SUPPLEMENTARY MATERIAL

Domino effect in allosteric signaling of peptide binding

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| Residue 1 | Residue 2 | Mutual Information | KS test value | KL divergence (Å) | Correlation |
|----------|----------|--------------------|---------------|-------------------|-------------|
| Gly23    | Lys79    | -                  | 0.59808       | -                 | 0.31662     |
| Phe24    | Lys79    | 0.03008            | 0.49532       | 18.07             | 0.32063     |
| Asn25    | Asp31    | -                  | 0.61054       | -                 | 0.28958     |
| Asn25    | Glu33    | -                  | 0.57146       | 22.27             | 0.29099     |
| Asn25    | Ile35    | -                  | 0.64436       | 18.93             | 0.35772     |
| Asn25    | Phe36    | -                  | 0.66421       | 48.25             | 0.38374     |
| Asn25    | Ile37    | -                  | 0.54305       | 35.11             | 0.32529     |
| Asn25    | Ser38    | -                  | -             | 26.37             | 0.32914     |
| Asn25    | Leu41    | 0.04424            | 0.70128       | 32.49             | -0.34922    |
| Asn25    | Ala42    | -                  | 0.6361        | 29.20             | -0.33893    |
| Asn25    | Gly43    | 0.03840            | -             | 33.92             | -0.36436    |
| Asn25    | Gln57    | -                  | 0.64150       | 35.32             | 0.33154     |
| Asn25    | Lys79    | 0.03268            | 0.50425       | -                 | -           |
| Asn25    | Lys92    | -                  | 0.54884       | 19.53             | 0.34175     |
| Asn25    | Glu94    | -                  | 0.55058       | 25.86             | 0.34518     |
| Asn25    | Glu95    | -                  | 0.62693       | 34.93             | 0.36738     |
| Asn25    | Tyr96    | -                  | 0.61221       | 32.07             | 0.36486     |
| Asn25    | Ser97    | -                  | -             | -                 | 0.33147     |
| Ile26    | Lys79    | 0.03855            | 0.49003       | -                 | -           |
| Ile37    | Lys79    | 0.03073            | 0.48914       | -                 | 0.31323     |
| Ser38    | Lys79    | 0.03792            | 0.48722       | -                 | -           |
| Ile40    | Lys79    | 0.03197            | 0.50300       | -                 | 0.30330     |
| Arg53    | Lys79    | 0.02928            | 0.48817       | -                 | 0.26781     |
| Gly55    | Lys79    | 0.03123            | 0.49293       | -                 | -           |
| Asp56    | Lys79    | 0.03118            | 0.49490       | -                 | -           |
| Gln57    | Lys79    | 0.03239            | 0.50499       | -                 | -           |
| Ile58    | Lys79    | 0.03021            | 0.48665       | -                 | 0.26505     |
| Leu78    | Glu9     | -                  | -             | 27.23             | 0.28767     |
| Leu78    | Leu41    | -                  | -             | -                 | -0.33846    |
| Leu78    | Gln57    | -                  | -             | 21.74             | -           |
| Leu78    | Ile58    | -                  | -             | 19.43             | -           |
| Leu78    | Leu59    | -                  | -             | 19.24             | 0.28927     |
| Leu78    | Leu66    | -                  | -             | 18.00             | -           |
| Leu78    | Arg67    | -                  | -             | 18.12             | -           |
| Leu78    | Gln90    | -                  | -             | 20.57             | 0.28648     |
| Leu78    | Tyr91    | -                  | -             | 27.79             | -           |
| Gln90    | Lys79    | 0.03744            | 0.49704       | -                 | -           |
| Tyr91    | Lys79    | 0.03955            | 0.49671       | -                 | 0.31159     |
| Lys92    | Lys79    | 0.03691            | 0.49695       | -                 | -           |
| Pro93    | Lys79    | 0.03654            | 0.48892       | -                 | -           |

*Table 5.1.* PDZ3 residue pairs for which Mutual Information, Kolmogorov-Smirnov (KS) test value, or Kullback-Leibler (KL) divergence between first (bound) and last (unbound) basins, or Pearson correlation to $u_1$ reaction coordinate is on the 99.9 percentile of all distances.
Figure S1. Definition of binding events. Threshold of peptide RMSD = 5 Å (top) and eigenvector projection \( u_2 = -0.75 \) (bottom) for binding and reseeded trajectories. Binding events considered are shown with a red star. Reseeded trajectories were not considered if the trajectory was initially bound (below threshold in the first frame), and therefore some trajectories seem below the threshold but no red star is shown. Vertical lines separate the independent trajectories.

Figure S2. Kinetic analysis of binding events. (a) Fitting of fraction of trajectories with peptide unbound to a one-parameter model where \( f(t) = \exp(-t/3606) \). Probability was obtained by averaging binary vectors of length 1050 ns. Corresponding to the seven binding events observed, seven vectors were flipped to zero (bound) at the time of binding. (b) Fitting according to a two-parameter model where \( f(t) = \alpha \exp(-t/3352) \).
Figure S 3. Robustness of the kinetic analysis with respect to the definition of the threshold for bindings. From top to bottom: RMSD of peptide Cα < 3 Å, RMSD < 7.5 Å, optimized RC $u_1$ < 0.1, optimized RC $u_1$ < -0.75.

Figure S 4. Projection of trajectory on natural reaction coordinates. These plots are similar to those in Figure 7, but with the coordinates transformed such that the diffusion coefficient is unitary. (a) Projection of the free energy on the slowest-relaxation eigenvector $u_1$. The Free Energy Profile shows the different basins from the bound state at around $u_1 = -25$ to the unbound states at about $u_1 = 60$. (b) Distribution of distances between the Cα of residues Glu94 and Ala101 in the C-terminal region projected on the $u_1$ natural coordinate. (c) Two-dimensional histogram of trajectory frames. (d) Projection of the free energy on the second eigenvector $u_2$. 
Figure S5. SAPHIRE plot of trajectory projected on natural reaction coordinate. Analogous to Figure 8. From top to bottom: structural annotation with interatomic distances. Value of $\bar{u}_1$: first eigenvector RC. Minimum distance between peptide and protein (Å), RMSD of peptide Cα with respect to crystal structure (Å). Distance between residues Glu94-Ala101, Kinetic and temporal annotation.

Figure S6. Reaction coordinate (RC) quality metrics for the natural coordinate $\bar{u}_1$. (a) Eigenvector optimality criterion $\theta(\bar{u}_1, \Delta t)$. (b) Free energy profile projected on the natural RC. (c) Committor optimality criterion $Z_{C,1}$.
Figure S 7. SAPPHIRE plot of RC $u_2$. From top to bottom: Structural annotation, $u_2$ RC, minimum distance (Å), KETWV RMSD (Å), distance between Glu94-Ala101 (Å), temporal and kinetic annotation.
Figure S8. Definition of bound and unbound states. Top: selected stretches of 20 ns with RMSD below 2.5 Å. Bottom: stretches of 20 ns where the peptide is completely unbound, meaning a minimum distance greater than 10 Å.

Figure S9. Protein-peptide interactions in the bound state ($u_i < -0.912$) Top: peptide/PDZ3 contact map. Bottom: sequence profile of cumulative number of contacts. The bottom panel shows the sum of each column on the contact map.
Figure S 10. Same as Figure S 9 for the non-natively bound sections of the trajectory, with $0.573 < u_1 < 0.621$.

Figure S 11. Same as Figure S 9 for the $u_2 > 0.441$ off states. The basin consists of a nonnative association of the peptide to the C-terminal region of the protein.
Figure S12. Free Energy Profiles along peptide-protein distance Reaction Coordinate. Figure shows the histogram-based and cut-based free energy profiles of the trajectory along the distance between the V Cβ and the Leu78 Cγ atoms. Both profiles show that the main barrier is around a distance of 8 Å, in consistence with the profile shown by [20].

Figure S13. Structural features of Ile37Ala-PDZ3 simulations. First panel: dssp analysis of α3 helix region. Middle panel: V-Cβ to Leu78-Cγ distance, marker of peptide position, white (unbound) or pink (bound). Last panel: Glu94-Ala101 distance
Figure S 14. Structural features of C-terminal region of WT PDZ3 unbound trajectory segments. Top panel: dssp analysis of C-terminal region. Middle panel: V-Cβ to Leu78-Cγ distance reporting on peptide unbound (white). Bottom panel: Glu94-Ala101 distance

Figure S 15. Structural features of C-terminal region of WT PDZ3 bound trajectory segments. Top panel: dssp analysis of C-terminal region. Middle panel: V-Cβ to Leu78-Cγ distance reporting on peptide bound (red). Bottom panel: Glu94-Ala101 distance