple conformational selection and population shift events, taking place at each step along the allosteric pathway. To picture such scenario, consider that the conformational change taking place at the binding site has to be recognized by a complementary conformation at that site, with a subsequent shift in the ensemble; similarly, the conformational change at the second step is recognized by complementary site again with a subsequent population shift, etc., across the structure, as the allosteric 'wave' proceeds. Thus, while to date a ligand-binding event has been perceived as correlating to a *single occurrence* of conformational selection and population shift, it can be described by a large number of conformational selection and population shift occurrences along the propagation pathways. Each occurrence translates to flipping of conformational states, similar to the event taking place upon ligand binding at the binding site. Stepwise conformational selection has been advanced for catalysis, where conformational selection and substate population shift at each step of the catalytic turnover can accommodate enzyme specificity and efficiency [65]. There too, NMR, X-ray crystallography, single-molecule FRET, and simulations clearly demonstrate that the free enzyme dynamics already encompass all the conformations necessary for substrate binding, preorganization, transition-state stabilization, and product release. Here we point out the generality of multiple step-wise occurrences.

It is intriguing to note that while conformational selection and population shift taking place upon ligand binding have been recognized as the origin of the alloteric effect [62], including in dynamic allostery in the absence of conformational change [44], and that allostery can similarly result from population shift induced by post-translational modifications (PTMs) [66] and from photon absorption [67], these have not been put together. Here we argue that as in binding, the allosteric effects induced by PTMs and light relate not only to population shift; but to conformational selection as well. Conformational selection and population shift take place conjointly.

2. Conformational selection and population shift

In 1999 we suggested that conformational selection and population shift are the major molecular recognition mechanism [7] and that it underlies the allosteric effect [5,62]. That proposition was inspired by the free energy landscape of Frauenfelder, Sligar and Wolynes which was published eight years earlier [68]. Even though this concept was founded on fundamental physical principles, we had great difficulties in publishing our first manuscript [7], with the critique being that there is no experimental validation for a large number of pre-existing states, and that the Protein Data bank (PDB) houses only two states: open and closed. It is fascinating to reflect on the change in the perception of the scientific community: from broad disbelief to being taken for granted. Conformational selection among protein ensembles of states

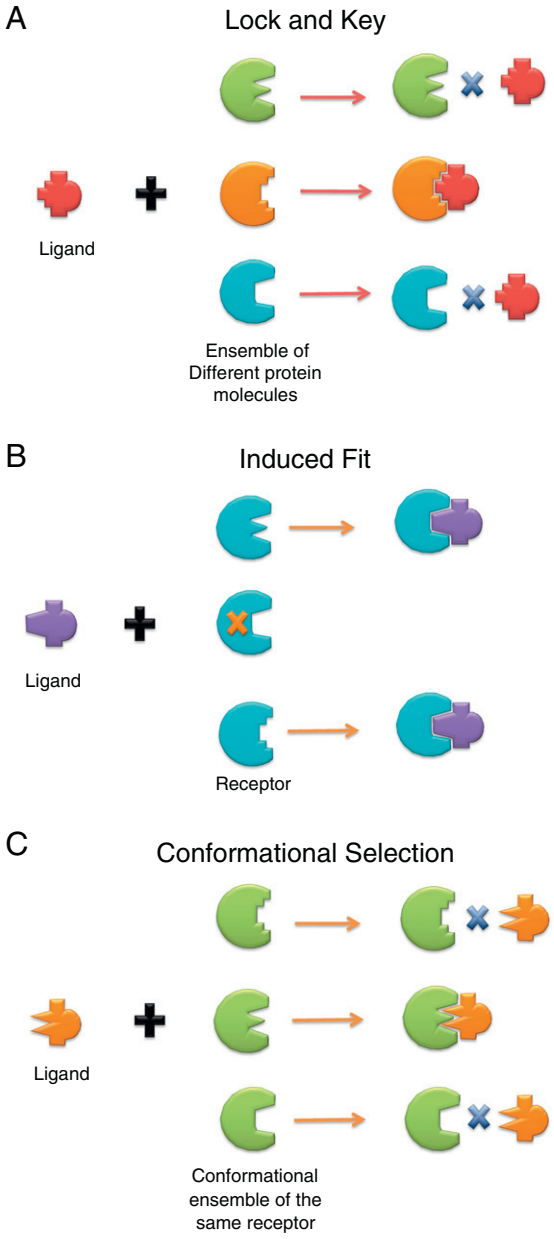

Fig. 1. Schematic illustrations of binding by 'lock and key' (Panel A), 'induced fit' (Panel B) and 'conformational selection' models (Panel C). According to the 'lock and key' model (Panel A), binding takes place when there is an exact geometric fit between the ligand and receptor. The cross sign denotes the absence of binding when the shapes do not match. Thus, among the pool of protein molecules (each protein type shown in a different color) the ligand selects the one whose shape is complementary. According to the 'induced fit' model (Panel B) there is no exact fit between the ligand and receptor before binding (shown by the cross on the receptor whose shape is complementary to the ligand). The ligand binds a protein molecule, inducing changes in the protein shape to fit the ligand. In the 'conformational selection' model (Panel C), the ligand selects a conformer from a pool of conformers of the same protein, whose shape is complementary via the same lock and key criterion. The different conformations of the same receptor are in the same color (green). The figure is adapted from Fig. 3 in Allosteric conformational barcode direct signaling in the cell. Nussinov R, Ma B, Tsai CJ, Csermely, P. Structure, 2013, September issue [67], with permission.

was proposed by Foote and Milstein for conformational isomerism in antibody diversity at the combining site [69]; however, it was not coupled with the concept of population shift. Population shift is the fundamental paradigm underlying conformational selection. This link was rooted in the free energy landscape which maps all possible conformations of a molecular entity or the spatial positions of interacting molecules in a system, and their energy levels on a two- or three-dimensional Cartesian coordinate system. Population shift is the more basic concept than conformational selection. Conformational selection (Fig. 1C) reflects the pre-existence of molecules as ensembles of states. However, as we have suggested then [3–8], conformational selection could not have taken place had it not been for the population shift. To retain the equilibrium and continue the binding reaction there needs to be a shift of the ensemble towards the binding-favored state. Population shifts explain the cooperative nature of allostery between residues lying on the allosteric pathway [70,71], which can only be understood in terms of conformational ensembles.

Induced fit [2] can only involve minor conformational change following binding. For larger conformational changes, induced fit is possible only if the match between the interaction sites is strong enough to provide the initial complex enough strength and longevity so that induced fit takes place within a reasonable time [11]. Induced fit alone cannot explain well binding where proteins undergo large conformational changes.

3. Multiple conformational selection and population shift events in multiprotein assembly resemble multiple conformational selection events in hierarchical folding

That conformational selection takes place not once in a given binding/allosteric event, but at every step along the allosteric pathway, can be

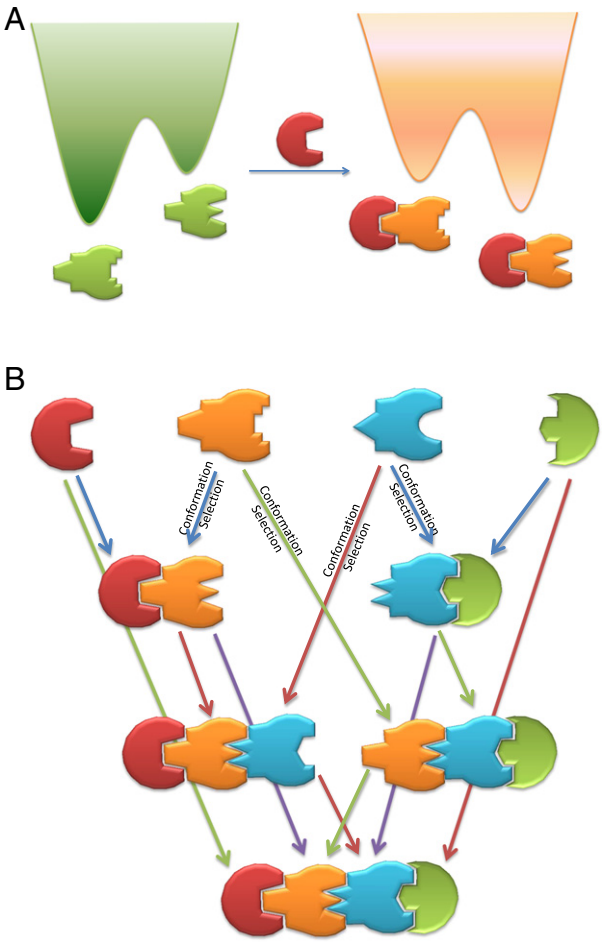

Fig. 2. A schematic illustration of the role of conformational selection in a binding event. Panel A emphasizes that conformational selection via a binding event is coupled with a population shift, which allosterically helps in guiding the next binding event. The free energy landscape illustrates that the relative stabilities of the conformers change following binding. In Panel B, a complex with four entities in a linear association is used here to illustrate three possible association pathways shaped by conformational selection events in a hierarchical process.

seen in multiprotein formation, which involves multiple binding events. Multiprotein complexes assemble hierarchically [72]; each binding event takes place via conformational selection coupled with a population shift (Fig. 2A), which allosterically helps in guiding the next binding event. The formation of multiprotein assemblies resembles protein folding, which is also a hierarchical process (Fig. 2B). In protein folding, the first step involves formation of preferred, meta-stable 'building blocks' which are highly populated, contiguous fragments [73]. Via conformational selection these hierarchically associate to form hydrophobic folding units, which are the basic unit from which a fold is constructed. A hydrophobic folding unit is an independent thermodynamically stable unit with a buried hydrophobic core. The hydrophobic folding units in turn associate via conformational selection and population shift which allosterically help create intramolecular domains, that subsequently similarly assemble to build either an intramolecular multidomain protein fold or further proceed to form an intermolecular quaternary structure. The difference between binding and folding is the absence, or presence, of chain connectivity. It is well known, however, that cleaving the polypeptide chain to create two molecules usually results in a dimer association having a structure similar to that of the monomer and using a linker to join two separate subunits generally results in a similarly folded monomer. With an extra connection in the intact chain the folding process is favorable kinetically and energetically, if the native structure is unaltered. This advantage is a result of favorable entropy. Chain cleavage results in two opposing factors. The unfavorable factor is the loss of entropy, which results from the splitting of the polypeptide chain. However, the removal of the linkage constraint may lead to a more favorable binding orientation. Thus, binding and folding are similar processes, governed by similar principles [3,74]. In folding, small stand-alone units with some population times hierarchically assemble via combinatorial assembly through conformational selection; in multimolecular associations it is molecules. Going back to the free energy landscape description, folding and binding can both be described by folding funnels. Folding can be viewed as going down the funnel-like free energy landscape toward the bottom of the funnel [75,76]; binding, which proceeds along a rugged free energy surface around the funnel bottom, can be viewed as microfunnel fusion [6,7]. Such extension of the free energy well provides a thermodynamic interpretation for the binding models [4]. Allosteric propagation, which follows the description of protein folding around the funnel bottom, can thus be portrayed by multiple intramolecular binding events, each taking place by conformational selection, with optimization by induced fit.

4. Conformational selection and population shift events in allosteric post-translational modifications and photon absorption

Similar to binding events, allosteric effectors such as PTMs and light also lead to conformational perturbations, and these also propagate in the structure via conformational selection and population shift. Simply put, the propagation does not care which effector induced it; once perturbation is incurred, it will spread in the structure, and spreading is via multiple conformational selection and population shift events. This also holds for dynamic allostery [44]. Nitrogen regulatory protein C (NtrC) phosphorylation provides a nice example of how proteins shift from one conformation to another following a PTM event [77,78]. There is a pre-existing equilibrium between a major inactive and a rarely populated active state, and phosphorylation shifts their relative abundance by stabilizing the active conformation rather than destabilization of the inactive form [77,79]. The data show that the transition between the two conformations involves loss of native stabilizing contacts which are compensated by non-native transient hydrogen bonds during the transition. While the allosteric propagation can be explained by induced fit which induces the opening of these hydrogen bonds and the loss of other possible stabilizing interactions, it can also be described by population shift in terms of the free energy landscape. Since the bottom of the folding funnel is not comprised of just one state, but of many thermally accessible substates, flipping between these states can involve conformational selection with a population shift similar to a ligand-binding reaction. Such landscape view is consistent with the premise that evolution has exploited the pre-existing states, and adapted them to play roles in protein function.

Light-triggered conformational changes have been observed [80]. The conformational change and signaling in light-oxygen-voltage (LOV) domains of Avenasativa phototropin 1 (AsLOV2), a member of the Per-Arnt-Sim (PAS) family, is mediated by a flavin mononucleotide chromophore that forms a covalent bond with a cysteine upon illumination. This event releases the carboxy-terminal Jα helix, which is the biological output signal, while the amino-terminal helix is a control element in AsLOV2's light-activated conformational change. This allosteric light-activated conformational change can proceed through the same conformational selection and population shift mechanism.

5. Conformational selection and population shift can specify signaling pathways

Proteins can assemble combinatorially to specify distinct cellular pathways. The number of major signaling pathways is limited and the diverse cellular outcomes are achieved via pathway cross-talk. Cross-talk takes place through shared signaling proteins whose binding sites express 'conformational barcodes' [67]. Each barcode is the result of a specific transient combination of co-occurring allosteric effects (prior binding events, allosteric PTMs, etc.) and orthosteric PTMs (PTMs at the binding site). A barcode has distinct shape and dynamics which recognize and conformationally select a partner molecule. Because the partner protein is a component of a pathway, selection of a partner specifies the pathway. The signal propagates through conformational selection and population shift events across protein–protein interactions throughout the pathway [81].

One example of such pathway cross-talk relates to transforming growth factor-beta (TGF-β)/bone morphogenic protein (BMP) signaling, which is involved in the vast majority of cellular processes. Deregulation of the pathway leads to developmental defects and disease, including cancer. Among the signaling pathways which cross-talk with TGF-β/BMP are the mitogen-activated protein kinase (MAPK), phosphatidylinositol-3 kinase (PI3K)/Akt, Wnt, Hedgehog, Notch, and the interleukin/interferon-gamma/tumor necrosis factor-alpha cytokines. The MAPK and PI3K/Akt pathways influence TGF-β/BMP signaling through Smad [82]. The linker region of Smad proteins is highly flexible and rich in serine, threonine and proline residues. The complexity of the patterns of phosphorylation and their outcomes can be seen from the observations that while phosphorylation of these sites inhibits Smad3 transcriptional activity, it does not block Smad3 from entering the nucleus, suggesting additional recognition scenarios for Smad3 inhibition mediated by linker phosphorylation barcodes. Further, Smad3 Thr178, Ser203, and Ser207 are Erk1/2 phosphorylation sites; in human breast cancer cells, Rho-dependent kinase (ROCK) and p38 MAPK phosphorylate Ser203/207, but not Thr178. These facilitate, rather than inhibit, TGF-β-induced growth inhibition. Together, these suggest that Smad2/3 linker recognition by a specific kinase phosphorylation can yield distinct outcomes. MAPKs (especially Erk1/2) also phosphorylate the linker of Smad1/5, which blocks its nuclear translocation. As a result, BMP function can be suppressed by signals that activate RTK/MAPK. In the Smad1 linker, Ser/Thr residues can be sequentially phosphorylated by Erk and then GSK3-β, creating a docking site for the Smad1/5-specific E3 ubiquitin ligase, Smurf1. Smurf1 binding not only causes ubiquitination and degradation of the Smads but also occludes their interaction with the nuclear pore complex, thereby preventing Smad nuclear translocation. Wnt signaling, which inactivates GSK3-β, reduces Smad1 ubiquitination. Fibroblast growth factor, which signals to the nucleus by binding to FGFR and activates multiple signal transduction pathways, including those involving Ras and MAPKs relieves the repression.

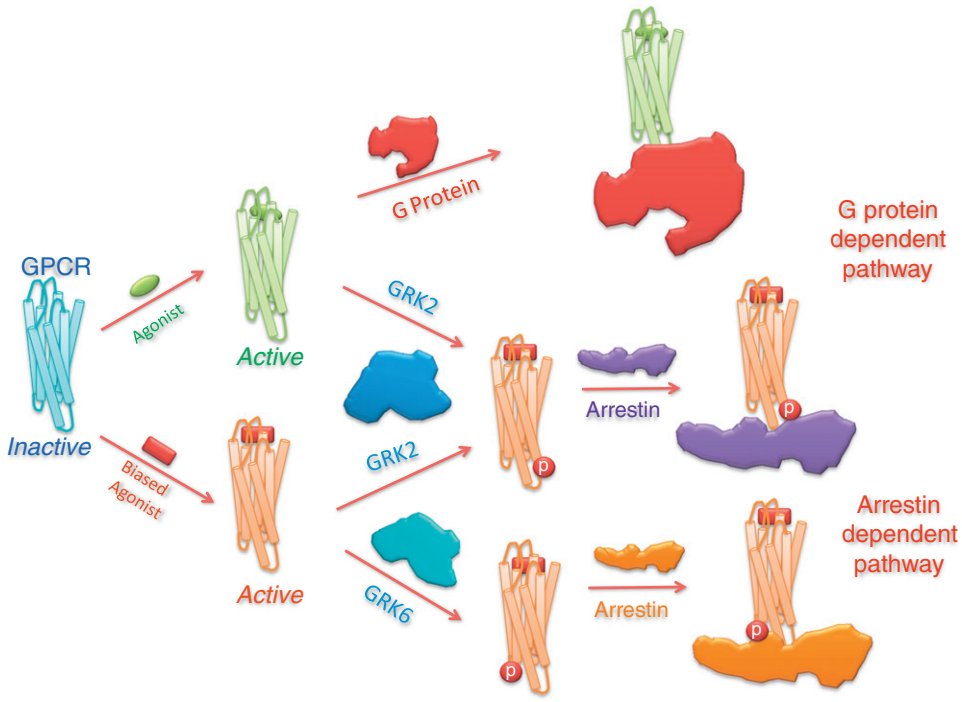

Fig. 3. An illustration of the role played by allostery on diversified cellular pathways with signaling through a single G protein-coupled receptor (GPCR). Initially, the two main branching pathways divide through an allosteric conformational selection of distinct GPCR active states by various types of agonist binding. In the agonist (G protein-dependent) pathway, the activated GPCR either activates the heterotrimeric G proteins which then promote the consequent signaling of the second messenger such as cyclic AMP, or recruits the GPCR kinases (GRKs) to phosphorylate Ser/Thr in the cytoplasmic loops and tail of the GPCR which in turn enable the recruitment of β-arrestins to mediate receptor desensitization and internalization. In the biased agonist (arrestin-dependent) pathway, distinct active GPCR conformations activate different sets of GRKs to create distinct phosphorylation patterns (barcodes) on GPCR. It is the barcode [67,103] that imparts distinct conformations, which in turn recruit arrestins with diverse conformations (illustrated in different colors in the Figure) to mediate different signaling pathways such as the ERK 1/2 activation. Recruitment can be either through orthosteric or allosteric interactions.

Together, these provide a compelling example for pathway cross-talk via shared proteins, mediated by patterns of phosphorylation. A key factor in binding of shared proteins is prior allosteric events and orthosteric PTMs at the binding sites. This can be seen in the sequential activation of Smad1/5 through successive binding of Erk and GSK3-β, and the different phosphorylation patterns of the Smad3 linker by different kinases. An additional well-studied example relates to the GPCR/arrestin pathways. Fig. 3 illustrates that different active GPCR conformations are recognized by specific ligands, which shift the ensemble toward these recognized states. In turn, these ligand-bound active conformations get selected by specific G protein-coupled receptor kinases (GRKs), which tag them with different phosphorylation patterns. The resulting phosphorylation barcodes are selected by β-arrestins, setting them onto the arrestin-dependent pathway. Alternatively, specific ligand-bound GPCR can be recognized by G proteins, to initiate the G protein dependent pathway [83,84]. In cross-talk, the combinatorial signal from two pathways, A and B, has to produce a different response than that triggered by A or B separately. Such cross-talk scenario can take place when components of the two pathways interact, as above; or when one signal modulates, or competes, with a key mediator of the other. Both scenarios work through population shift. However, cross-talk may also take place indirectly, when components of one pathway are enzymatic or transcriptional targets of the other [82].

6. Conformational selection and population shift and the spatial structure of cell signaling systems

Signaling pathways which span long distance scales in and between cells exploit the cellular spatial organization. This interplay between signaling and the spatial organization of the cell has not been fully appreciated. The cell is organized: from small complexes and assemblies, to local membrane-associated and cytoplasmic dynamic clusters (which can be on the order of tens to hundreds of molecules), to the long scale filamentous cytoskeleton. This dynamic multi-scale organization also mediates signaling and coordinates cellular responses. Cellular organization is adjusted by signaling states; in turn, it influences the conformational distribution of signaling molecules by acting as conduits of conformational redistributions. Understanding the interplay across multiple scales, linking it to the physicochemical basis of the conformational behavior of single molecules, and ultimately relating it to cellular function are the major challenges. We view cell signaling primarily not as a sequence of diffusion-controlled molecular collisions, but instead as transient, allostery-driven cluster re-forming interactions, within and across scales [85]. Signaling spanning this cellular spatial organization, within and between clusters, proceeds via multiple sequential conformational selection and population shift events. As we illustrate in the examples below, they take place at protein–protein interfaces; protein–RNA (and DNA), protein–lipid and lipid–lipid.

One example which illustrates this principle for long scale organization relates to signal transduction where RNA-binding induces the melanoma differentiation-associated protein 5 (MDA5) to form filaments consisting of helical arrays. In turn, these protein arrays nucleate the assembly of the signaling adaptor mitochondrial antiviral signaling protein into its active fibril form [86]. This example points to a signaling scenario in which the regular arrangement of the MDA5 protein N-terminal caspase recruitment domains, positioned on the outside of the filaments, provides a conformational barcode for the scaffolding mitochondrial antiviral-signaling protein fibril assembly. Thus, signaling proceeds via a series of conformational selection and population-shift events: RNA binding induces a population shift in the MDA5 protein which results in self-assembly-favored conformations. Population shift within these creates state favoring recruitment of the adaptor

mitochondrial antiviral signaling protein, with the allosteric signal propagating to form the active fibril state. Scaffolding proteins typically work via conformational biasing mechanism [87].

Going down in scale-sizes, our next examples relate to signaling through micro/nanoclusters. Clusters are common, and contain multiple dynamically preorganized copies of a signaling protein, and its partners. The clusters are transient, forming and dispersing. They are preferentially associated with membrane domains, anchored through lipophylic PTMs and often cationic charges on a protein terminal and hydrophobic tails. This spatial domain organization, together with the mediating membrane lipids stabilize the clusters, and facilitate allosteric signaling through cluster members and their partners. Population shift can either take place directly between the proteins, or indirectly, through lipids [88–90], such as cholesterol and sphingolipids. We reason that clusters enhance signaling not only because of the robustness incurred by protein concentration; in particular, because of their cooperative allosteric behavior. The prevalent occurrence of signaling proteins in clusters is testament to the advantages inherent in such organization, which we believe are largely due to allostery [85]. This is further supported by the observation that BRaf inhibition enhances nanoclustering of K- and N-Ras [91] which cluster in disordered membrane domains, but has no effect on H-Ras clustering in the fluidic organized domain [92]. BRaf is a partner of Ras protein. BRaf side-by-side asymmetric dimers provide two Ras docking sites, essentially crosslinking two Ras molecules, resulting in a cross-linked Ras mesh and cooperativity between Ras monomers. However, in addition, current data indicate that Ras also directly dimerizes as well.

The Eph/ephrin cluster signaling system [93] provides another interesting example. Eph/ephrin clusters possess several unique features differentiating them from those of other receptor tyrosine kinases. Prominent among these are the initiation of bi-directional signaling cascades and ligand and receptor subclasses with promiscuous intra-subclass interactions, but rare inter-subclass interactions. Eph/ephrin clusters at the sites of cell–cell contact are associated with Eph signaling initiation. Importantly, these clusters also provide an example of how signaling crosses cellular size scales: the physical reorganization of EphA2 clusters on the cell surface allosterically induces changes in the dynamic cytoskeleton morphology, revealing a mechanism for spatio-mechanical regulation of the Eph signaling pathways [94] via multiple conformational selection and population shift events. Another recent striking example relates the conformational states of the kinase Lck with the regulation of clustering in early T cell signaling [95]. Thus, both Eph/ephrin and Lck/T-cell receptors, provide clear examples of our premise that the dynamic spatial conformational organization of the clusters is related to the specific cellular signaling state as we detail below for the Lck/T-cell example.

The T cell antigen receptor (TCR) exists in a range of conformational states [95]. Signaling initiates by selective binding of antigenic peptides bound to major histocompatibility complexes on antigen-presenting cells. Lck, a tyrosine kinase belonging to the Src family of kinases, recognizes specific bound conformations and phosphorylates TCR at the tyrosine-based activation motif (ITAM). Multiply-phosphorylated ITAMs recruit and activate the tyrosine kinase Zap70. In turn, Zap70 phosphorylates signaling scaffolding proteins, like Lat. Phosphorylation of TCR by Lck is an essential step in the signaling cascades of T cells activation. The myristoylated and palmitoylated residues at the Lck amino terminal anchor the kinase to the plasma membrane. SH3 and SH2 domains adjoin the lipid anchor. Nearby are the tyrosine kinase catalytic domain and the carboxy-terminal. Lck's activity is regulated by trans-autophosphorylation and dephosphorylation of an activating tyrosine (Tyr394) in the catalytic domain and a carboxy-terminal inhibitory tyrosine (Tyr505). Tyr505 phosphorylation stabilizes the closed state where the phosphorylated carboxyl terminus of Lck binds its SH2 domain. In this closed conformation Lck is inactive. Dephosphorylation by the CD45 phosphatase of Tyr505 shifts the equilibrium toward the open, active Lck conformation. CD45 can also dephosphorylate Lck at the activating Tyr394, which inhibits Lck activation. There is no evidence of direct activation of Lck by the TCR or by the coreceptor, arguing for equilibrium shifts controlled by phosphorylation/dephosphorylation [95]. As typical for kinases in the cell, the equilibrium favors the inactive state; however, still with ~40% of Lck being constitutively active, which suggested that the Lck dynamic spatial reorganization may regulate TCR signaling. Lck distributions on the molecular level are controlled by the conformational states of Lck, with the open, active conformation inducing clustering and the closed, inactive conformation preventing clustering. Quantification of the distribution of Lck points to a mechanism for Lck clustering in which the active/inactive conformational states of Lck, rather than the kinase activity itself, control the degree and dynamics of Lck clustering. Clustering is regulated intrinsically, with the open conformation inducing clustering and the closed conformation opposing clustering. The distribution is regulated mainly by self-association and the clustering dynamics determine the phosphorylation state of the TCR [95–97]. It has been suggested that self-association could be charge-mediated interactions [95]. Electrostatics has been linked to the distribution of proteins in membranes [98] and to the sequestration of the positively charged TCR ITAM domains into negatively charged lipids in the inner leaflet of the plasma membrane [99–101]. For the signaling to take place, the interactions – whether direct or lipid-mediated – are likely to take place via conformational selection. The population shift which follows is the origin of the redistribution of the cluster ensemble of conformational states. Allostery crosses molecular boundaries upon binding [102]; and these events can propagate across clusters and pathways [85].

7. Allosteric propagation and allosteric efficacy

Throughout the paper we related to allosteric propagation. In this context, it behooves us to add a clarifying note about the popular concept of allosteric propagation pathways. Intuitively, propagation fits well into a description of allosteric phenomena, and helps to understand how two far-away sites are coupled to each other. However, the coupling through a propagation pathway does not determine the allosteric efficacy; it is merely a necessary condition for allostery. Efficacy is the capacity to produce an effect. In allostery, the efficacy is determined by the sum of the relative stabilization of the active conformation and the destabilization of the inactive conformation following ligand binding. The propagation pathway describes how the two sites are connected in the structure.

8. Observing population shift

Quantitative experimental measurements of the free energy of the predominant state following distinct allosteric events or their combination can provide an indication for population shift. Traditionally, measuring changes in the affinity of receptors has also been a popular venue; however, more recently, effects on both the affinity and the efficacy have also been considered ([104,105]; reviewed in [16]). One such example is the seven transmembrane (7TM) receptors with a modulator that interacts and transmits information through population shift to a guest. In this case population shift has been considered both as vectorial transfer of information from ligand-binding domains to the cytosol and along the plane of the membrane (receptor dimerization) [16]. NMR is also powerful in following population shift [35,77,78,106], even when taking place through high energy, low-populated states [77]. From the computational standpoint, it is possible to detect population shift through molecular dynamic simulations, for example, either through analysis of clusters of conformational states across trajectories [107,108] and comparisons between unliganded and liganded stat ≈ es, or via dihedral angle dynamics observed in extended simulations [108]. This last approach is also able to derive the time scales and pathways of signal transmission [25]. Such analyses can be applied to complexes of proteins in a pathway, as in the SUMOylation pathway

[107,108] or the ternary MLL:KIX:c-Myb complex which plays a role in Pol II-mediated transcription [40]. Since population shift takes place across a protein–protein interface boundary, it may also be exploited in allosteric drug discovery where drugs ('allo-network' drugs) would target a protein upstream of the mutant protein [109]. The beneficial drug effects would reach its 'diseased protein' target via population shift across intermolecular boundaries.

9. Conclusions: generalization of the multi-conformational selection and population events

Induced fit explains why biomolecules can bind even if they are not optimized for binding [2]. However, induced fit requires certain prior molecular match to impart sufficient affinity before inducing conformational adaptation [11]. In the absence of this prerequisite, induced fit slips into a kinetic bottleneck, even if overall the reaction is thermodynamically feasible. This requisite pre-existing match is provided by pre-selected interacting species. Following selection between fitting structures, induced fit can complete the binding reaction via minor readjustments. The selected conformer is picked from a conformational pool around the native state, as described by the free energy landscape. Hence, induced fit cannot be the major recognition mechanism.

Above, we differentiated between induced fit and conformational selection based solely on an operational definition, foregoing the biological significance. However, considering that we are dealing with protein function, surrendering the functional implications is not justified. Consider an allosteric two-state (open and closed) model and switching between the states. In principle, to switch from one conformational state to the other a protein can follow either the induced fit or the conformational selection route. The induced fit route can dominate the switch from an open to a closed state, while the conformational selection route can dominate the switch from a closed an open state. However, such a conjecture overlooks the fact that the pre-existing conformational states in the ensemble have been optimized by evolution, which is not the case for induced fit, which has not been tendered across evolutionary time. To fulfill its function, a protein has been optimized by evolution not only to populate a single active conformation, but two or more switchable states. The most important biological implication of conformational selection is that the various conformational states have been pre-specified by evolution for a protein and co-optimized with its binding partner [8]. Induced fit cannot generate arbitrary states as implicitly implied, pointing to the biological significance of conformational selection. A multi-step conformational selection mechanism is advantageous for enzymes; it may also be conducive in binding, particularly in cases with large conformational changes. In such mechanistic light, it has recently been suggested that directional selection may precede conformational selection [110].

Over the last few years, experiment and computations have observed multiple pre-existing states. These support conformational selection in receptor-ligand recognition. Further, these pre-existing ensembles are now also recognized to have been adapted for function, including those sparsely populated transition states. This begs the recognition that these states may similarly be selected, which argues for multiple successive conformational selection and population shift events. It holds for catalysis where enzyme dynamics point to stepwise conformational selection steps [65]; it is also likely to hold for other conformational events in the cell. Here we related to multiple conformational selection and population shift events in multiprotein assembly which resemble multiple conformational selection events in hierarchical folding; to conformational selection and population shift events in allosteric post-translational modifications and photon absorption; to conformational selection and population shift events in specifying signaling pathways, and as key factors controlling the spatial structure of cell signaling systems, altogether providing a broad outlook of the cell.

Currently, recognition is taken as involving a single conformational selection event. Here we suggest that not one — but many conformational selection events are involved. This view which is based on the free energy landscape, generalizes conformational selection and population shift across all allosteric scenarios; it argues that all follow the same mechanism and exploit the conformational ensemble around the bottom of the folding, and binding, microfunnels.

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