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Dynamically Driven Protein Allostery Exhibits Disparate Responses for Fast and Slow Motions

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ABSTRACT There is considerable interest in the dynamic aspect of allosteric action, and in a growing list of proteins allostery has been characterized as being mediated predominantly by a change in dynamics, not a transition in conformation. For considering conformational dynamics, a protein molecule can be simplified into a number of relatively rigid microdomains connected by joints, corresponding to, e.g., communities and edges from a community network analysis. Binding of an allosteric activator strengthens intermicrodomain coupling, thereby quenching fast (e.g., picosecond to nanosecond) local motions but initiating slow (e.g., microsecond to millisecond), cross-microdomain correlated motions that are potentially of functional importance. This scenario explains allosteric effects observed in many unrelated proteins.

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Allostery, whereby ligand binding at a distal site affects binding affinity or catalytic activity at the active site, is traditionally considered as mediated through conformational transition (1). In 1951, Wyman and Allen (2) described a scenario of cooperative oxygen binding to hemoglobin in terms of conformational entropy. Later it was proposed that an allosteric ligand may produce changes in protein dynamics within a given conformational state without a switch in conformational states (3). A growing list of proteins has been characterized as exemplars of such dynamically driven allostery (4–12). Our molecular-dynamics (MD) simulations (12) along with the NMR data of Namanja et al. (7) suggest that a main consequence of substrate binding to the WW domain of Pin1 is rigidification of the loops around the catalytic site of the PPIase domain. Previous theoretical work of dynamically driven allostery has focused on free energy, not dynamics per se, and indeed this type of allostery has been referred to as entropic allostery (3,13,14). Here we examine generic features of dynamics in dynamically driven allostery.

For the purpose of considering conformational dynamics, we may simplify a protein molecule into a number of relatively rigid microdomains connected by joints. For example, allosteric network methods use data from MD simulations to partition proteins into communities and edges between them (8,10,12). Our premise is that the binding of an allosteric activator adds physical coupling between nearby microdomains and strengthens other intermicrodomain coupling. The stronger coupling, as illustrated on a simple model, leads to disparate responses for fast and slow motions, which are actually observed in Pin1 and many other proteins.

Our simple model, inspired by community analysis on Pin1 (12), represents three main microdomains of Pin1 as springs for describing local motions (Fig. 1). In apo Pin1, intermicrodomain coupling is weak. We extremitize this situation by assuming that the three springs are totally uncoupled. The potential energy of the model is then

$$U_0(x_1, x_2, x_3) = \sum_{i=1}^{3} (k_i/2) x_i^2, \quad (1)$$

where x_i denote the internal coordinates for the local motions and k_i denote the spring constants. Upon binding to the WW domain, a phosphopeptide with sequence FFpSPR acts as a bridge between microdomains 1 and 3 via direct physical contact. In addition, the coupling between the other two pairs of microdomains is also strengthened. Representing the intermicrodomain coupling by harmonic potentials, we obtain the total potential energy

$$U(x_1, x_2, x_3) = \sum_{i=1}^{3} (k_i/2) x_i^2 + \sum_{i=1}^{3} (J_i/2)(x_{i+1} - x_i)^2, \quad (2)$$

where J_i denote the strengths of intermicrodomain coupling, and $x_4 \equiv x_1$ (for the circular coupling pattern). We are interested in the strong coupling regime, where $J_i > k_i$.

We assume that the motions are diffusive in nature. In apo Pin1, the local motions are uncoupled and each internal coordinate is assigned its own diffusion coefficient d_i. Each

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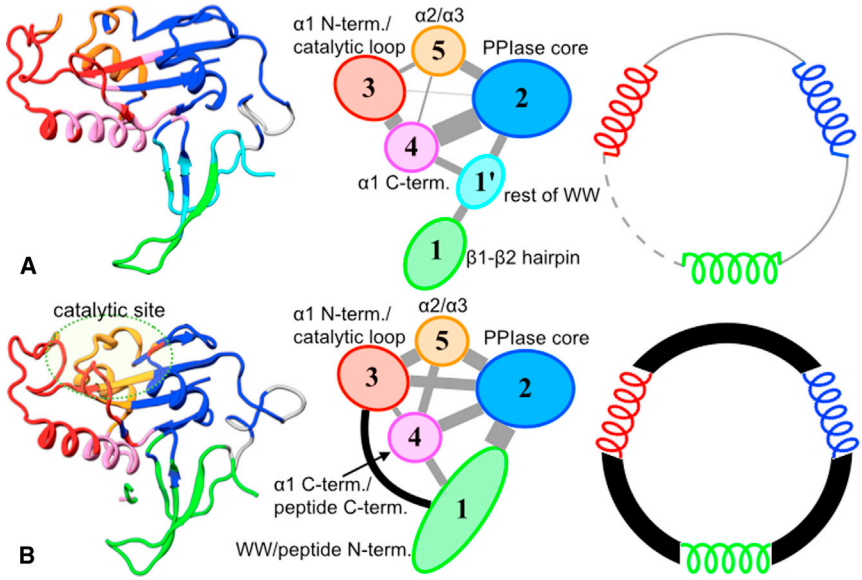

FIGURE 1 Dynamic model of protein allostery. The community analysis results for (*A*) apo and (*B*) FFpSPR-bound Pin1 from Guo et al. (12) are shown as motivation for the dynamic model. The communities are shown in different colors as cartoon structures (*left*) or as circles (*middle*). Intercommunity connections are shown as lines, with width proportional to the cumulative betweenness of intercommunity edges (*middle*). The three largest communities are represented in the dynamic model, each by a spring (*right*). The springs are weakly coupled in the apo form but are strongly coupled in the FFpSPR-bound form. Motions of the internal coordinates are assumed to be diffusive. To see this figure in color, go online.

coordinate then undergoes diffusion in a harmonic well. This situation is similar to the diffusion-in-a-cone model for bond vector internal (e.g., picosecond to nanosecond) motions in interpreting NMR relaxation data (15). For the diffusion in a harmonic well, the time correlation function is a single exponential: $\langle x_i(t)x_i(0)\rangle = \langle x_i^2\rangle \exp(-t/\tau_i)$, where $\langle x_i^2\rangle = k_BT/k_i$, with k_B denoting Boltzmann's constant, T denoting the absolute temperature, and relaxation time $\tau_i = k_BT/k_id_i$.

When the microdomains become strongly coupled as occurs upon FFpSPR-WW binding, a major consequence on the dynamics is that the effective diffusion coefficient, D, for the coupled motions is orders-of-magnitude smaller than the diffusion coefficients, d_i, for the local motions. Intuitively, one certainly expects $D/d_i < 1$, but there is also direct experimental support. By measuring residue-residue contact formation rates in unstructured peptides, it was found that relative diffusion of two residues in a peptide chain is an order-of-magnitude slower than the relative diffusion of free amino acids (16). Even more dramatically, the intramolecular diffusion coefficient in unfolded protein L was found to decrease 2–3 orders of magnitude as the concentration of the denaturant GdnHCl was reduced from 6 to 0 M (17). Removing the denaturant had the effect of increasing intramolecular coupling, but still the diffusion studied was in the unfolded (though compact) protein. Because d_i describe local motions of essentially free amino acids whereas D describes coupled motions within a folded protein, it would not be surprising if the values of D/d_i are even smaller than implicated by these experiments.

The problem of diffusion in the potential of Eq. 2 can be solved analytically. When all the spring constants k_i are identical ($=k$) and all the coupling constants J_i are identical ($=J$), the time correlation functions of all the coordinates are $\langle x_i(t)x_i(0)\rangle = (k_BT/3k)\exp(-t/\tau_s) + (2k_BT/3(k+3J))\exp(-t/\tau_f)$. The first exponential has a relaxation time $\tau_s = k_BT/kD$ that is orders-of-magnitude longer than those ($\tau_i = k_BT/k_id_i$) for local motions in the apo protein. Compared to the first exponential, the second exponential has a much shorter relaxation time $\tau_f = k_BT/(k+3J)D$ as well as a much smaller amplitude (note that $J > k$). The normalized cross correlation, $\langle x_ix_j\rangle/(\langle x_i^2\rangle\langle x_j^2\rangle)^{1/2}$, is $J/(k+J)$ and approaches 1, indicating highly synchronous motions of the internal coordinates. So the binding of the allosteric ligand strengthens intermicrodomain coupling, and consequently quenches fast, asynchronous motions but initiates slow, synchronous motions.

These predicted dynamic consequences are precisely what had been found by Namanja et al. Their side-chain methyl ^{2}D relaxation data indicated quenched picosecond-to-nanosecond dynamics upon FFpSPR binding (7). On the other hand, microsecond-to-millisecond exchange dynamics, as captured by the product of the longitudinal and transverse relaxation rates of side-chain methyl ^{13}C, was enhanced (18). In our MD simulations, the allosteric action of FFpSPR-WW binding is manifested by rigidification of the catalytic-site loops (12). To further dissect the allosteric mechanism, we investigated whether the effects of FFpSPR-WW binding could be mimicked by artificially enhancing intra- or intermicrodomain coupling in the form of conformational restraints in MD simulations. Artificial enhancement of coupling within microdomain 1 or 2 or between microdomains 2 and 3 was ineffective, but artificial coupling of microdomains 1–3 together produced the same allosteric action as FFpSPR-WW binding. This observation provides support to our modeling of allosteric binding as strengthening intermicrodomain coupling.

Our model predicts that allosteric action reduces with weakening of ligand-induced intermicrodomain coupling. Namanja et al. (7,18,19) found that a second phosphopeptide, from the mitotic phosphatase Cdc25C, produced less

prominent effects on both picosecond-to-nanosecond and microsecond-to-millisecond dynamics than FFpSPR did. To see whether the reduced allosteric action is mediated by weakened intermicrodomain coupling, here we carried out an MD simulation of Pin1 with the Cdc25C peptide bound at the WW site. Compared to FFpSPR, the Cdc25C peptide has more extensive interactions with microdomain 1 but minimal interactions with microdomain 3 (Fig. 1 *B* and Fig. S1 *A* in the Supporting Material). The catalytic loop and neighboring residues do not become rigidified (Fig. S1 *B*), indicating reduced allosteric action. A community network analysis confirms that the intermicrodomain coupling is not as strong as in the case with FFpSPR bound (Fig. S1 *A*).

The disparate responses for fast and slow motions predicted by our model are also observed in many other proteins that involve a strong dynamic component in allosteric regulation. When thrombomodulin (specifically TM456) is bound to the anion binding exosite 1 (ABE1) of thrombin, MD simulations of Gasper et al. (8) showed that the picosecond-to-nanosecond dynamics of the 30_{CT} loop (part of the ABE1) and the neighboring 60_{CT} insertion is quenched but microsecond-to-millisecond dynamics of the loops around the active site is initiated (Fig. S2 *A*). In agreement with model prediction, the motions of these loops are strongly correlated. Community analysis showed that the ligand (i.e., TM456), the distal binding site (i.e., ABE1), and active-site loops form strongly connected communities, similar to what we found for Pin1.

Srivastava et al. (10) studied the dynamic effects of a mutation, Y204A, in the catalytic subunit of the cAMP-dependent protein kinase. The mutation is far away from the active site (Fig. S2 *B*) but still renders cAMP-dependent protein kinase catalytically inefficient. The mutant can be viewed as an apo protein whereas the wild-type protein is bound with an allosteric ligand. Tyr^{204} forms a hydrogen bond with Glu^{230} on the F helix and also van der Waals contacts with the F helix. The wild-type protein undergoes synchronous slow (millisecond) motions (possible opening and closing between the small and large lobes separated by the active-site cleft). In contrast, in the mutant, motions are faster and uncorrelated. Community analysis showed that the structural elements around Tyr^{204}, the rest of the large lobe, and the small lobe, form tightly coupled communities, but the coupling is significantly weakened in the mutant. The mediation of the allosteric action of Tyr^{204} through strengthening intermicrodomain coupling is reminiscent of the situations with Pin1 and thrombin.

Popovych et al. (4) studied the conformational and dynamic effects of sequential cAMP binding to the two distant sites in the catabolite activation protein N-terminal domain dimer. In the singly-liganded species, the subunit with the bound ligand undergoes significant conformational perturbation but the conformation of the unliganded subunit is minimally affected. On the other hand, correlated microsecond-to-millisecond motions are initiated throughout the whole dimer while picosecond-to-nanosecond motions of some residues are dampened, consistent with our model prediction. Further support of the dynamic model is provided by experimental observations on other proteins, including quenching of picosecond-to-nanosecond dynamics by a distal helix appendage in a PDZ domain (5); enhancement of millisecond motions by the binding of an allosteric activator to imidazole glycerol phosphate synthase (6); appearance of microsecond-to-millisecond dynamics at the Flim binding interface of CheY upon phosphorylation of Asp^{57} (9); and the initiation of concerted microsecond-to-millisecond motions by an N-terminal sequence in the catalytic domain of CheA (11). (An allosteric inhibitor could weaken intermodule coupling by, e.g., wedging into tightly interacting structural elements (20).) Interestingly, in a case where allostery is apparently mediated by significant conformational transition, microsecond-to-millisecond dynamics is suppressed upon allosteric activation (21).

In conclusion, the dynamic model and the many supporting examples suggest that dynamically driven allostery has generic features. This type of allosteric action is manifested by quenching of fast (e.g., picosecond-to-nanosecond) motions but initiation of slow (e.g., microsecond-to-millisecond) motions. Such slow dynamics has often been suggested to be functionally important ((6,8,10); see the Supporting Material for further discussion of this and other pertinent issues). The disparate responses of fast and slow dynamics come about due to strengthening of intermicrodomain coupling by allosteric activators, and may represent a defining departure from traditional conformationally driven allostery.

SUPPORTING MATERIAL

Supporting Discussion and two figures are available at http://www.biophysj.org/biophysj/supplemental/S0006-3495(15)00453-1.

AUTHOR CONTRIBUTIONS

J.G. and H.-X.Z. designed and performed research; H.-X.Z. wrote the article.

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Supporting Material

SUPPORTING DISCUSSION

We emphasize that the focus of this paper is the dynamic effects elicited by allosteric binding. Specifically our simple model predicts that allosteric activators produce disparate responses for fast and slow motions: quenching of ps-ns dynamics but initiation of μs-ms dynamics. These disparate dynamic responses have been directly observed in many proteins that apparently involve a strong dynamic component in allosteric regulation. The analysis through our model suggests that this dynamic behavior is perhaps inevitable.

Below we discuss several related issues. Note that, as we extend further and further from what our model actually predicts, the conclusions become more and more tentative. We hope that they nevertheless can be useful for stimulating more thoughts on the dynamic aspect of allosteric regulation.

Cooperative binding through reduction in conformational entropy cost

While we have emphasized its dynamic aspect, our model of course also predicts effects of the allosteric activator on the thermodynamics, i.e., free energy, of the protein. Given the potential energies in Eq. 1 for the apo protein and in Eq. 2 upon binding the ligand, the ratio of the partition functions after and before ligand binding is

$$\frac{Z}{Z_0} = \frac{\int dx_1 dx_2 dx_3 \, e^{-U(x_1,x_2,x_3)/k_{\mathrm{B}}T}}{\int dx_1 dx_2 dx_3 \, e^{-U_0(x_1,x_2,x_3)/k_{\mathrm{B}}T}}$$

Evaluating the integrals, we obtain

$$\frac{Z}{Z_0} = \frac{k}{k+3J}$$

Recall that our interest is the strong coupling regime, where $J \gg k$. Then the ligand, via strengthening inter-microdomain coupling, results in a significant reduction in the partition function, i.e., $Z/Z_0 \ll 1$. Correspondingly the allosteric binding is accompanied by an increase in free energy, $k_{\mathrm{B}}Tln(Z/Z_0)$, arising from a decrease in conformational entropy.

We may consider the decrease in conformational entropy due to binding at the allosteric site as a reduction in conformational entropy cost for subsequent binding at the active site (1). In other words, if binding at the active site were to occur without prior binding at the allosteric site, the cost in conformational entropy would be higher, and correspondingly the binding free energy would be higher by $k_{\mathrm{B}}Tln(Z/Z_0)$. Strictly speaking, our model deals only with the binding at the allosteric site; the benefit for the subsequent binding at the active site, in the form of reduced conformational entropy cost, is merely anticipated. Interestingly, the anticipated thermodynamic consequence of our model fits the scenario of cooperative oxygen binding to hemoglobin described by Wyman and Allen (2). These authors suggested: "as the first oxygen molecule enters a pair of hemes, there is some kind of ordering of the hemoglobin molecule leading to a more regular arrangement in space. When the second molecule of oxygen enters, no considerable further change of configuration is necessitated."

For our model, the ligand-induced decrease in conformational entropy can be most easily understood from the increase in the normalized cross correlations $< x_i x_j > /(< x_i^2 x_j^2 >)^{1/2}$, from 0 in the apo protein to $J/(k+J)$ upon allosteric binding. There is

also justification to associate the decrease in conformational entropy with the quenching of ps-ns dynamics. Sharp et al. (3) have proposed that the ligand-induced change in conformational entropy can be calculated from the changes in NMR-derived order parameters, which measure the degrees of motion of backbone amides and sidechain methyls on the ps-ns timescale. Accordingly, quenching of ps-ns dynamics would lead to a decrease in conformational entropy.

Potential contributions of ms-scale dynamics to catalytic efficiency

While the quenching of ps-ns dynamics may be associated with a role in enhancing binding affinity at the active site via reducing the conformational entropy cost, the initiation of ms-scale dynamics by an allosteric activator could contribute to the catalytic efficiency of an enzyme. An enzyme-catalyzed reaction proceeds through the basic steps of substrate binding, product formation, and product release. Each of these steps has the potential to be rate-limiting for the overall reaction. Again, our model deals only with allosteric binding and thus makes no direct predictions about events at the active site. However, we may anticipate that the ms-scale dynamics initiated by the allosteric activator influences the three steps of the enzyme-catalyzed reaction.

In fact, such ms-scale dynamics has been proposed to play a positive role for each of the three steps (4-6). Gasper et al. (4) suggested that ms-scale dynamics manifested by active-site loops of thrombin upon binding thrombomodulin could increase the binding rate constant of the substrate, i.e., protein C. Lipchock and Loria (5) found that the backbone ^{15}N sharp resonance of a residue at a catalytically critical site in apo imidazole glycerol phosphate synthase broadened to beyond detection upon titration with an allosteric activator. The implicated ms dynamics at this site was thought to facilitate the catalytic step. Srivastava et al. (6) posited that the synchronous ms-scale dynamics in wild-type PKA-C involved opening and closing between the small and large lobes, thereby accelerating product release, which was known to be the rate-limiting step.

An experimentally testable prediction on the allosteric regulation of Pin1

According to our model, the disparate dynamic responses of Pin1 at ps-ns and μs-ms timescales upon binding of the phosphopeptide FFpSPR to the WW site arise from strengthened inter-microdomain coupling. Specifically we assume that, via direct physical contact simultaneously with microdomains 1 and 3, FFpSPR acts as a bridge between them. If our reasoning is correct, then the physical form that serves as the inter-microdomain bridge should not matter. To test this idea, we introduced two histidine substitutions, one in microdomain 1 (residue Gln33) and the other in microdomain 3 (residue Lys97), and then used a zinc ion to coordinate the two histidines. Our molecular dynamics simulations showed that this mutant, free of FFpSPR, exhibited the same allosteric behavior, including rigidificaiton of the catalytic-site loops, as the wild-type protein with FFpSPR bound at the WW site. Experimental test of this predicted "constitutive activity" of the mutant is under way.

Further development of the dynamic model

In considering conformational dynamics, it certainly makes intuitive sense to partition a protein molecule into a number of relatively rigid microdomains connected by joints. Achieving the partitioning by a community network analysis seems to be a

reasonable option, but such an approach involves a number of choices (e.g., level of modularity) that could affect the outcome. This type of networks uses motional correlations for calculating inter-residue connections. An alternative might be to use physical distances for calculating inter-residue connections, as done in elastic network models. A way forward would be to "parameterize" the partitioning and other features of our model against the NMR data on the proteins discussed in this paper. The hope is that these efforts would lead to a robust model that makes new predictions (e.g., ms-scale) on ligand-elicited dynamic effects.

In particular, using identical k_i and J_i values, we have so far focused on overall trends in fast and slow motions. NMR data have shown that quenching of ps-ns dynamics and appearance of μs-ms are not uniform throughout a protein. It would be of interest to see whether our model, with varying k_i and J_i, can capture this regional non-uniformity in dynamic responses, thereby yielding deeper insight into allosteric regulation.

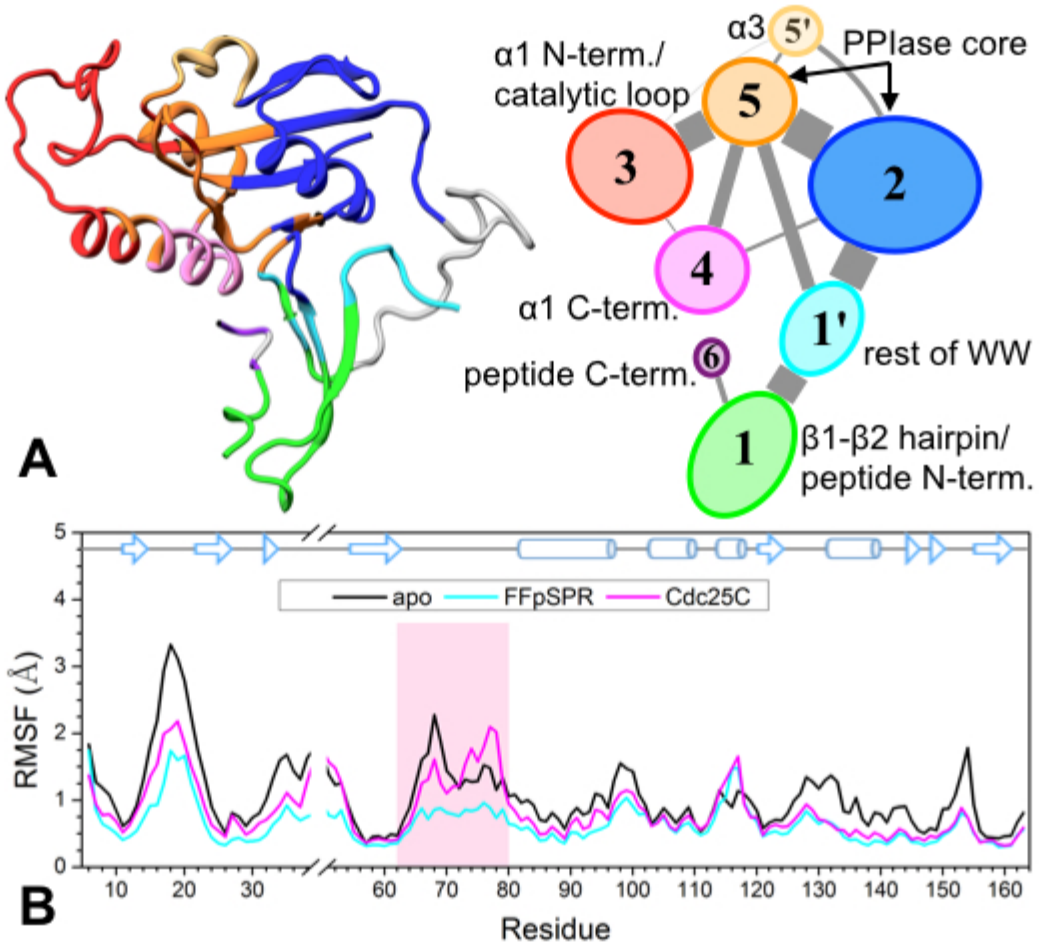

FIGURE S1 Allosteric effects of the Cdc25C peptide on Pin1. (A) Community analysis. The community network analysis was performed using the NetworkView plugin in VMD with default setting. (B) Root-mean-square-fluctuations (RMSFs) of apo, FFpSPR-, and Cdc25C-bound Pin1. Residues 62-80 containing the catalytic loop are shaded to highlight the difference between FFpSPR- and Cdc25C-bound Pin1. Results for Cdc25C-bound Pin1 were from analyzing the last 40 ns of a 100-ns simulation, with the initial binding pose of the peptide grafted from its complex with the isolated WW domain (Protein Data Ban (PDB) entry 1I8G). Results for apo and FFpSPR-bound Pin1 are from Guo et al. (1).

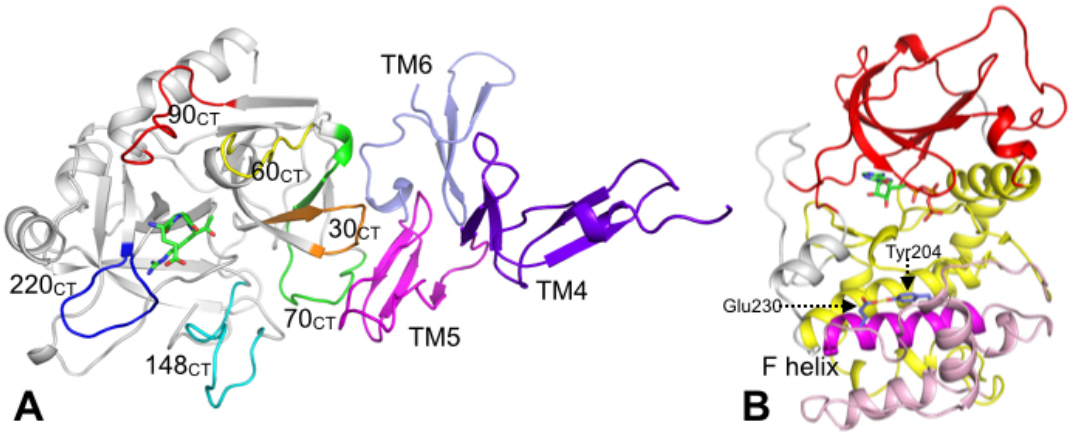

FIGURE S2 Other proteins that exhibit allosteric behaviors consistent with model prediction. (A) Thrombin, with TM567 as the allosteric ligand (PDB entry 1DX5). ABE1 consists of the 30_{CT} loop (orange) and 70_{CT} loop (green). The active site is indicated by a bound inhibitor (shown as stick); four surrounding loops are shown in color: 60_{CT} insertion (yellow), 90_{CT} loop (red), 148_{CT} loop (cyan), and 220_{CT} loop (blue). The colored elements form tightly coupled communities (4). (B) PKA-C, with Tyr204 as an allosteric ligand (PDB entry 1ATP). An ATP molecule is shown as stick to indicate the active-site cleft. The F helix (magenta), the surrounding structural elements (pink), the rest of the large lobe (yellow), and the small lobe (red) form tightly coupled communities (6).

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