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CONCERTED MOTIONS IN ALLOSTERIC MODEL PROTEINS AT TERAHERTZ FREQUENCIES

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ABSTRACT. PDZ3 is an allosteric protein that represents an ideal test system to analyze the molecular determinants at the basis of the allosteric mechanism. Recent computational studies of the terahertz (THz) fluctuations of this protein have highlighted a response nucleus for binding modulated by the ligand that is visible only at THz frequencies and that overlaps with the known allosterically responding residues (Conti Nibali *et al.* 2017). With the aim of further characterizing the changes in the terahertz dynamics of this allosteric protein following the binding event, we have carried out an analysis of the correlation motions of pairs of PDZ3 residues from molecular dynamic simulations. We identify concerted and non-random THz fluctuations in the main secondary structure elements of the PDZ3. Importantly, we highlight a concerted motion of some residues belonging to the allosteric response nucleus for binding.

1. Introduction

Allostery can be defined as any process in which an event at one site of a macromolecule, *e.g.*, the binding of a ligand, impacts the function, by altering the dynamics, or the distribution of conformations, of another site. This process, which involves a long-range communication between distant ligand-binding sites, regulates the protein function by modulating the affinity or binding modes for ligands. The events underlying this "action at a distance" phenomenon typically involve a group of amino acids that constitute a responsive subset, which has been investigated by numerous approaches.

The first models for allostery agreed on the importance of concerted structural changes between two binding sites. Although this structure-centric understanding of allostery had been the dominant paradigm ever since, it became increasingly evident the provided scenario was incomplete. In 1984 Cooper and Dryden introduced the term "dynamic allostery", presenting a general model whereby ligand-induced changes in protein dynamics (*i.e.*, changes in the frequency and amplitude of atomic motions) could produce allosteric communication between distinct binding sites, even in the absence of structural changes (Cooper and Dryden 1984).

Since this prescient work, the role of protein dynamics in allostery has progressively gained attention and has been widely investigated, with numerous studies focussing on the picosecond-nanosecond (Igumenova *et al.* 2006; Popovych *et al.* 2006) and the microsecond-millisecond (Akyuz *et al.* 2015; Roberts 2015) time scales, where the former time scales are mainly due by backbone and side-chain fluctuations and the latter involve large-scale conformational changes. Lately, the importance of fast thermal fluctuations occurring in biosystems on the sub-picosecond to picosecond time scale and the terahertz (THz) frequency range has been highlighted, *e.g.*, in assisting ion transport across phospholipid membranes (D'Angelo *et al.* 2008; Conti Nibali *et al.* 2014b; Rifici *et al.* 2014) and in providing possible vibrational channels through which proteins and membranes may couple to their solvent (Paciaroni *et al.* 2013; Conti Nibali *et al.* 2014a; Conti Nibali and Havenith 2014; D'Angelo *et al.* 2017). As far as allostery is concerned, these THz fluctuations may play a key role to enhance access to functional configurations (Niessen *et al.* 2015) and to form a coherent signal pathway from the orthosteric ligand-binding site to the activation region (Woods *et al.* 2016).

Recently, by means of a molecular dynamics (MD) simulations study of two model PDZ domains, we have investigated the relationship between the fast THz dynamics of these proteins and their allosteric behavior (Conti Nibali *et al.* 2017). Focussing on protein dynamics in the THz regime (0.1-3.0 THz) as opposed to lower frequency has allowed us to identify a response nucleus modulated by the ligand that is visible only in this frequency range. The overlap between the residues of this response nucleus and the known allostery in the investigated PDZ domains suggests that fast THz dynamics could play a role in the allosteric communication and be part of the hierarchy of time scales related to dynamic allostery. In this study, by means of MD simulations, we aim to further investigate the THz fluctuations in the PDZ domains, by quantifying correlated motions and thus identifying protein regions that move in a concerted fashion in presence of a ligand.

2. Methods

2.1. Constructs and MD trajectories. We have carried out MD simulations of the PDZ3 domain from the synaptic protein PSD-95 in its free state (PDB structure: 1BFE (Doyle *et al.* 1996)) and in complex with the pentapeptide CRIPT (PDB sequence: 1BE9; peptide sequence: KQTSV). We have performed the simulations with the GROMACS suite (Hess *et al.* 2008), using the GROMOS force field (van Gunsteren *et al.* 2002) and the SPC water model (Berendsen *et al.* 1987). Each system was solvated with 6000 explicit water molecules filling an octahedral box. The initial configurations of the trajectories here investigated have been extracted from two 400 ns simulations at constant pressure and temperature with a time step of 2 fs, carried out by Morra and coworkers for the unbound and the bound states (Morra *et al.* 2014). In greater detail, 9 snapshots were selected from each of these long trajectories at given time interval (every 10 ns between 90 and 180 ns): the snapshots were used as starting points for 100 ps microcanonical runs with a time step of 2 fs. The atomic positions and velocities, saved every 10 fs, were collected. In order to specifically investigate protein dynamics in the $0.1 - 3.0$ THz range, we have applied to these trajectories a Fourier filtering method that enables us to analyse motions in the selected frequency window (Conti Nibali *et al.* 2017).

The Fourier filtering method comprises three main steps: (1) A Fourier transform operation is applied to the trajectory of the system, yielding the amplitude spectrum in the frequency domain. (2) A specific frequency window is selected and the amplitude spectrum is set to zero outside this window; this operation yields a reduced spectrum. (3) The reduced spectrum is transformed back to the time domain, so as to obtain the filtered trajectory. Here, we have implemented Fourier filtering thanks to an efficient program, developed by Turton and coauthors (Sessions *et al.* 1989; Turton *et al.* 2014). The filtered trajectories were used for the analyses of the fast THz fluctuations: in the following we will refer to these dynamics as fast dynamics at THz frequencies. The quantities calculated in the following are averages over the full set of filtered trajectories.

2.2. Correlation analysis. We set out to quantify correlated motions in the PDZ3 domain and in particular to identify protein regions that move in a concerted fashion following to binding event. In order to address these questions, we have performed analysis of the cross-correlation coefficients of pairs of PDZ3 residues from MD simulations. This approach provides a convenient framework to identify concerted and non random fluctuations. The correlation matrix describes the linear correlation between any pairs of Cα atoms as they move around their average position during dynamics. The correlation matrix $Corr_{ij}$ is a N$\times$N array, whose i-j entry summarizes the correlation between the motion of atom i and of atom j, is defined as:

$$Corr_{ij} = \frac{< (\overrightarrow{r}_i - \overrightarrow{r}_{i,ave}).(\overrightarrow{r}_j - \overrightarrow{r}_{j,ave}) >}{\sqrt{< \overrightarrow{r}_i - \overrightarrow{r}_{i,ave} >^2 < \overrightarrow{r}_j - \overrightarrow{r}_{j,ave} >^2}}$$

At a qualitative level, a positive correlation between two atoms reflects a concerted motion along the same direction, whereas a negative correlation indicates an opposite direction motion.

3. Results and discussion

In order to highlight changes in the correlation motions of the PDZ3 domain upon ligand binding in the investigated THz frequency window, we have first computed the correlation matrix for the unbound and bound states (Fig. 1). In Fig. 1 the 3D structure of the bound PDZ3 is reported for reference: the secondary structures elements later discussed in the text, loop L12, loop 23, helices H1 and H2, have been highlighted with labels and correspond to the following residues: L12 res 13-17, L23 res 25-30, H1 res 40-46, H2 res $66-78$. We have then calculated the difference MATRIX between the cross-correlation matrices of the two states (bound-unbound): in this matrix positive values (red-to-yellow colors) describe residue pairs that move in a more concerted fashion in the same direction following the complex formation while negative values (black-to-cyan colors) indicate the residue pairs that strengthen their relative motion in opposite directions upon binding. The results of this analysis is shown in Fig. 2.

We find that, following the complex formation, the residues belonging to the main secondary structure elements that give rise to a highly coordinated motion along the same direction are 66-67 with 24-26 (first selection) and 38-41 with 43-47 (second selection), while those that move in a concerted way and in opposite directions are 13-17 with 39-43

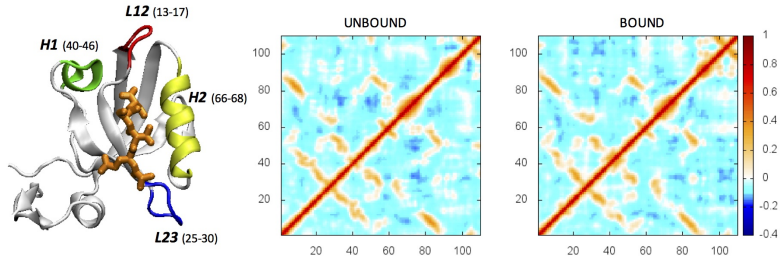

FIGURE 1. Cross-correlation matrices in THz frequency range, calculated considering the motion of Cα atoms around the average position. Left panel: The 3D structure of the PDZ3 in complex with the pentapeptide CRIPT (bound state) is reported for reference, with the main secondary structure elements highlighted by means of a different coloring. Middle panel: Cross-correlation matrix for the apo PDZ3 (unbound state). Right panel: Cross-correlation matrix for the PDZ3 complex (bound state). A correlation close to 1 (color code: red) corresponds to highly coordinated motion of the atom pair along the same direction, whereas a negative correlation (color code: blue) indicates motion in opposite directions.

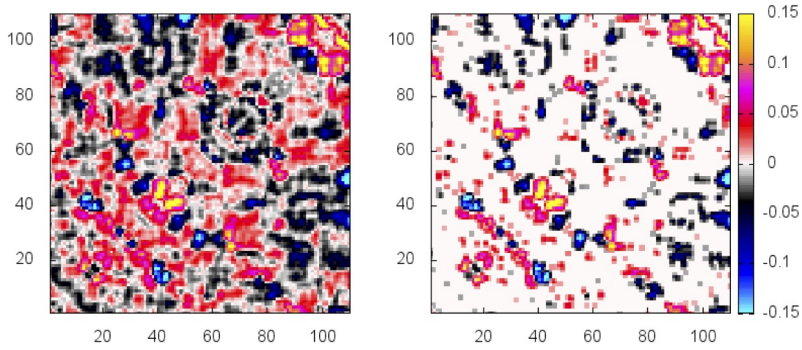

FIGURE 2. Left panel: The difference between the cross-correlation matrices of the two states (bound − unbound) for the Cα is shown. Positive (negative) values indicate increased (decreased) cross-correlation coefficients of pairs of PDZ3 residues upon ligand binding. Right panel: In order to highlight the most significative changes in the data shown in the left panel, we have set to 0 (white color) all the values between -0.05 and 0.05.

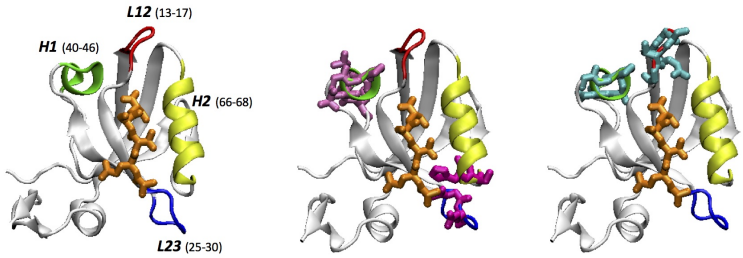

FIGURE 3. Left panel: The 3D structure of the bound PDZ3, with the different secondary structure elements highlighted. Middle panel: Residues that, following the binding event, move in a more coordinated fashion along the same direction are represented in magenta (first selection) and in mauve (second selection). Right panel: Residues that, following the binding event, give rise to a motion in opposite directions are represented in cyan (third selection). See main text for details.

(third selection). These selected residues are represented in Fig. 3. Focussing first on the first selection (Fig. 3, middle panel, magenta), residues 66-67 are part of the terminal part of helix H2 while residues 24-26 pertain to the region of loop L23. In a recent work we have found that residues 66-67 of H2 as well as some residues that preceed loop L23 are part of the response nucleus for binding defined at THz frequencies (Conti Nibali *et al.* 2017). Moreover, residues 24-25 are listed among the coevolving amino acids of the sector identified in the PDZ family by Lockless and Ranganathan (Lockless and Ranganathan 1999). Thus, both the subsets of residues in the first selection are related to the known allostery in PDZ3.

The cross-correlation analysis presented here has thus allowed us to better define the response nucleus for binding at THz frequency and characterize its dynamics. Concerning the second selection, its residues are mostly part or located next to helix H1. Thus, this selection describes a highly coordinated motion in the helix H1. More precisely, two different subsets of this helix increase their coordination in the bound state, moving in the same direction. Due to the complex formation, a higher flexibility at THz frequencies together with increased distance fluctuations relative to the rest of the protein (both at THz and at lower frequencies) have been reported for helix H1 (Conti Nibali *et al.* 2017). Also NMR relaxation measurements have reported an increased mobility of H1 (Petit *et al.* 2009). It is believed that the increased flexibility in helix H1 might be connected to the known functional properties of the PDZ region involving helix H1 and the nearby loop, as a site for protein- protein interactions that have been hypothesized to allosterically regulate the

ligand affinity at the orthosteric binding site (Peterson *et al.* 2004; van den Berk *et al.* 2007). Overall, these findings indicate that upon binding helix H1 increases its flexibility and markedly moves with respect to protein bulk while maintaining a high coordinated motion of its residues, moving all together in the same direction.

The third selection revealed by the cross-correlation analysis concerns residues belonging to the loop L12 and to the helix H1. The changes in dynamics in H1 have been described in the previous paragraph. Also for loop L12, both for THz frequencies and at lower frequencies, increased distance fluctuations relative to the rest of the protein have been reported (Conti Nibali *et al.* 2017). So both these secondary structure elements, loop L12 and helix H1, significantly move with respect to the protein. The cross-correlation analysis adds another piece of information: the observed anti-correlation of L12 and H1 indicates a relative motion towards opposite directions. Some other residues, that do not belong to the main secondary structure elements of the PDZ3, show a negative correlation: these are residues 54-56 with 27-29 (fourth selection) and residues $48-51$ with $108-110$ (fifth selection).

4. Conclusions

In this study we have characterized the correlation motions of the PDZ3 domain occurring in the THz frequency window for both the unbound and bound state, with the aim of highlighting the dynamical changes due to the complex formation. The analysis of the cross-correlation coefficients of subsets of PDZ3 residues has allowed us to identify concerted and non-random THz fluctuations in the main secondary structure elements of the PDZ3, indicating some regions of the protein moving in the same direction and others in opposite directions. Importantly, some of these residues have been related to the know allostery in PDZ3 and to the response nucleus modulated by the ligand that is visible only at THz frequency (Conti Nibali *et al.* 2017). This study has thus provided additional significative information on the THz dynamics of the allosteric model protein PDZ3 and of some of its residues involved in the allosteric mechanism.

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