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Protonation Induced Dynamic Allostery in PDZ Domain: Evidence of Perturbation Independent Universal Response Network

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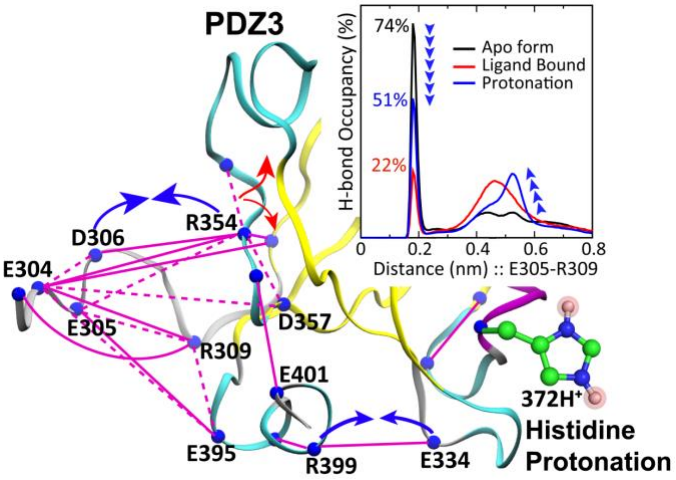
ABSTRACT

Dynamic allostery is a relatively new paradigm where certain external perturbations may lead to modulation of conformational dynamics at a distant part of a protein without significant changes in the overall structure. While most well characterized examples of dynamic allostery involve binding with other entities like small molecules, peptides or nucleic acids, in this work we demonstrate that chemical modifications like protonation may lead to significant dynamical allosteric response in a PDZ domain protein. Tuning the protonation states of two histidine residues (H317 and H372), we identify the allosteric pathways responsible for the dynamic response. Interestingly, the same set of residues that constitute the allosteric response network upon ligand binding, seem to be responsible for protonation induced dynamic allostery. Thus, we propose the existence of an inherent universal response network in signaling proteins, where the same set of residues can respond to varying types of external perturbations in terms of re-arrangement of hydrogen bonded network and redistribution of electrostatic interaction energies.

TOC GRAPHICS



KEYWORDS Dynamic allostery, pH-dependent allostery, PDZ domain

Allosteric regulation is a significant component of the biomolecular signaling circuitry.[1] In contrast to the classical structural view of allostery, "dynamic allostery" has emerged as a new paradigm, where dynamics in the distal parts of the protein gets modulated on point mutation or ligand binding.[2-5] This phenomena has been attributed to primarily entropic effects,[6] population-shift in pre-existing conformational sub-states through modulation in underlying free energy landscape,[1, 4, 7] and re-distribution of electrostatic interactions.[8] The known examples of dynamic allostery typically involve either binding of small molecules or peptides, or mutation at a distal site, or post-translational modifications. In this work, we explore manifestation of a rather simple chemical modification, namely protonation, on dynamic allostery in a PDZ domain protein.

It is now well-recognized that electrostatic interactions play a significant role in biomolecular function including enzyme catalysis, signal transduction through protein-protein interaction and allostery etc.[9-15] A crucial component of electrostatic control of protein function is modulation of the protonation state of functionally important amino acid residues.[16-17] pH varies significantly in different diseased states of tissues and thus altering the normal protonation states of the proteins that in turn affect the ligand binding affinity and/or functional activity of various allosteric enzymes. Moreover, the physico-chemical environment strongly influences the pK_a values of the ionisable groups, that includes electrostatic interaction with other residues, desolvation free energy, conformational changes associated with water penetration and so on.[18-21] In addition, ligand binding often leads to large pKa shifts and associated proton uptake/release. [10, 22-25] A thermodynamic basis of pH-dependent allostery establishes a link between protonation state and conformational state of proteins.[26-27] If the pK_a varies between two distinct conformational states, changes in solution pH may lead to cycling between two conformations.[21, 28-31]

PDZ domain proteins, involved in membrane localization of multi-protein signaling complexes, are classic examples of dynamic allostery upon ligand binding.[5] Nussinov and co-workers have suggested that proteins may have multiple pre-existing allosteric pathways and favor one depending on the perturbation events such as ligand binding, mutations, and changes in the cellular physiological conditions.[32] In this work, we consider the possibility that protonation of certain residues in PDZ domain may trigger similar response as ligand binding. Since majority of the pH-regulated biochemical processes occur within pH range of 5~8, histidine residues (pK_a=6.5 in water) often play an important role in regulating pH-sensing systems.[33-39] PDZ3 domain proteins considered in this work have two histidine residues at positions 317 and 372 situated at the β1-β2 loop and α2 helix, respectively (Fig. 1a). We have computed the pK_a values for the histidine residues in the PDZ3 domain (PDB: 3I4W) using the Delphi server.[40] The pK_a values for H317 and H372 are 6.46 and 6.80 respectively (Table S1). It has been shown earlier that protonation of H372 can modulate the ligand binding affinity of PDZ domain.[41-42]

We have elucidated the role of specific electrostatic interactions and the population shift in hydrogen bonded network towards observed dynamic allostery in PDZ3 domain upon ligand binding.[8] We expect that the protonation at certain key residues would create a similar perturbation in the electrostatic interaction network, and might result into a dynamical allosteric response. Moreover, our hypothesis is that various perturbation events such as ligand binding, or mutation, or protonation may trigger a pre-existing universal response network in this system. In order to investigate the effect of protonation of H317 or H372, we have performed all-atom molecular dynamics (MD) simulations of PDZ3 domain (PDB: 3I4W) in three different states: (1) Neutral (without ligand): both H317 and H372 are uncharged, (2) 317H: protonation of H317

only and (3) 372H: protonation of H372 only. We also present results for PDZ3 domain in ligand bound state to compare the effect of protonation vis-à-vis ligand binding.

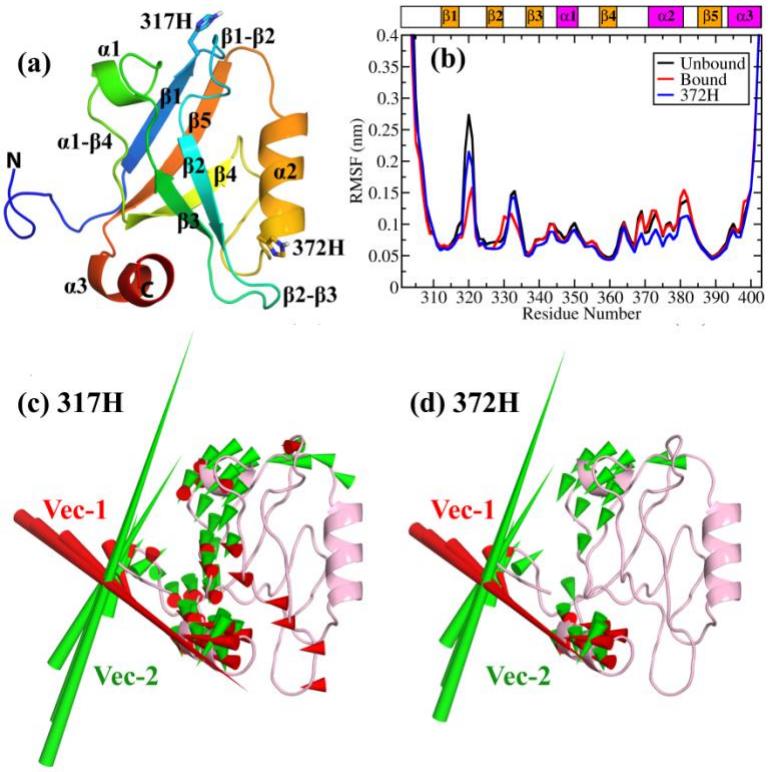


Figure 1. (a) Structure of PDZ3 domain protein (PDB: 3I4W). Histidine residues at positions 317 and 372 are highlighted. Ligand binds in the cavity between β2 and α2 where 372H forms a hydrogen bond with threonine at position -2 of Class-I peptide ligands. (b) Residue-wise root mean square fluctuations (RMSF) of apo form (black line), ligand bound state (red line) and apo form with protonated H372 (blue line). Figs. (c) and (d) illustrate the motions along the 1st and 2nd eigenvectors for 317H and 372H protonation states in the form of porcupine projections on the PDZ domain. The length of the porcupines represents the local amplitude of the motion.

To elucidate the effect of protonation on the conformational plasticity, we examine the root mean square fluctuations (RMSF) and principal component analysis about the mean conformation with (317H and 372H protonation) and without protonation. Previous studies show that PDZ domain

exhibits greater flexibility and plasticity in the loop regions (β1-β2 and β2-β3) in the unbound state (neutral) which is suppressed upon ligand binding introducing rigidity in the domain.[5, 43-47] Our principal component analysis (PCA) results highlight a collective mode that shows significant dynamics in the N-terminal regions, α2 helix and other loop regions (Figs. 1c-d, S3). Surprisingly, we observe change in the conformational flexibility of the PDZ domain upon protonation (Fig. 1b) without discernible changes in the backbone structure (Figs. S1b, S2). The β1-β2 loop shows the least structural deviation and reduced fluctuations upon protonation of 317H, which suggests that protonation selectively enhances the rigidity of the β1-β2 loop situated next to the protonation site. In contrast, upon protonation of 372H, there is a decrease in fluctuations in the β1-β2 loop region and towards the C-terminal of α2 helix (α2-β5 loop) as compared to the unbound state. This trend tends to be similar to the fluctuation profile obtained for ligand bound state (Fig. 1b). Such modulation of conformational flexibility upon protonation in the binding pocket may alter the ligand binding activity as well.[48] The observed modulation in distal dynamics due to introduction of extra positive charges on the histidine residues represents clear signature of protonation induced dynamic allostery.

Earlier we have established that the electrostatic interaction energy provides a sensitive yardstick to capture the allosteric effects in PDZ domain.[8] To understand the effect of protonation on the underlying energy landscape, we demonstrate the modulation in the residue-wise interaction energy upon protonation at 372H and 317H (Figs. 2, S4). We compute the change in the average electrostatic interaction energy of each residue as: $\Delta \mathrm{E_i} = \langle \mathrm{E_{i,protonated}} \rangle - \langle \mathrm{E_{i,neutral}} \rangle$. It is expected that a major component of this perturbation will be due to direct interaction with the positive charge introduced at the histidines. Hence, in order to identify allosteric response in terms of perturbation of other intra-protein interactions, we calculate $\Delta E_{i,rest} = \Delta E_{i,protein} -$

$\Delta E_{i,His}$, where $\Delta E_{i,rest}$ represents the effect of protonation on the i^{th} residue with respect to the rest of the protein excluding direct interaction with the charged H372.

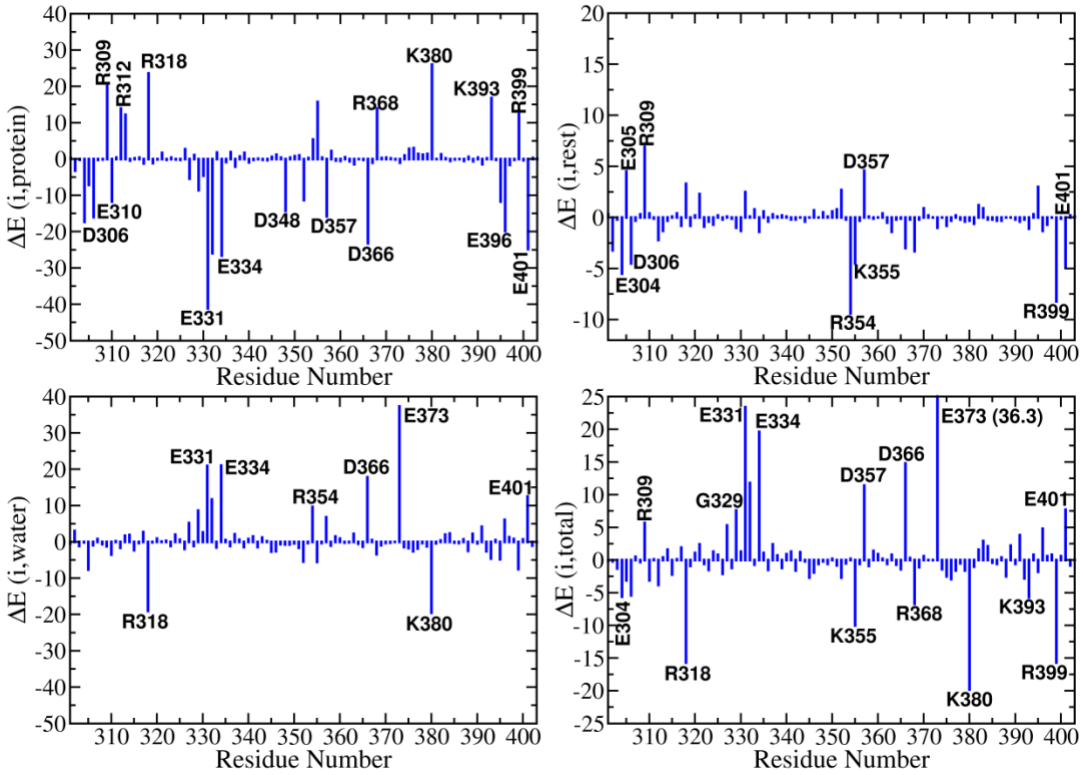


Figure 2. Residue-wise change in average electrostatic energy (kcal/mol) between the protonated and neutral state upon protonation at 372H $\left(\mathbf{\Delta E_i = \langle E_{i,protonated}\rangle - \langle E_{i,neutral}\rangle}\right)$. The change in interaction energy due to protein with and without contribution from the protonated histidine residue are shown separately in figures (a) and (b) as $\mathbf{\Delta E_{i,protein}}$ and $\mathbf{\Delta E_{i,rest}}$, respectively. Contribution due to water only $\left(\mathbf{\Delta E_{i,water}}\right)$ is shown in figure (c). Figure (d) shows the change in total interaction energy i.e., $\mathbf{E_{i,total} = (\Delta E_{i,protein} - \Delta E_{i,His}) + \Delta E_{i,water}}$. Numerical values of these data are provided in SI (Table S2, S3 and S4).

In Fig.2, we observe significant perturbation in residue-wise electrostatic energy ($|\Delta E_{i,protein}|$ >15 kcal/mol) for residues that are located far from the protonation site H372, e.g. D306, R318,

E331, R399 etc. (additional data provided in Fig. S4). Interestingly, we observe more favorable protein-protein interactions ($\Delta E_{i,rest} < 0$) for residues, E306, D306, R354, K355, R399 etc. upon protonation at 372H indicating tightening of electrostatic interactions at these positions. Protonation also alters the local solvation environment around distal residues ($\Delta E_{i,water}$) indicating possible role of water in modulating the allosteric response (Figs. 2c, S4c). Figs. 2d and S4d show the changes in total electrostatic interaction energy ($\Delta E_{i,total}$) that indicates that protonation may generate both favorable ($\Delta E_{i,total} < 0$) and unfavorable ($\Delta E_{i,total} > 0$) energetic perturbations. This proves that although the overall enthalpic change might be small, there exists large local cancellation and redistribution of electrostatic energy similar to the effect of ligand binding.[8]

In order to identify the network of energetic perturbation that would connect the site of protonation with the distal allosteric sites, we have dissected the residue-wise energetic perturbation ($\Delta E_{i,protein}$) into pair-wise contributions $\left(\Delta E_{ij}\right)$, where $\Delta E_{ij} = \langle E_{ij}\rangle_{protonated} - \langle E_{ij}\rangle_{neutral}$. The residue pairs with large perturbation ($\left|\Delta E_{ij}\right| > 2$ kcal/mol excluding direct interaction with protonated residues) are used to construct the perturbation map (Fig.3). As mentioned before, the protonated histidine will trivially contribute to the energetic perturbation of each residue by direct interaction. Hence, to capture the re-arrangement in intra-protein interactions we exclude contribution of histidine ($\Delta E_{i,His}$) for constructing the network.

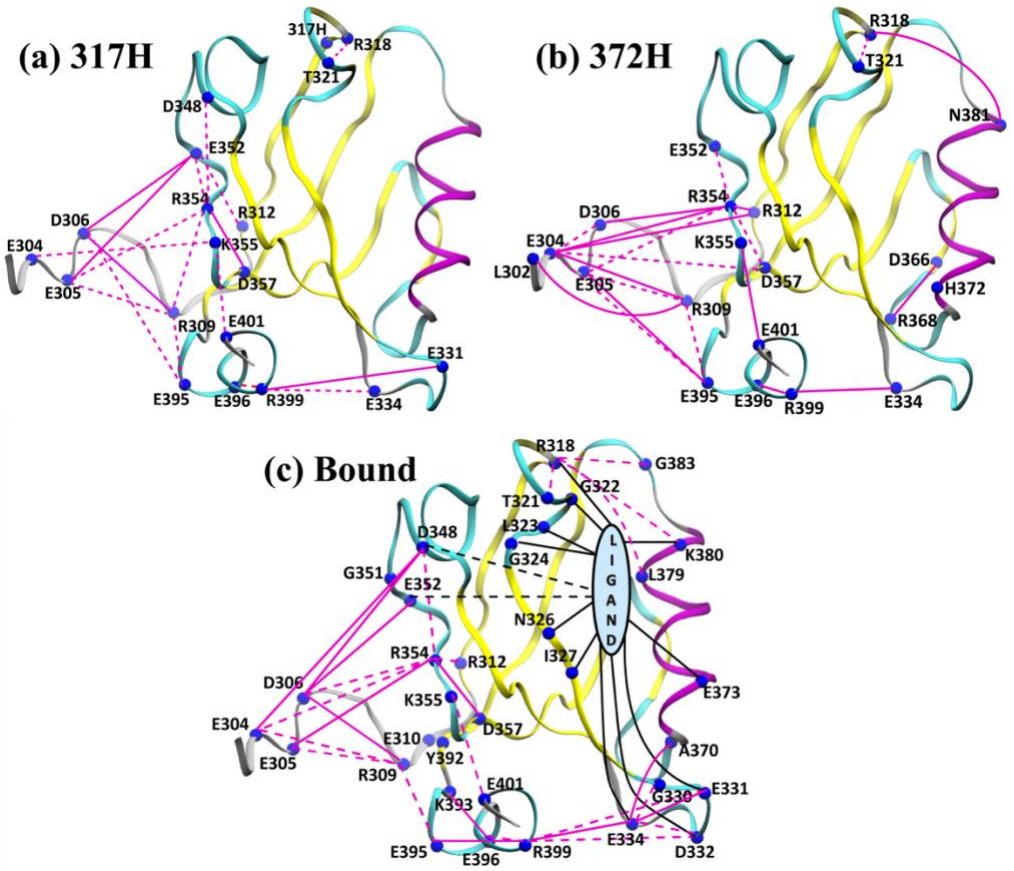


Figure 3. Network representation of the perturbation in pairwise interaction energies: The network is mapped onto the three-dimensional protein structure (3I4W). Figure (a) and (b) show perturbation network upon protonation. The blue spheres represent residues with $|\Delta E_{i,(Pro-His)}| \geq 5$ kcal/mol or $|\Delta E_{i,Pro}| \geq 10$ kcal/mol, but exclude residues with direct and exclusive interaction with the protonated residues. The connections in magenta color indicate $|\Delta E_{ij}| > 2\ kcal/mol$. Connections with negative and positive ΔE_{ij} values are indicated with solid and dashed lines, respectively. The ΔE_{ij} values for all significant pairs have been reported in Table S5. Figure (c) shows perturbation map upon ligand binding, and the blue spheres indicate residues with $|\Delta E_{i,Pro}| \geq 5$ kcal/mol or $|\Delta E_{i,ligand}| \geq 7$ kcal/mol. The black lines represent connections between peptide ligand and residues with $|\Delta E_{ij}| > 7\ kcal/mol$. The

connections in magenta color indicate $|\Delta E_{ij}| > 4\ kcal/mol$. The connections based on $|\Delta E_{ij}| > 2\ kcal/mol$ were considered only for residues that are directly perturbed on ligand binding.

Fig. 3 presents a comparison between the energetic perturbation networks in three systems with different kinds of perturbation, namely protonation at (a) 317H and (b) 372H, and (c) ligand binding to the neutral state. Clearly, all perturbations show extensive energetic redistribution at the distal region (towards N-terminal) from the protonation/ligand binding sites with a differential pattern. Interestingly, we observe significantly more negative ΔE_{ij} values (solid lines) for 372H protonation as compared to 317H. In particular, a few salt-bridge pairs such as E334-R399, K355-E401, D306-R354 etc. are significantly stabilized for 372H protonation, but destabilized for 317H (dashed lines). In addition, there are certain connections that become more unfavorable upon both protonation, e.g. E305-R309, R309-E395, R318-T321, E352-R354 and E305-R354 (Fig. S5). Interestingly, connections like E305-R309, R309-E395, E334-R399, K355-E401, and E305-R354 also appear in energetic perturbation network identified due to ligand binding (Fig. 3(c)).

Thus, we observe that all the perturbation maps have certain common features. Particularly the effect of 372H protonation has significant resemblance to the ligand binding effect. These results suggest that there exists a set of residues that constitute the nodes/hubs of the energetic perturbation network in terms of specific interactions like H-bonding and salt bridges irrespective of the nature of the perturbation. But the edges connecting the nodes may depend on the specific nature of the perturbation. Thus, we propose that PDZ domain has an intrinsic and universal response network of nonbonded interactions independent of the perturbation, whereas specific perturbations would activate/deactivate specific regions of this response network.

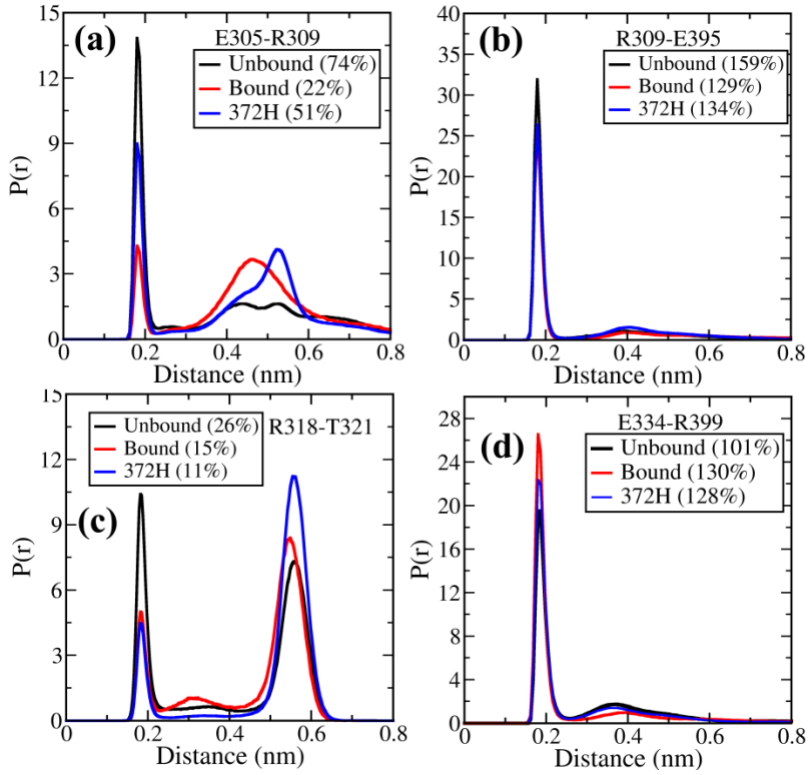


Figure 4. Probability distribution of pairwise minimum distance between representative salt-bridge pairs. The H-bond occupancy (%) values for unbound, ligand bound and 372H protonation states are indicated in the plot. More examples and data are provided in Fig.S6 and Table S6.

Our earlier work has established that on ligand binding in PDZ3 domain, there is a significant population shift in the H-bonded interactions between the residue pairs identified through the energetic perturbation map.[8] Here we demonstrate a very similar population shift of the specific interactions upon protonation. Figs. 4 and S6 show the population distribution of the minimum distance between all atoms of certain residue-pairs constituting an edge of the network/graph for neutral/unbound, ligand bound and protonated states. A peak around 0.2nm would indicate an H-bonded state. For most of the cases, we observe that the overall shape of the population distribution does not change, but the H-bond occupancy can vary significantly across various

states. Interestingly, for the residue pairs shown in Fig.4, we clearly see that population shift due to 372H protonation is in the same direction as the ligand bound state. The networks in Figs.3(b) and 3(c) also show rather similar features indicating that 372H protonation illicit similar response as the ligand binding in the neutral state. These results establish that the small population shift in H-bonded network, can lead to significant modulation in the underlying energy landscape. More examples of such population shift depending on the protonation state are provided in Fig. S6.

In summary, this work demonstrates the evidence of protonation induced dynamic allostery in the PDZ3 domain protein. Protonation of different histidine residues lead to differential modulation of conformational plasticity in the distal part of the protein without significant changes in the backbone structure. We demonstrate that the fluctuations/dynamics in the loop regions (β1-β2 and β2-β3) and α1-β5 coiled region are suppressed upon protonation at positions H317 or H372. We establish a connection between the dynamic allostery and the perturbation in the residue-wise electrostatic interaction energies. We highlight that there exist an extensive interaction network in PDZ3 domain spanning several common residues, and the allosteric modulation can be observed in terms of propagation of energetic perturbation through any of this network depending upon the perturbation factor. We have identified the response network based on residue pairwise interaction energy that highlights extensive rearrangement and redistribution of interactions at the distal regions of PDZ3 domain. The subtle changes in the population distribution of H-bonded interactions in the network manifest into long range modulation in free energy landscape and dynamics. Response due to 372H protonation has surprising similarity to the effect of ligand binding. Hence, we propose that there exists an inherent pre-existing universal response network of interactions in PDZ domain proteins that may undergo population

shift upon perturbation by different factors such as ligand binding and protonation. There exists a common set of residues constituting the response network, but depending on the specific perturbation, the nature of the response might vary. This phenomenon might be quite general among all proteins involved in biomolecular signaling processes, and they might have evolved in such a fashion to be able to respond to wide range of external perturbations based on a universal response system.

ASSOCIATED CONTENT

Supporting Information.

The Supporting Information is available free of charge on the ACS Publications website. MD simulation details, comparison of numerical values for H-bond occupancy, average change in the residue-wise (ΔE_i) and pairwise (ΔE_{ij}) interaction energies for different systems.

AUTHOR INFORMATION

Notes

The authors declare no competing financial interests.

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