Supporting Information

Structure and Dynamics Analysis on Plexin-B1 Rho-GTPase Binding Domain as a Monomer and Dimer

Liqun Zhang,[*],[†] Thomas Centa,[‡] and Matthias Buck[†]

Department of Physiology and Biophysics, Medical School of Case Western Reserve University, Cleveland, OH, 44106, and University of Cincinnati, 2600 Clifton Ave., Cincinnati, OH, 45221

E-mail: liqun.zhang2@case.edu

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[*]To whom correspondence should be addressed

[†]Department of Physiology and Biophysics, Medical School of Case Western Reserve University, Cleveland, OH, 44106

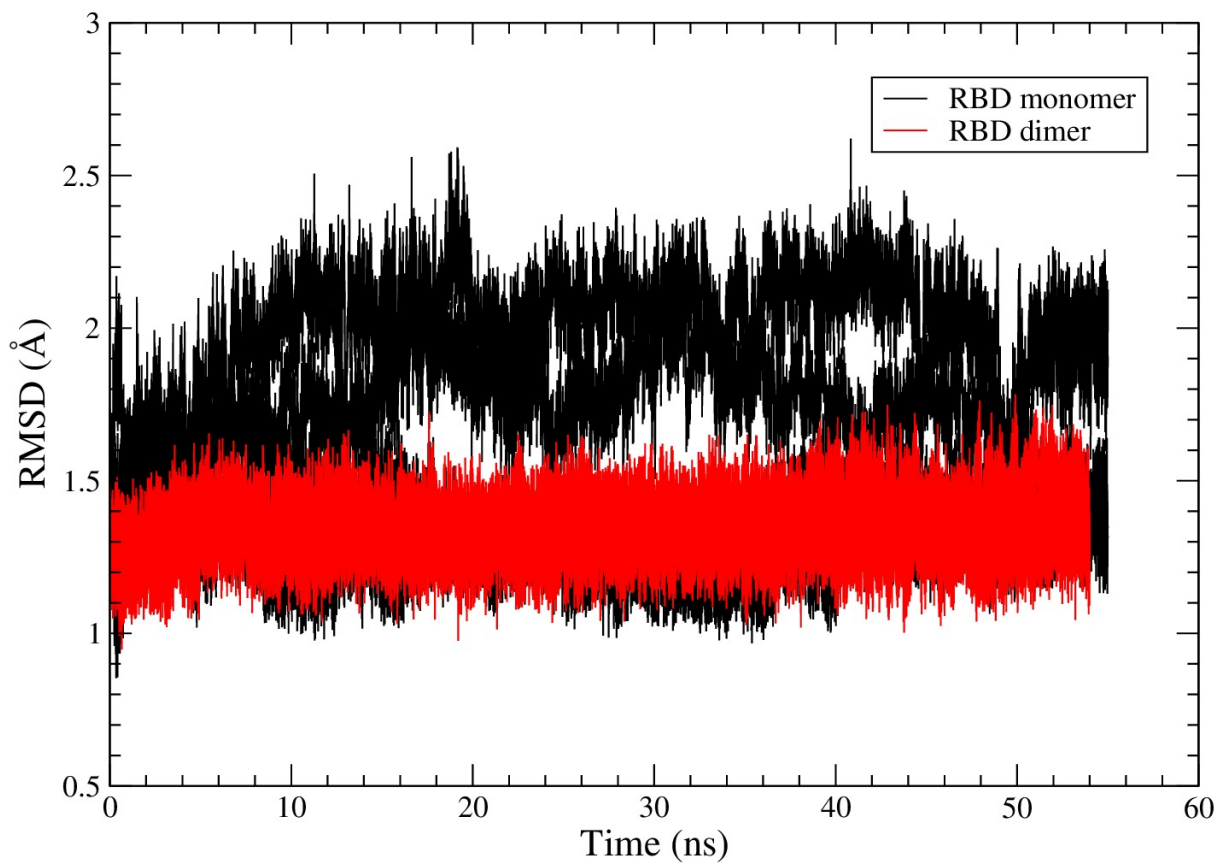[‡]University of Cincinnati, 2600 Clifton Ave., Cincinnati, OH, 45221

RMSD Figure

Figure S1: RMSD of heavy atoms comparison between RBD monomer(in Black) and dimer (in Red) based on all monomer and dimer simulations. The loop regions and termini are not considered, and each monomer trajectory and each unit in dimer trajectories are aligned on the crystal structure of RBD initially. For the RBD dimer simulations, all RMSD overlapped.
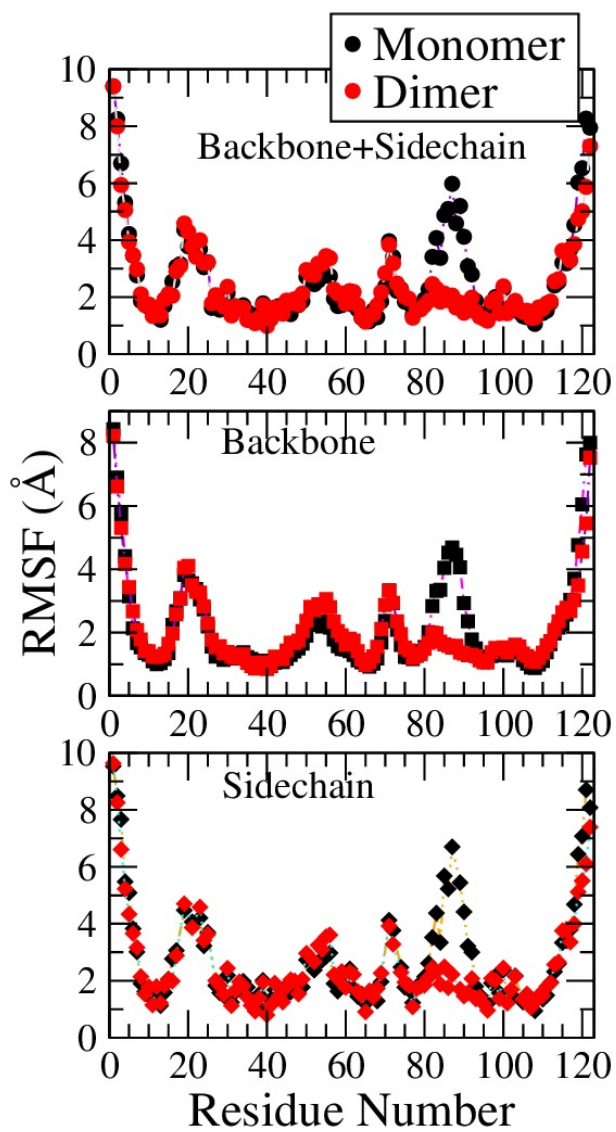
RMSF Figure

Figure S2: RMSF comparison between plexin RBD monomer(in Black) and dimer (in Red) simulations based on all (including backbone and sidechain atoms) (top panel), backbone atoms only(middle panel), and sidechain atoms only(bottom panel).

Dynamical Network Analysis Results for RBD Dimer

From the dihedral angle cross-correlation calculation, no signal pathways were detected for RBD dimer. Using the source and sink residue numbers from the RBD monomer as input, the network pathways for RBD dimer were predicted with results shown below. It is obvious that in the dimer form, the signal pathways can transmit from one unit to another unit through the L4 loop. Although it is always believed that the two monomers should be symmetric in structure, the signal pathways predicted are not symmetric for two units, with more signal pathways in unit 1 (on the left of both side view and top view figures) than unit 2.
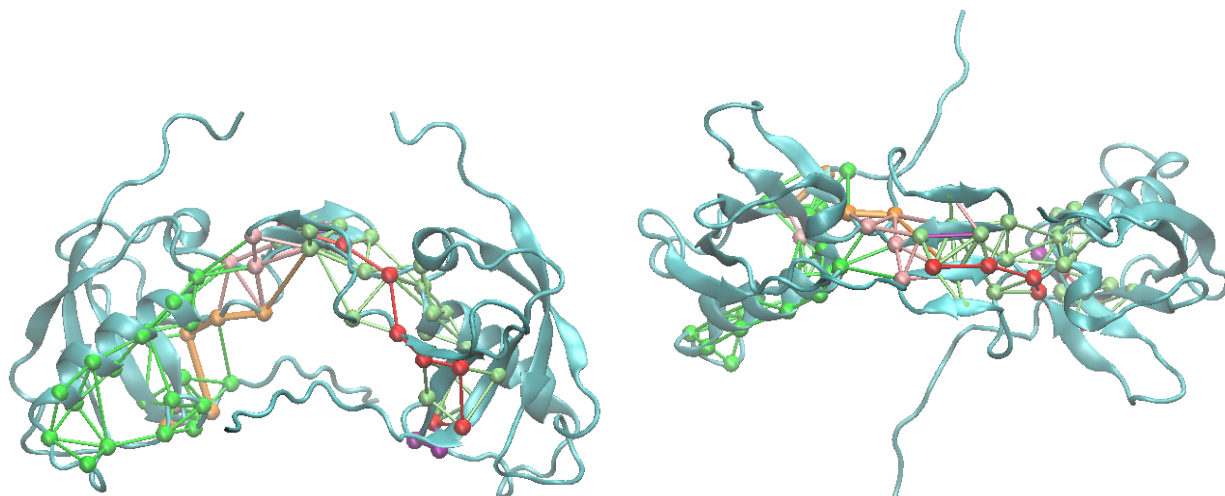

Figure S3: Dynamical network pathways predicted for RBD dimer in side view (Left) and in top view (Right). The signal pathway 1 is shown in Green for unit 1 (left), signal pathway 2 in pink which is mostly overlapped by signal pathway 1, and with the shortest pathway shown in orange. The signal pathway 1 is shown in Lime for unit 2, signal pathway 2 in purple which is mostly overlapped by signal pathway 1, and with the shortest pathway shown in red.

Principle Component Analysis Results

Principle Component Analysis (PCA) is a mathematical procedure converting a number of possibly correlated variables into a number of uncorrelated variables using orthogonal transformations. In order to find out which structure was kept the same after plexin-B1 RBD binded, we compared the configurational space between monomer trajectory and dimer trajectory by calculating first 5 major principle components through the Wordom[1] program.

Comparing the configurational distribution of RBD in monomer and dimer forms, we have results shown in Figure S4. We can see that there is considerable structural overlap between monomer and dimer forms. During the binding, only part of the RBD structure changed to facilitate the process. This is consistent with previous RMSF results, which also showed extensive similarities in structure between the monomer and dimer forms.
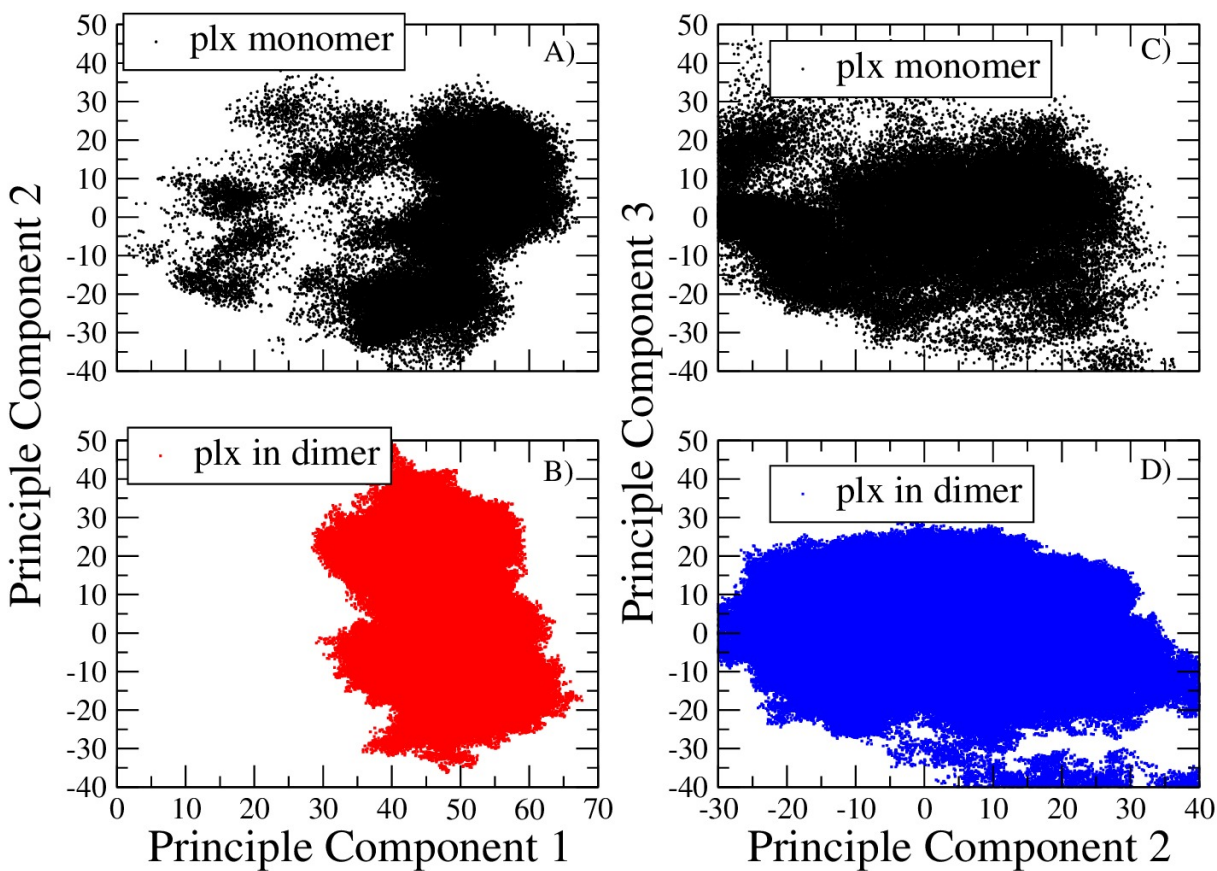
Figure S4: PCA comparison between combined plexin-B1 RBD monomer trajectories and each RBD in the combined dimer trajectories. Principle component 2 vs. principle component 1 comparison between plexin RBD monomer in A) and dimer forms in B); Principle component 3 vs. principle component 2 comparison between plexin RBD monomer in C) and dimer forms in D). The plexin RBD monomer data is shown in black dots in A) and C), plexin RBD dimer data is shown in red in B) and blue in D).

Binding Entropy Results

Based on S^2 results for RBD in monomer and dimer forms, the plexin RBD binding entropy was estimated. The binding entropy is due to changes in bond motions of mainchain and sidechain N-H, C-H and C=O groups using Lipari-Szabo order parameters, S^2, following the approach of Yang and Kay.[2] S^2, reflecting the amplitude of bond fluctuations on the ps-ns timescale, and correlation times were calculated using CHARMM over the same time intervals as we did on the previous project[3] for S^2 calculations.

The conformational entropy change can be calculated from the change in S^2 using Equation 1 and 2.[2]

$$S_p/k = A + \ln \pi [3 - (1 + 8S_{LZ})^{1/2}] \tag{1}$$

Here S_{LZ} is the Lipari-Szabo order parameters, has the relationship with S^2 calculated from simulation as:

$$S_{LZ} = \sqrt{S^2} \tag{2}$$

An order parameter describes the amplitude of the internal motion of a bond vector and depends on the distribution of orientations of the vectors. Entropy also depends on the distribution of the orientations of the bond vectors. This method has been applied to analyze the protein binding with ligand,[4] and protein folding.[2] In this project, the conformational entropy change in dimerization process was calculated based on ΔS^2.[2]

Calculating entropy change based on order parameter change during the binding process, binding entropy data are shown in Table S1. It can be seen that surface groups contributed more to the entropy decrease than core groups, and sidechain groups contributed more to the entropy decrease than backbone atoms because of the larger amount of bonds involved in the sidechains and surface residues. The entropy per bond is similar for the three kinds of bonds, and at different locations.

Mapping the specific binding entropy for each bond types on each heavy atom in RBD

Table S1: Summation of binding entropies for different bond types at different locations

bonds	CH		
	entropy (kcal/mol)	number	avg (kcal/mol)
backbone-core	-71.84	38	-1.89
backbone-surf	-154.81	73	-2.12
sidechain-core	-349.98	201	-1.74
sidechain-surf	-842.48	492	-1.71
bonds	NH		
	entropy (kcal/mol)	number	avg (kcal/mol)
backbone-core	-90.78	41	-2.21
backbone-surf	-106.48	58	-1.84
sidechain-core	-96.19	43	-2.24
sidechain-surf	-157.17	139	-1.13
bonds	CO		
	entropy (kcal/mol)	number	avg (kcal/mol)
backbone-core	-61.86	39	-1.59
backbone-surf	-168.79	67	-2.52
sidechain-core	-80.02	43	-1.86
sidechain-surf	-230.16	106	-2.17

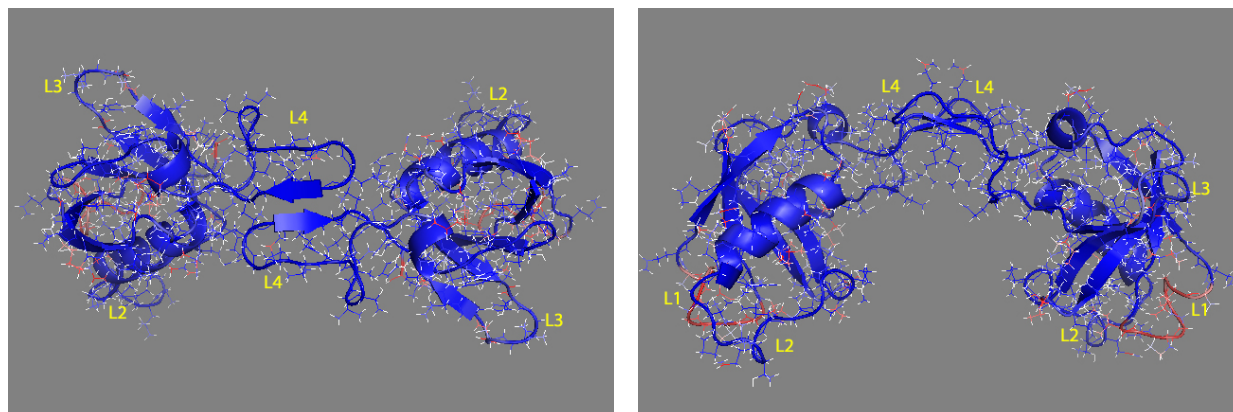dimer structure, results are shown in Figure S5.

Figure S5: Plexin-B1 RBD monomer to dimer entropy change shown as b-factor in structure in top view at Left and in side view at Right. Color range from blue to white to red (with white represents no change, blue represents entropy decreasing while red represents entropy increasing). The head 9 residues and tail 9 residues are not shown.

As can be seen from both the left and the right figures, after binding, some regions become partially flexible on both backbone and sidechain: residue R7 and L14 on head and tail of $\beta1$, reside 17-19 on head of the L1 loop, D33 on tail of $\beta2$, residue 42-49 on tail of $\alpha1$, R61 and R68 on head and tail of $\beta3$. Some residues become flexible on sidechain bonds only, like residue 8-9 on head of $\beta1$, 42-47 on tail of $\alpha1$. Some residues become partially flexible on backbone only, like residue 20-26 on tail of L1, 48-49 on tail of $\alpha1$, S69 on tail of $\beta3$, and so on. The L4 loop only becomes rigid. In correlated motions, if some regions on protein network become rigid while other regions become more flexible, then there could be an allosteric mechanism involved. Based on the entropy change in structure, the L4 loop, part of $\alpha1$, the L2 loop, $\beta2$, and $\beta5$ should be correlated, but anti-correlated with L1 and the tail of $\beta2$, and some spot residues on the L1 loop, $\beta1$-3, and $\alpha1$. The allosteric mechanism should involve some residues in both the former group and the latter group.

Comparing the entropy change and order parameter change, consistent results are observed. With sidechain atoms on the surface showing an increase in order parameters as shown in the right of Figure 6, they become more rigid, thus have an entropy decreasing as shown in the right of Figure S5. Meanwhile, the backbone atoms on the core only showed

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slight increase in S^2, thus slight decrease in ΔS. The C-terminal and the L1 loop become more flexible with a decrease in S^2, thus an increase in ΔS, comparing the results shown in the left of Figure 6 and the left of Figure S5.

References

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