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Transmembrane Communication: General Principles and Lessons from the Structure and Function of the M2 Proton Channel, K⁺ Channels, and Integrin Receptors

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Abstract

Signal transduction across biological membranes is central to life. This process generally happens through communication between different domains and hierarchical coupling of information. Here, we review structural and thermodynamic principles behind transmembrane (TM) signal transduction and discuss common themes. Communication between signaling domains can be understood in terms of thermodynamic and kinetic principles, and complex signaling patterns can arise from simple wiring of thermodynamically coupled domains. We relate this to functions of several signal transduction systems: the M2 proton channel from influenza A virus, potassium channels, integrin receptors, and bacterial kinases. We also discuss key features in the structural rearrangements responsible for signal transduction in these systems.

Keywords

signal transduction; allostery; transmembrane signaling; receptor; ion channel

INTRODUCTION

In this review, we explore how the binding of ligands to proteins induces functional conformational changes. Ligand-induced conformational changes are fundamental to many aspects of protein function; for example, when an enzyme binds its substrate, the act of binding induces changes in the protein's structure and dynamics to enable catalysis (1–3). Thus, it is often difficult to separate binding from catalysis. Similarly, binding of an allosteric ligand can lead to the propagation of conformational changes over long distances. We focus primarily on the generation and transmission of conformational changes in membrane proteins; we first introduce the types and limits of coupled, ligand-induced conformational changes. Next, we examine in quantitative terms the effects of different coupling mechanisms.

We then explore how ligand binding affects the structures and functions of various membrane proteins. We compare how the binding of multiple protons and potassium ions to the M2 proton channel of influenza A and the potassium channel, respectively, is used to generate high specificity, to organize the conduction pathway, and to orchestrate

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functionally essential large-scale conformational changes. We then explore the mutual influence and signaling between the transmembrane (TM) domains and other membrane-associated or water-soluble domains or proteins in the context of these channels, bacterial histidine kinases, and integrins, exploring the mechanisms by which multiple proteins, small molecules, metal ions, phospholipids, and posttranslational modifications influence their activation and signaling.

COUPLING OF LIGAND BINDING AND ENVIRONMENT TO CONFORMATION

Ligand binding generally leads to changes in both structure and dynamics, with increases and decreases in dynamics often occurring at different sites of the same protein (4). The effect of ligand binding on protein activity can be viewed in light of classical thermodynamic concepts of allostery, induced fit or conformational selection, and cooperativity (1–6). For simplicity, we consider a two-state transition between an active and a resting state. The experimentally observed change in activity depends on several factors including: (a) the free-energy difference or “energy gap” between the active and resting states, which determines the fraction of the protein in the activated state in the absence of the ligand; (b) the affinity of the protein for the ligand and their concentrations; and (c) the ability of the ligand to induce a transition when fully bound. Less than complete activation occurs, even when the ligand is fully bound if it binds to both the activated and the resting conformations. In this case, the extent of activation relates to the relative affinity of the ligand for the activated versus resting state. These concepts of coupled equilibria are well studied and understood. Relating these to such phenomena as partial activation, potentiation, and synergistic ligands is a simple matter of algebraic book keeping, as shown below.

In the next section, we explore in more quantitative terms how these simple binding phenomena lead to a variety of activation curves when there are multiple ligands capable of activating the protein of interest. In such cases, distinct ligand-binding sites at different locations can “couple” to give a variety of outcomes; the binding of one can alter the affinity of the other for the protein, and they can have additive or nonadditive effects on the degree of maximal activation upon ligand saturation.

The coupling of binding and functional transitions refers to the phenomenon by which the binding of a ligand to one site influences the structure, dynamics, and binding affinity at a second site, which relates to the protein's energy gap and the ratio of affinities of the regulatory ligand for the activated versus the resting states. Given these simple requirements, it is not surprising that coupling can be achieved in different ways. One special case is when the binding of a ligand induces a change in association state. When no change in association is observed, it is often implicitly assumed that signaling occurs via “hard connections” in which helices shift, tilt, or change register, mechanically driving a conformational change that propagates across a bilayer. A second model involves communication between subunits, which can entail large-scale or subtle changes in interactions between abutting domains or ones connected by flexible linkers. These different modes of communication can lead to different outcomes when one measures the effects of mutations at positions between the binding sites. For example, if a domain shifts as a rigid body, the search for a “pathway” from one location to the other might give rise to different results than if the transition of structural and dynamic information occurs by correlated motions of side chains that propagate in a two-state manner between the two sites.

Finally, both the magnitude as well as the sign of the energy gap of a TM protein depend on the membrane environment. Any shift in structure that changes the exposure of specific residues or the width (cross-sectional surface area) of a protein at a given position in the membrane will make the energy gap sensitive to the physical properties of the bilayer.

Hence, mechanosensitive channels switch on in response to changes in bilayer tension (7). Potassium channels respond to the TM electrical potential, and the location and coupling of cytoplasmic loops and helices change in response to the fraction of phosphoinositides, sphingomyelin, and cholesterol in the bilayer (8–11). Indeed, the hydrophobic length of TM helices is reflected in the width of the bilayers in the organelles to which they localize. Additionally, once located in a given membrane, there is tight coupling between the lipid environment and the conformation of the protein as it, for example, partitions into cholesterol/sphingomyelin-rich phases (12, 13).

SIMPLE MODELS FOR ENERGETIC COUPLING

Energy Gap

It is convenient to think of functional proteins as having at least two thermodynamic states—a resting state and an active state. The free-energy gap between these, $G_{gap} = G_{active} - G_{inactive}$ (where G_{active} and $G_{inactive}$ are the free energies of the active and inactive states, respectively), determines the equilibrium fraction of active protein as $f_{active} = \frac{1}{1 + e^{\Delta G_{gap}/RT}}$. R is the gas constant, and T is the absolute temperature. Because the largest possible fraction of the protein in the active state is 1, this also determines the maximal attainable degree of activation owing to binding of any ligand or perturbation, as $A = 1 + e^{-G_{gap}/RT}$. For example, if G_{gap} is 0.41 kcal/mol, then at room temperature, the background fraction of the active state is 1/3. Therefore, even the best activating agent can only result in a threefold increase in activity. However, if G_{gap} is 6.82 kcal/mol, the maximal degree of activation is 10^5 .

A ligand causes activation when it binds with higher affinity to the active relative to the inactive state (see **Figure 1a,b**). If K_A and K_I are the dissociation constants for the ligand binding to the active and inactive states, respectively, then the ligand is activating if $K_A < K_I$. Moreover, the fraction K_I/K_A determines the activating potential of the ligand. When the system is saturated with ligand, the free-energy difference between active and inactive states becomes: $\Delta G_{gap}^{eff} = -RT \ln(K_I/K_A) + \Delta G_{gap}$. For an ideal activating ligand, K_I approaches infinity, such that ligand saturation leads to complete activation, and the degree of maximal activation (relative to background) is only limited by the intrinsic G_{gap} . If a ligand binds to

both states, the maximum degree of activation is $A = (K_I/K_A) \cdot \frac{1 + e^{-\Delta G_{gap}/RT}}{1 + (K_I/K_A)e^{-\Delta G_{gap}/RT}}$, and is limited by both the intrinsic G_{gap} and the ratio of equilibrium binding constants K_I/K_A (see **Figure 1b**). For a given protein (and a fixed G_{gap}), this ratio of equilibrium binding constants could differ for different ligands, leading to varying degrees of activation upon ligand saturation. The specifics of a biological process determine how large G_{gap} needs to be and how it is modulated by binding. Large G_{gap} and K_I/K_A lead to a high degree of maximal activation (**Figure 1b**). A large G_{gap} is also useful in situations with multiple effectors that act in combination.

Environment

G_{gap} can be modulated by environmental variables, such as the membrane composition. Although it may be possible to treat membrane effects as ensembles of binding sites, we treat them as environmental factors modulating G_{gap} in the simplest case. For example, voltage-dependent potassium channels exist in equilibrium between “on” and “off” conformations that differ not only in their conductance characteristics, but also in the number of charged groups on the two sides of the electrically impermeable membrane. The on-to-off conformational change involves the movement of 12 charged groups across the membrane, corresponding to ~0.28 kcal/mol/mV. Thus, abrupt switching occurs over a 10

mV increment in the membrane potential (14), such that if at resting state the population of active channel is 1%, a 10-mV change in potential leads to a 50-fold activation.

Coupling

The activation states of adjoining protein domains can be coupled. For example, once the receptor domain of a TM signaling protein shifts into its active state, this information is transmitted onto the functional cytoplasmic end of the molecule. This is sometimes referred to as allostery and is equivalent to stating that one end of the molecule is thermodynamically coupled to the other. This coupling depends on the specifics of the molecule's structure and dynamics but can be thought of as arising from differences in interdomain interaction free energies when in different states. This interdomain coupling or allostery can therefore be captured with a thermodynamic coupling free energy parameter, G_c , which determines the extent to which the different domains experience each other's states.¹

Simple Model 1

Consider the simple model of a TM signaling molecule in **Figure 1c**. A two-state extracellular receptor domain, with an energy gap ΔG_{gap}^R , receives input from a signaling ligand, which binds preferentially to the active state of the receptor. The receptor domain is thermodynamically coupled, via the coupling free energy parameter $\Delta \Delta G_c^R$, to the cytoplasmic domain, which carries out the function of the system in its active state. The intrinsic activity of the cytoplasmic domain is controlled by its own energy gap, ΔG_{gap}^C . **Figure 1d** shows how the activity profile of this system (e.g., level of activation as a function of ligand concentration) depends on ΔG_{gap}^C and $\Delta \Delta G_c^R$. Because the function of the overall system is based on the activity of the cytoplasmic domain, high values of its gap lead to low levels of background activity, thus more switch-like behavior (compare profiles at low and high ΔG_{gap}^C in **Figure 1c**). However, because the two domains are coupled, the energy gap of the receptor domain also influences the level of activation. In fact, the energy gaps of the two domains can be thought of as jointly modulating the overall effective energy gap of the system, as shown in **Figure 1c**.

Simple Model 2

The TM domain may have two or more states, especially because many TM domains are dimeric, naturally leading to the possibility of multiple low-energy conformations or changes of local association. In **Figure 2**, we consider the case of the TM domain having its own active and inactive states, with the state of the receptor coupled to that of the TM, which is in turn coupled to the state of the cytoplasmic domain. **Figure 2b** illustrates how the activation profile depends on the new parameters in this model—the energy gap of the TM domain and its coupling to the cytoplasmic domain. Whereas larger energy gaps of the cytoplasmic domain always lead to larger overall energy gaps of the system (i.e., a larger degree of maximal activation), this is not necessarily the case for the energy gap of the TM domain (see **Figure 2b**). The effect of increasing ΔG_{gap}^{TM} can go in either direction, depending on the other parameters. Intuitively, this is because unlike ΔG_{gap}^C , ΔG_{gap}^{TM} does not directly modulate the activity of the functional cytoplasmic domain, but rather does so through thermodynamic coupling. Increasing ΔG_{gap}^{TM} leads to lowering of the absolute active fraction of the cytoplasmic domain by virtue of the two domains being coupled. This serves to

¹Coupling free energy is the effective free energy of interaction between two domains of a protein when both are in the same state of activation (both active or both inactive) relative to their interaction free energy when they are in different activation states (one active and the other inactive).

silence background activity in the resting state, whereas in the presence of an activating ligand, it limits the maximal fraction of active protein. The overall consequence depends on which of these two effects dominates. Because the TM domain lowers activity of the resting state, it may provide higher levels of activation compared to a system without a two-state TM domain (**Figure 2b**). This illustrates the advantage of having multiple loosely coupled domains—it allows for strong switching behavior without resorting to large energy barriers between active and inactive states. Intermediate domains also provide additional opportunities for modulation of the signal. Additional environmental conditions, such as the state of the membrane or the presence of specific ligands, can be integrated into the final output of the signaling system.

Simple Model 3

Extracellular and intracellular signals can be integrated by the same receptor. The VirA/VirG two-component system from *Agrobacterium tumefaciens* integrates multiple inputs with both its extracellular and cytoplasmic domains, including plant phenolic signaling molecules, monosaccharides, and pH (15). This can be modeled with the addition of a two-state cytoplasmic linker domain (**Figure 3a**). An intracellular ligand, such as a phenolic molecule, binds stronger to the active state than the inactive one (ligand *C* in **Figure 3a**), whereas a different ligand, such as a sugar, modulates the states of the receptor domain (ligand *L* in **Figure 3a**). Both the maximal activation as well as the concentration of half-maximal activity (IC₅₀) change for one ligand in response to different concentrations of the other (**Figure 3b**). Such cumulative modulation of activity by both ligands effectively amounts to signal integration. It can be used by the cell to perform certain actions only in cases, for example, where both ligands are present at certain concentrations. In this case, both ligands were chosen to bind more tightly to the active states of their respective domains, but repressing signals can also modulate receptor function.

THERMODYNAMIC AND STRUCTURAL COUPLING UNDERLYING ION BINDING SPECIFICITY, CONDUCTION, AND GATING OF THE M2 PROTON CHANNEL AND K⁺ CHANNELS

In this section, we explore the mechanism by which environmental variables and permeant ion binding couple to the structural, dynamic, and functional properties of two protein families: (*a*) the K⁺ channels typified by KcsA and the voltage-gated K⁺ channels (16–18) and (*b*) viroporins typified by the M2 proton channel of the influenza A virus (19, 20). Despite large differences in overall structure, they share many similarities.

1. Both have dual gates to regulate ion diffusion into versus through the channel.
2. They achieve high selectivity by binding multiple copies of the permeant ions.
3. Motions of the gates are thermodynamically coupled.
4. Both M2 and K⁺ channels are blocked by positively charged hydrophobic alkylamines.

The structure and conduction properties of K⁺ channels have been extensively reviewed (16–18). However, the structural basis for the energetic coupling associated with the opening and closing of its two gates is only now becoming clear (21–34). Similarly, the structural mechanism of the activity of the M2 proton channel has progressed dramatically in recent years (35–40).

Biological Functions and Structural Biology of Influenza A Virus M2

M2 is a small multifunctional membrane protein, which is essential to the life cycle of the influenza A virus (41–44). This proton channel was originally discovered as the target of the antiviral channel-blocking drugs amantadine and rimantadine. The channel conducts protons into the interior of the virus entrapped within acidifying endosomes and is essential for the uncoating of the viral RNA from the matrix protein. M2 delays acidification of the late Golgi in some strains of the virus. Its channel activity and other independent functions are compartmentalized into remarkably compact sequences consisting of a short N-terminal region that is important for incorporation into the virion (45); a TM helix that is necessary and sufficient for tetramerization, drug binding, and proton channel formation (46–49); a C-terminal amphiphilic helix that is not required for channel formation or drug-binding (46), but is involved in membrane localization, budding, and scission (43); and a C-terminal tail that interacts with the matrix protein, M1 (50). Mutagenesis and electrophysiological measurements of full-length M2 in oocytes showed that drug-resistant mutations occur at pore-lining residues of the N-terminal portion of the TM helix (51–53). A recent crystal structure of the amantadine-bound M2 TM domain (37) showed electron densities in the N-terminal pore region. A secondary site on the outside of the protein can be observed when the drug is present at very high concentrations in micelles (38) or bilayers (19), but electrophysiological studies and the drug sensitivities of reverse-engineered viruses showed that this site does not contribute to the pharmacological inhibition of the channel (51–53).

The membrane-interactive domain of M2 is a 40-residue region (47) consisting of the TM pore-forming domain, followed by the basic amphiphilic helix (43, 54). The structure of the pore domain has been studied by X-ray crystallography (37, 40) and solid-state NMR (SSNMR) (19), resulting in structures of varying resolution. The highest-resolution structure is a 1.65-Å crystal structure (40) of the TM domain of M2 from micelles, which complements earlier structures obtained under different conditions at intermediate resolutions (2.0 to 3.5 Å) (37). This peptide has also been studied by aligned and magic-angle-spinning NMR (49, 55), culminating in a structure of the amantadine-peptide complex in phospholipid bilayers (19), with a resolution on par with that of solution NMR. Solution NMR (38, 56) and recent SSNMR studies (35) have focused on the structure of a fragment that includes the TM and the cytoplasmic helix. Although these studies are in reasonable agreement on the overall arrangement of the helices in the TM region, they place the cytoplasmic helices in different orientations; in the solution structure (56), the cytoplasmic helix forms a tetrameric bundle extending into the cytoplasm, whereas the SSNMR structure places the cytoplasmic helix against the C-terminal end of the bundle exposed to the head-group region of the bilayer (35). Extensive mutagenesis (43, 46, 54) has shown that the cytoplasmic helix contributes to the localization and budding of viruses; however, simultaneous mutation of five residues that mediate the interactions proposed to be formed by the cytoplasmic helix in the SSNMR or the solution structures have no discernable effect on the expression, insertion, conduction, selectivity, or drug binding of the full-length proteins (43, 46, 54). This is consistent with the fact that the cytoplasmic helical segment has a function distinct from channel formation and acts in a largely autonomous manner (43). A synthetic 16-residue peptide with this sequence alone is capable of inducing budding in giant unilamellar vesicles (43) and mutation of this sequence inhibited budding of transfected M2 protein *in vivo*.

Ion Binding and Flux through M2 and K⁺ Channels

The flux of ions through channels is very tightly regulated to prevent leakage, which would be disastrous for the life of an organism. For example, voltage-gated channels are tightly regulated over a very narrow range of TM electrical potential (16), and both the pH dependency of activation and the maximal conduction of the M2 channel are closely

matched to not exceed the requirements for a given strain of virus to minimize toxicity to its host cell (51).

Figure 4 illustrates the ion-conducting pores of the K^+ channel, KcsA (57, 58), in comparison with the M2 proton channel (40). In both, ions first encounter a hydrophobic gate formed by the coalescence of pore-lining helices; the activation or “A gate” of KcsA is in a closed conformation, while the Val27 gate of M2 is partially open. In KcsA, an ion next encounters a central aqueous cavity, followed by a selectivity filter with sites that can bind four ions, albeit not simultaneously. Finally, a water-lined cluster of water molecules marks the exit.

At subsaturating ion concentrations, the rate of flux through the open form of a pore generally scales with the ion concentration on the side from which it is diffusing, multiplied by a second-order rate constant (16). Because ions can flow in both directions, a net flux of a given species is observed only in the presence of a TM chemical or electrical gradient. The second-order rate constant for diffusion of ions through K^+ channels is near the diffusion-controlled limit ($\sim 10^8 \text{ M}^{-1} \text{ s}^{-1}$), resulting in rates approaching 10^8 ions/s under physiological electrochemical gradients (16). The selectivity filter determines the ability of K^+ channels to exhibit such high rates while maintaining exquisite selectivity for K^+ ions over Na^+ . Two potassium ions (separated by one molecule of water) can stably occupy the four binding sites at a given time, and they oscillate between “1 and 3” and “2 and 4” configurations with nearly equal occupancy (57, 59–62). The conducting conformation of the selectivity filter binds these K^+ relatively tightly, with a dissociation constant about two orders of magnitude lower than that of the physiological ion concentration (59), leading to specific stabilization as in **Figure 1a,b**. The binding interaction with K^+ provides a sufficiently strong driving force to induce a conformation featuring a convergent and periodic array of close-packed carbonyl groups (and the side chain of Thr75), creating four sites with nearly perfectly matched energetics in the 1 and 3 versus 2 and 4 configurations. Movement of a third K^+ from a site in the aqueous cavity just below the selectivity filter (lower gray ion in **Figure 4c**) into the selectivity filter leads to a repulsion and rapid expulsion of an ion to the cellular exterior. The entire process takes place in approximately 10 ns, indicating the absence of high-energy barriers along the path. Thus, the negative cooperativity between two tight-binding sites and a third weak site provides both high selectivity as well as very rapid K^+ diffusion. The site is also highly selective; when there is only Na^+ in solution, the channel defaults to a nonconductive conformation, which is collapsed at sites 2 and 3. The theoretical basis for this process in terms of the relative contributions of electrostatics, hydration, sterics, and entropy remains an area of active investigation (63–67).

The M2 proton channel conducts protons with an overall second-order rate constant of about 10^7 to $10^8 \text{ M}^{-1} \text{ s}^{-1}$ near pH 6 (68–71). Given the very low proton concentration at this pH, this rate constant translates to a turnover rate of about 100 ions/s. Although seemingly slow, this rate is approximately that expected for the diffusion-controlled flux of a proton through a pore with the dimensions of the M2 channel (20, 68). The precise rate of proton flux through M2 varies in a sequence-dependent manner within the viral genomic context (51, 72, 73). The rate of proton flux through M2 reaches an upper limit at low pH (74, 75), indicating that a process other than proton diffusion into the channel becomes rate limiting when the diffusion of protons into the channel is rapid. Equilibrium studies show that M2 binds two protons per tetrameric channel at His37 with high affinity (76). The pK_a of these two histidines is around 8 or approximately two orders of magnitude higher than the physiological pH under which the channel functions. Thus, binding of the first two protons specifically drives the conformational ensemble toward the conducting state (**Figure 1a,b**). The third pK_a of His37 is near 6, matching the midpoint of the pH-proton flux profile (74, 75). In the shuttle model of proton conduction (77), the protein alternates between the +2

and +3 states, and the saturation of proton flux at low pH has been associated with the rate-limiting deprotonation of His37 (and any accompanying conformational changes). An order of magnitude estimate of the rate of deprotonation can be obtained on the basis of the above-mentioned fact that protons arrive at a rate of approximately $10^8 \text{ M}^{-1}\text{s}^{-1}$, and the third pK_a of His37, which predicts an off rate of approximately 100 s^{-1} in good agreement with experimental values (68–71). M2 promotes diffusion of protons from the acidic environment of an endosome (pH_{out}) into the viral interior (pH_{in}), and electrophysiological measurements show that proton flux is greater with low pH_{out} and high pH_{in} than in the reverse situation for M2 from most strains of influenza A virus (70, 74, 75, 78). Trp41, which interacts with His37, is required for this asymmetric conductance (78). At longer times when pH_{in} and pH_{out} are both low, a low level of cation flux is observed, preventing the buildup of the electrical gradient, which would otherwise result as protons accumulate in the interior of the virus (71).

Structural studies of M2 in the neutral to +2 state provide an explanation for M2's asymmetrical proton flux. The high-resolution structure (1.65 Å of the protein in what appears to be the +2 state) shows a pore filled with cascading layers of water molecules, interleaved with amino acid side chains, which provide a pathway for Grotthuss diffusion of protons through “wires” of hydrogen-bonded waters and side chains (40). Protons gain access to the channel through the Val27 gate. Passing through a region of disordered water, a proton arrives at an “entry cluster” of water molecules, consisting of a triangular prism of six waters whose square base is anchored through hydrogen bonds to the N of His37 and the carbonyl oxygen of Gly34 (Ala34 in the crystallized mutant). The His residues engage in a “His-box” interaction similar to aromatic boxes, with adjacent rings interacting in an edge-face manner. There is no direct hydrogen bonding between the imidazoles, but instead, they are connected via a highly structured network of water molecules.

On the cytoplasmic side of the His box, its N nitrogens bind a tight “bridging” dimer of water molecules that are well situated to mediate a π -cation interaction with the electron-rich rings of Trp41 residues. The Trp41 rings are stabilized in a basket-like structure via a third cluster of water molecules, which bridges the indole NH of Trp41 with the carboxylate of Asp44 and completes the path to the viral interior. The basic arrangement of the His box was first predicted in a model of the protein based on mutagenesis of the pore-forming region (77), and it is also seen in the solution NMR structures (38) as well as in a high-resolution SSNMR structure of the amantadine complex (19). An alternate orientation of the His residues has been suggested, but this arrangement was based on an SSNMR model built exclusively from measurements of the orientations of backbone amides, which did not incorporate any experimental distance restraints or information concerning the geometric arrangement of side chains (35). In most structures, the Trp41 basket is well oriented to inhibit reverse flow of protons out of an acidified virus.

Based on their high pK_a s (8), the binding of the first two protons to His37 thermodynamically stabilizes the His37 box in much the same way that binding of the first two K^+ ions stabilize the selectivity filter of KcsaA, whereas binding of a third permeant ion occurs with a lower affinity ($\text{pK}_a \approx 6$) and increases the lability of the protons attached to the His box. SSNMR studies show that this third protonation event increases the dynamics of His37 and the hydrogen-bonding of its N to water molecules on the cytoplasmic side of the His box (36). Solution NMR studies show increased mobility of the overall structure and more rapid dynamics of Trp41 (38). Finally, crystal structures that appear to be representative of the intermediate- and low-pH states show a dramatic increase in the aqueous accessibility of the site (37).

Although these features are now clear, important mechanistic questions remain. One is how protons diffuse to the His box, and another is how the protein stabilizes a high degree of charge in such a narrow enclosure. In the predominantly neutral state, the upper portion of the channel is lined by hydrogen-bond accepting carbonyl groups of Ala30, Gly34, and the deprotonated lone pairs of His37. Thus, water molecules in the channel experience a dearth of hydrogen bond donating groups and are highly polarized to bring protons into the channel, kinetically and thermodynamically favoring protonation of His37. As charge density accumulates, it is stabilized by the clusters of waters bound to hydrogen-bond accepting carbonyls of Gly34 and the electron-rich ring of Trp41. A second question is how protons exit from the His box through the Trp basket into the virus interior. The mechanism of transfer of the third proton from the water-His cluster depends on whether the third proton is fully transferred to His37 as in the shuttle model (35, 36, 79). In this model, the addition of a proton from the outside to the N of His37 is followed by release of the proton to the interior of the virus. A ring flip or water-mediated tautomerization repositions a lone pore on His37 to accept another proton from outside the virus, beginning another step of a cyclic process. A gated channel model instead envisions opening of a pore lined by a chain of water molecules (80), and a hybrid model involves rapid delocalization of the proton throughout the water/His cluster (40). Experimental data favor formal transfer of the proton to and from the His box/water cluster during each turnover, and this remains a topic of current investigation.

Coupling between Drug and Permeant Ion Binding and Large-Scale Conformational Changes

In the classical model for nerve impulse transmission, now largely defined at atomic resolution (81), a perturbation of the TM electrical potential induces a change in the G_{gap} and a concomitant conformational change of a “voltage sensor,” which places a mechanical force on the K^+ channel inner activation gate. This force leads to a shift in the A gate inner pore helix (strong coupling or large magnitude of G_c , **Figure 2b**), opening the lower section of the pore (**Figure 4**). Prior to this step, the selectivity filter is already in its conducting state, loaded with two K^+ ions. Thus, opening of the A gate results in rapid conversion of the channel from the “C,O” to the “O,O” state (the letters respectively designate the state of the A gate and the selectivity filter) that enables ion transmission. The channel then spontaneously closes by proceeding through two other conformational states (8, 21–24, 26, 31, 82, 83); opening of the helices at the lower end of the bundle places a strain on the open conformation of the selectivity filter, promoting its deformation to a nonconducting open-inactivated (O,I) conformation in a process known as C-type inactivation (a second N-type inactivation process involves binding of an N-terminal peptide to the open pore, which blocks transmission by impeding proton transport and promoting C-type inactivation). As the voltage sensor to the hyperpolarized conformation, the A gate relaxes too, generating the C,I state, which now promotes reorganization of the selectivity filter, regenerating the resting C,O form of the channel. Because these transitions are kinetically and thermodynamically coupled, inactivation is highly modulated by permeant ions and pore blockers. Also, although motion through the cycle occurs in response to changes in membrane polarization for the voltage-gated channel, in the case of the KcsA channel, the transitions occur spontaneously at acidic pH, driven only by thermal energy (84, 85).

Recently, a variant of the KcsA channel was crystallized in a number of different conformations, providing a view of the same channel in various states of activation (23, 24) and extending earlier structural work on the Na^+/K^+ channel (32). As expected from the energetic coupling, opening of the A gate at the lower end of the channel induces a nonconducting (inactivated) conformation of the selectivity filter and vice versa (as expected

from the model in **Figure 1c**). We have used a generalized geometric model (86) to examine the conformational transitions of the channel helical bundle over the entire family of 10 structures (see **Figure 5**). The pore-forming bundles can be described to less than 1-Å resolution by simple motions representing a helical bending motion and a change in the “pitch angle” representing the tilt of the helix relative to the bundle axis, as anticipated from previous studies (28, 32). Both a change in bending by up to 25° and rigid-body tilting of up to 26° occur. These motions occur within a plane parallel to the perimeter of the bundle. The pivot point occurs near the top of the selectivity filter, resulting in dramatic motions near the A gate. More subtle—but nevertheless significant—shifts near the pivot point destabilize the packing of the helices against the open selectivity filter, promoting a conformational change of the filter to its inactivated state. The change involves a network of side chains (29, 31), including Phe103, which undergoes a rotameric shift toward Thr75 of the selectivity filter and Ile100 in the neighboring subunit. All interactions appear to be necessary to maintain a large value of G_{gap} , but Phe103 appears to help translate changes in the structure of the inner helical bundle to changes in the kinetic and thermodynamic stability of the selectivity filter (and vice versa). Indeed, this coupling is lost in mutants in which Phe103 is replaced by a small side chain, and the mutations of residues at homologous positions in mammalian channels have similar effects (24). Other regions of key importance include a carboxyl-carboxylate pair, which directly modulates the selectivity filter, found between Glu71 and Asp80 (82, 87) and a patch of ionic residues, which modulates the stability of the inner pore helix in the open versus closed conformations, located near the cytoplasmic end of the bundle (88). Interestingly, the transition occurs in a nearly fully cooperative process involving a coordinated movement of the four subunits (27, 89).

The recent structure of KcsA with its N-terminal cytoplasmic helix intact (33) revealed a well-defined four-helix bundle that projects approximately 70 Å toward the cytoplasm, confirming an earlier site-directed electron para-magnetic resonance study (90, 91). This helical bundle stabilizes the protein and modulates gating by stabilizing the closed form of the channel. Of particular interest is the relationship between the stability of the bundle and the gating of the channel (92, 93).

A similar structural analysis of the entire set of crystallographic, solution, and SSNMR structures of the pore-forming segment of the M2 proton channel showed similar motions, although the shifts in the helices were less extreme than in KcsA. The pivot point occurs near the top of the bundle, such that tilting primarily affects the C-terminal end. A slight (up to 12°) bending of the helices tends to cause variable left-handed supercoiling, minimizing the divergence of the helices in the most bent structures. Although the structures were solved in a variety of environments, at varying pH and with different length constructs, they vary in a continuous manner along a single trajectory, suggesting movement along a smooth functionally relevant energy landscape.

There is a general trend for the structures to diverge near the C terminus with decreasing pH, and hence protonation of His37. A small dilation of the N terminus accompanies the C-terminal dilation. A potential caveat is that the protonation states are not always unambiguously defined by the pH and the pK_a s of His37. Thus, it is reassuring that molecular dynamics simulations from the groups of Klein, Cross, and Zhou showed the same trend, as the charge state of the four His residues was varied (94–96). Furthermore, solution and SSNMR studies show a greater degree of conformational mobility and hydration of the bundle as the degree of protonation increases (36, 97, 98). A second potential caveat is that the micelle environment used for the crystal structures might allow larger-scale fluctuations than observed in bilayers (99). However, the high-resolution crystal structure is the most tightly packed of the various structures, and the motions between the

structures occur along a common trajectory, suggesting that protonation is strongly coupled to motions of the TM helices.

Finally, it is interesting to examine the amplitude and timescale of the motions of M2 in comparison to the gating motions of KcsA. Overall, the range of conformational changes seen for M2 are smaller than those of the K⁺ channel. For M2, the diameter of the channel measured at Trp41 increases by 7 Å from the most compact to the widest structure, whereas the corresponding change is in excess of 15 Å for the K⁺ channel. Moreover, the timescale of conformational gating in the K⁺ channel depends on the membrane environment, pH, and voltage (26, 31, 100), but at low pH, the gating transitions occur on the millisecond time frame (84, 85). Finally, the KcsA is structurally more complex than M2, having not only a membrane-binding helix at one terminus but also an extended helical bundle at the other terminus (33). By comparison, M2 has a short unstructured N-terminal segment (which is entirely deleted in the corresponding M2 protein from the influenza B virus) and a C-terminal surface helix, which has no discernable effect on channel activity (43).

NMR studies of M2 show that in bilayers and micelles the amides' resonances are broadened near physiological pH (38, 101, 102), indicative of helical motions on the same microsecond to millisecond time regime as the gating seen in K⁺ channels. Side chain dynamics also show motions in the microsecond regime (36, 38, 103). However, an important distinction between the two proteins is the faster flux of ions through KcsA. During a 10-ms opening, approximately 10⁵ ions are conducted through KcsA, whereas M2 would pass approximately a single proton during this same time period (69, 71). Thus, proton conduction through M2 appears to occur amid conformational fluctuations, suggesting a transporter-like mechanism in which N-terminal opening in the underprotonated state encourages the entry of exterior water, while conformational changes following protonation promote release of the proton into the virus interior (37, 94, 95). Thus, it is important to devise experiments that test the dynamical changes associated with protonation/deprotonation under conditions in which the channel can be shown to be conducting protons.

Finally, alkylammonium drugs are known to inhibit both the K⁺ channels as well as M2. Because channel-blockers bind preferentially to specific states of the channel, they have significant effects on the extent and kinetics of various inactivation processes, as well as directly blocking the pathway of ion conduction (104–106). Thus, they have at times been considered to act via conformational selection, allosteric binding, or physical occlusion, but the latter is a dramatic and immediate mechanism by which they can achieve complete inhibition. Tetrabutylammonium (TBA) binds at the entry to KcsA's selectivity filter (**Figure 4e**) in a manner quite similar to the binding of amantadine (adamantylamine) to M2, which lies proximal to the entry water cluster/His box (19). In both cases, there is little voltage dependence to the inhibition, which is consistent with the location of the drugs just prior to their respective selectivity filters over which the electrical potential is known to decay quite steeply (46, 104, 107). In KcsA, TBA displaces the K⁺ hydrate near the entry of the selectivity filter (**Figure 4e**), and drug binding displaces protons from His37 in M2 (46, 76). **Figure 4d** illustrates the SSNMR structure of the M2 channel with the water molecules seen in the high-resolution structure superimposed to show their approximate positions. The ammonium group lies within hydrogen-bonding distance of the entry cluster, such that binding not only blocks access of protons to the center, but also lowers the pK_a of His37 (46, 76, 101). Interestingly, it has been possible to design higher-affinity drugs on the basis of this analysis (108, 109).

INSIDE-TO-OUTSIDE COUPLING IN INTEGRIN RECEPTORS

Integrins are $\alpha\beta$ heterodimeric adhesion receptors that mediate interactions between the extracellular environment and the intracellular cytoskeleton (110–114), demonstrating how bidirectional TM signal transduction is fine-tuned by multiple coupled equilibria in the extracellular, TM, and cytosolic domains (**Figure 6a**). Integrin ectodomains contain a large globular N-terminal ligand binding “head” and distinct C-terminal α - and β -“legs” that cross the plasma membrane as single TM domains and terminate in short cytoplasmic domains that bind intracellular molecules. The inactive integrin adopts a bent conformation, placing the head in proximity to the membrane and the distal legs. Upon activation, an extended conformation is favored and the α - and β -legs, TM, and cytoplasmic domains separate.

Interactions between the α - and β -chain legs, TM, and membrane-proximal cytosolic domains stabilize the inactive integrin. Single point mutations can result in receptor activation (115–118), and membrane-proximal α -disulfide cross-linking completely abrogates TM signal transduction in either direction (119, 120). Although structural studies suggest that there is a distinct TM interface, the membrane-proximal regions are dynamic (118, 120–123), befitting the need for rapid signal transduction.

The “loose” dynamic interactions between the α - and β -chains near the membrane give way to an extensive interface between globular portions of the extracellular domain, which can be perturbed via mutagenesis (124). Thermodynamic coupling occurs throughout the globular ectodomain. Crystallization with and without ligand resulted in active-extended (125) and inactive-bent (126, 127) conformations, respectively. Soaking the inactive crystals with a ligand resulted in only small-scale changes in the binding site without leg extension owing to crystal contact constraints (126). Without such constraints, anti-integrin drugs can generate adverse side effects by activating the receptor and generating immune reactive epitopes or ligand-induced binding sites (LIBSs) in the integrin legs (114). Small-molecule drugs can uncouple subdomains as well. Allosteric inhibitors that compete for interdomain contacts generate functionally split integrins in which the head is locked into an inactive conformation, but distal regions generate active LIBSs (127). LIBS antibodies also shift the integrin equilibrium to an active conformation. Constraining the integrin C termini stabilizes the bent conformation and the inactive ligand binding site, but the addition of LIBS antibodies and Mn^{2+} (a second allosteric activator) shifts the head domain equilibrium to an active state despite the distal constraints (128). Demonstrating bidirectionality, LIBS antibodies induce TM separation when not constrained (116). The combination of a LIBS antibody and a second activator, Mn^{2+} , further increased activation, but Mn^{2+} alone did not significantly affect TM separation. Therefore, as in the case of VirA/VirG (**Figure 3**), multiple stimuli are integrated to produce different intracellular responses (modeled in **Figure 6b**).

Although the integrin cytoplasmic domain is much shorter (~5–6 kDa for α -integrins) than the ectodomain, it integrates multiple signals to extracellular function. In spite of the fact that there is convincing evidence of a defined α IIb β 3 TM interface, the membrane-proximal and cytoplasmic domains have not provided consistent structures (118, 122, 129–132). Recently, to capture the α IIb β 3 inactive conformation, a disulfide cross-link was introduced between the α IIb and β 3 membrane-proximal domains (133). The α IIb cytoplasmic domain was disordered, but caused a kink in β 3 that was predicted to stabilize the interaction of two β 3 cytoplasmic helices with the bilayer. Significantly, these dynamic membrane interacting helices contain the primary motifs that bind the evolutionarily related talin and kindlin families of proteins (reviewed in References 113 and 134). **Figure 7** outlines a simple linked equilibrium model, which is quantitatively described in **Figure 6c,d**, illustrating how coupling between talin- and kindlin-binding motifs allows differentiation between ligands

with varying affinities for the receptor, effectively recapitulating an intermediate affinity state without the need for distinct extracellular conformations (128, 135).

KINETIC CONSIDERATIONS

The extensive conformational changes involved in integrin activation illustrate how coupled equilibria can affect the kinetics of receptor-ligand interactions. Platelets are surrounded by a large excess of soluble fibrinogen, yet they do not spontaneously activate $\alpha\text{IIb}\beta_3$, whereas a small-molecule Arg-Gly-Asp (RGD) ligand can shift the integrin equilibrium toward activation. This active state is long-lived, on the order of hours, in purified integrin (136), suggesting that, although the small-molecule binding site is kinetically accessible, the bent conformation is not kinetically accessible to a large fibrinogen molecule. Recent surface plasmon resonance analyses demonstrate that inactive $\alpha\text{IIb}\beta_3$ does not bind soluble fibrinogen without kinetic priming by a small-molecule ligand (137). Even in its primed state, the on rate is relatively slow and entropically disfavored, which the authors attribute to the fact that $\alpha\text{IIb}\beta_3$ binds to disordered regions in addition to the central RGD sequence. The local equilibria that regulate activation of an individual integrin participate in larger monomer-multimer equilibria driven by polymeric ligands, the cytoskeleton, and mechanical force (138, 139), potentially giving integrin clusters unique kinetic properties under tension as well.

The control of the oligomeric state and conformation is crucial to a range of signaling receptors, including cytokine and growth factor receptors (140, 141), and has been proposed to play a role in fine-tuning G protein-coupled receptor signal transduction (142). Receptor oligomerization can be simply accounted for as a thermodynamic state in a larger equilibrium. However, it may also produce kinetic effects by potentially kinetically uncoupling ligand-induced conformational states from signal transduction until oligomerization has occurred. Different signaling events can be encoded in such equilibria as in the case of members of the epidermal growth factor receptor family, which equilibrate to populations of monomers and preformed dimers (143) and demonstrate allosteric regulation that may allow discrimination of various ligands to produce different signals (144).

CONCLUSIONS

With the expanding number of high-resolution membrane-protein structures, especially in different functional states, structural decomposition of the events involved in signal transduction is increasingly possible. Common structural mechanisms have emerged. For example, despite large structural and functional differences, the M2 proton channel and the KcsA K^+ channel share striking similarities in their mechanisms of ion selectivity and rearrangements responsible for gating and switching between different states of activation. Similarly, although the details of signaling through various two-component bacterial kinases and adhesion protein receptors differ, common principles underlie recurring themes. These systems can be modulated with effector molecules, both natural and synthetic, that perturb the native equilibrium and elicit manifold responses. Chemical probes, in combination with biophysical, structural, cellular, and organismic approaches, are being increasingly used to deconstruct these complex equilibria ultimately toward the goals of engineering new therapeutics or biotechnological processes.

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M2: the M2 proton channel from influenza A virus

Energy gap: the difference in free energy between active and inactive states

A gate: activation gate of the potassium channel, formed by the C-terminal helix that lines the pore

SUMMARY POINTS

1. Binding of ligands to membrane proteins induces functional conformational changes that are thermodynamically and kinetically communicated between different protein modules.
2. There are significant commonalities in the structural features, often involving perturbations in helix-packing geometries, that carry the signal across the membrane.
3. The coupling between extracellular, TM, and intracellular domains can be understood with thermodynamic models, using basic concepts, such as the energy gap between active and inactive states and interdomain coupling energies.
4. Communication can be mediated with subtle structural rearrangement between states that are in equilibrium with one another and does not require hard connections with protein domains mechanically driving a conformational change that propagates across a bilayer.
5. Thermodynamic coupling of different domains allows for the sensing and integration of multiple inputs and generation of distinct signals. This can produce either switch-like behavior or result in more graded responses, as appropriate for the biological system.

FUTURE ISSUES

1. Emerging experimental findings concerning the structural basis of gating and selectivity in K⁺ channels are now laying a firm foundation for in-depth theoretical and computational investigations of these processes.
2. Systems and single-molecule level studies can be integrated to provide quantitative descriptions of signal transduction.
3. What are the amplitudes of side chain and backbone fluctuations in M2 and how do they couple to its pH activation and proton transport?
4. Design of new M2 inhibitors that address the problem of drug resistance is of critical importance, as this will be aided by the availability of amantadine-M2 structures.
5. The C-terminal cytoplasmic helix of M2 is emerging as a functionally rich element, with the recent discovery that it acts to aid in localization and viral filament formation as well as in promoting budding and scission (43, 54). The structural and biophysical bases for these activities are interesting topics for further investigation.

Figure 1.

Simple linked-equilibrium models. (a) A thermodynamic cycle illustrating the coupling between activation of a two-state protein and ligand binding. (b) Activation profiles of a two-state protein as a function of an agonist ligand concentration. Ratio of dissociation constants for the inactive and active states, K_I/K_A , and the intrinsic energy gap, G_{gap} , determine maximal attainable activation. (c) A diagram of a simple signal-transduction molecule. Two domains, a receptor domain and a cytoplasmic functional domain, each with two states, are thermodynamically coupled, with the receptor modulated by an activating ligand. All eight states of the system are explicitly shown. Free-energy differences between ligand-bound states (back side of the cube) are omitted for simplicity, but follow simply

from thermodynamic cycles. L indicates the concentration of the cytoplasmic ligand. In a and c , equilibrium free-energy differences correspond to the direction of the arrow with which they are shown. (d) Activation profiles of the system in panel c as a function of the energy gap of the cytoplasmic domain, ΔG_{gap}^C , and coupling free energy of the receptor to the cytoplasmic domain, $\Delta\Delta G_c^R$. K_A and K_I are set to 10 nM and 20 μ M, respectively, and the energy gap of the receptor domain, ΔG_{gap}^R , is set to 3.0 kcal/mol. The effective energy gap for this system can be shown to be

$$\Delta G_{gap}^{eff} = \Delta G_{gap}^C + RT \ln \left(\frac{e^{(\Delta G_{gap}^R - \Delta\Delta G_c^R)/RT} (1+L/K_I) + (1+L/K_A)}{e^{(\Delta G_{gap}^R + \Delta\Delta G_c^R)/RT} (1+L/K_I) + (1+L/K_A)} \right) - \Delta\Delta G_c^R$$
 Thus, if $\Delta\Delta G_c^R$ is zero (i.e., there is no coupling between the receptor and cytoplasmic domains), the overall gap is just ΔG_{gap}^C , but as long as $\Delta\Delta G_c^R$ is negative (i.e., activation of the receptor promotes the active state of the functional domain), higher values of ΔG_{gap}^R contribute to a higher overall energy gap. Similarly, tighter coupling (lower $\Delta\Delta G_c^R$) leads to a higher overall gap and larger degree of maximal activation.

Figure 2.

A more complex model of a signaling molecule. (a) In addition to the receptor and functional cytoplasmic domains as in **Figure 1**, here the transmembrane (TM) domain is explicitly modeled as having two states. The intrinsic fraction of TM domain in the active state is defined by ΔG_{gap}^{TM} , and it is coupled to the cytoplasmic domain via ΔG_c^{TM} . The 16 states of the system along with the corresponding free energies are shown in the table on the right. (b) The dependency of activation profiles on ΔG_{gap}^{TM} and ΔG_c^{TM} . K_A and K_I are set to 10 nM and 20 μ M, respectively, and values of other parameters are shown. The line with circles

demonstrates the case where no two-state TM domain is present, and the receptor domain couples directly to the cytoplasmic domain with the same ΔG_{gap}^R as in other models.

Figure 3.

A realistic model of signal transduction, inspired by the VirA protein of the VirA/VirG two-component system. (a) In addition to the domains and states in the model presented in **Figure 2**, there is a cytoplasmic linker domain, with its own active and inactive states, which is modulated by the signaling ligand *C*. A different ligand, *L*, modulates the receptor. The total number of states in this system is 64, and for clarity only the fully inactive and the fully active states are diagrammed. (b) Activation, relative to the resting state, is plotted against an increasing concentration of ligand *L* in the presence of different concentrations of *C*. This illustrates the ability to integrate signals from multiple ligands. Though this model does not explicitly treat the receptor as being dimeric (as is the case for the VirA protein), it can be easily extended to do so. In this case, the binding of a ligand may or may not be coupled with receptor dimerization, and there may exist cooperativity (negative or positive) between the two ligand-binding sites. Potential scenarios that can be explored include the following: Binding of a single ligand may push the system toward the active state, whereas binding two ligands symmetrically may favor the inactive state; alternatively, the receptor may signal only when two ligands are bound; or binding of the second ligand may proceed independently of the first one.

Figure 4.

The ion-conducting pores of M2 and KcsA. Both are drawn with their cytoplasmic domain oriented downward. The physiological flow of ions is inward (down) for M2 versus outward (up) for KcsA. (a) A high-resolution crystal structure of M2 [Protein Data Bank (PDB) code 3LBW] showing the overall structure, and (b) a blow-up of the ordered pore-lining side chains and water molecules. In all five panels, water molecules are shown as small magenta spheres, and the identity of the various side chains are annotated in panel b. (c) The structure of KcsA (PDB code 1K4C) with the potassium ions in the selectivity filter shown in dark gray. Additional potassium ions are shown in gray, and waters of hydration in magenta. (d and e) The structures of amantadine and tetrabutylammonium (TBA) bound to M2 and KcsA (PDB code 2HVK for the latter), respectively. The structure of M2 in panel d is from the solid-state NMR structure (2KQT) with the water from 3LBW docked in to show the approximate locations of water molecules.

Figure 5.

(*a,b*) Parametric views of structural changes in the pore-forming helices of the M2 protein channel and (*c,d*) the KcsA channel. Pore-forming helix bundles (residues 25–46 for M2 and 86–122 in KcsA; for the latter, some structures lacked the N-terminal section of this region) were described via the arched bundle parameterization (outlined in Reference 86). In total, 8 structures were analyzed for M2 [Protein Data Bank (PDB) codes 2RLF, 3C9J, 2KQT, 2L0J, 3LBW, and 3 symmetric structures derived from 3 different helices of the asymmetric structure 3BKD], and 10 structures were analyzed for KcsA (PDB codes 1R3J, 2HVK, 3EFF, 3F5W, 3F7V, 3F7Y, 3FB5, 3FB6, 3FB7, and 3FB8). Essential features were identified by forcing progressively more of the parameters to have the same values for all structures. Panels *a* and *c* illustrate the accuracy of the parametric representation by comparing true backbone structures (*cyan*) with idealized templates (*green*) for representative bundles from M2 and KcsA, respectively. An advantage of the mathematically modeled structures is the unambiguous alignment frame they provide. (*b*) Axial and side views (for the latter, only two opposing helices shown) of superposed parameterized models of all eight M2 structures clearly illustrate the dominant modes of motion (illustrated with *arrows*). Essential parameters of variation for these structures are the radius of curvature of helical arches, helix tilt angle, and bundle radius (86), with the possible exception of the solution NMR structure 2RLF [whereas all structures were fit within 1.0-Å root mean square deviation (RMSD) with just these three parameters, the first model in 2RLF produced an RMSD of 1.66 Å]. (*d*) Axial and side views (for the latter, only two opposing helices are shown) of superposed parameterized models of all 10 KcsA structures. The essential parameters of variation for these structures are radius and direction of curvature of helical arches, and helix pitch angle (86).

Figure 6.

Thermodynamic model of integrin signaling. (a) At the coarsest level, extracellular, transmembrane (TM), and intracellular domains of integrins can be thought of as being either active or inactive, giving rise to eight coupled states as illustrated. The various effector molecules modulate integrin state by preferentially binding to select domain conformations. “Inside-out” signaling refers to modulation of the extracellular domain conformation via interactions between an integrin’s cytosolic tails and intracellular signaling molecules. “Outside-in” events sometimes refer to the activation of oligomerization-dependent signaling pathways subsequent to binding polyvalent ligands. However, keeping with our model, here it refers to the ability of extracellular ligands to affect the conformation and function of intracellular domains. (b) Using reasonable values for the energy gaps and coupling constants associated with these states as well as equilibrium binding constants for the active and inactive states of different effector molecules, one can interrogate the thermodynamics of integrin signaling in a coarse, semiapproximate manner. Shown here is the level of integrin activation (separation of TM/tail regions) in response to increasing amounts of an activating antibody as a function of Mn^{2+} concentration. This illustrates outside-in signaling (the level of activation is shown on the z-axis and also indicated by the pseudocolor). (c,d) Inside-out signaling as illustrated by the fraction of extracellular domain bound to native ligand as a function of different amounts of active intracellular talin and kindlin. The binding affinity of the ligand as well as the amounts of talin/kindlin all couple to determine the activation state of the integrin.

Figure 7.

Coupled equilibria linking integrin transmembrane (TM) interactions to cytosolic events. Integrin TM heterodimers (*left*) stabilize inactive ectodomain conformations, and TM separation (*right*) stabilizes active ectodomain conformations. The heterodimer stabilizes interactions between the -cytoplasmic domain and the inner leaflet of the bilayers (*top*). Cytosolic proteins talin and kindlin shift the equilibrium toward an active conformation by stabilizing the extended cytoplasmic domain and separated TM domains (*bottom right*). Loss of either talin-1 or kindlin-3 in mouse platelets results in bleeding and nearly complete loss of inside-out integrin activation (138, 139). Similarly, patients who lack kindlin-3 suffer from bleeding and immune impairments due to loss of IIb 3 and 2 integrin functions, respectively (140–144). Although platelets that lack talin and kindlin-3 are physiologically impaired, they are capable of partial integrin activation in the presence of very strong agonists. Also, large excesses of talin can activate integrins in the absence of kindlins (145), coexpression of kindlins and talin result in significantly increased activation (146, 147), and overexpression of kindlins alone does not significantly activate integrins. This equilibrium can further be expanded via phosphorylation of integrin interaction motifs (148, 149) or competition with other signaling molecules, such as filamins (150, 151).