Disordered linkers in mutli-domain allosteric proteins: entropic effect to favor the open state or enhanced local concentration to favor the closed state?

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Supporting Information

1. Definition of the effect of disordered linkers

Here, we chose the linker length to exhibit the effect of disordered linkers (Fig. S1). In the initial ensemble, the stabilization effect was weakened by shorter or longer linkers (columns A and E). The entropic effect of the disordered linker's behavior is shown at the optimal length for a maximum $\Delta G_{\text{Closed-Open}}$. After allosteric activated binding, the free energy curve of the final ensemble as a function of the linker length showed a parallel decrease. Moreover, the strength of the allosteric ligand can be reflected as the free energy gap during the allosteric process. Once the protein pair and the allosteric ligand are chosen, the free energy curve is fixed. If the linker renders the protein too stable to open, the protein will lose its allosteric ability especially when the allosteric ligand is weak (column C). Thus in Fig. S1 only columns B and D are switchable as allosteric pathways. With this method, we can elucidate the entropic magnitude between different linker lengths.

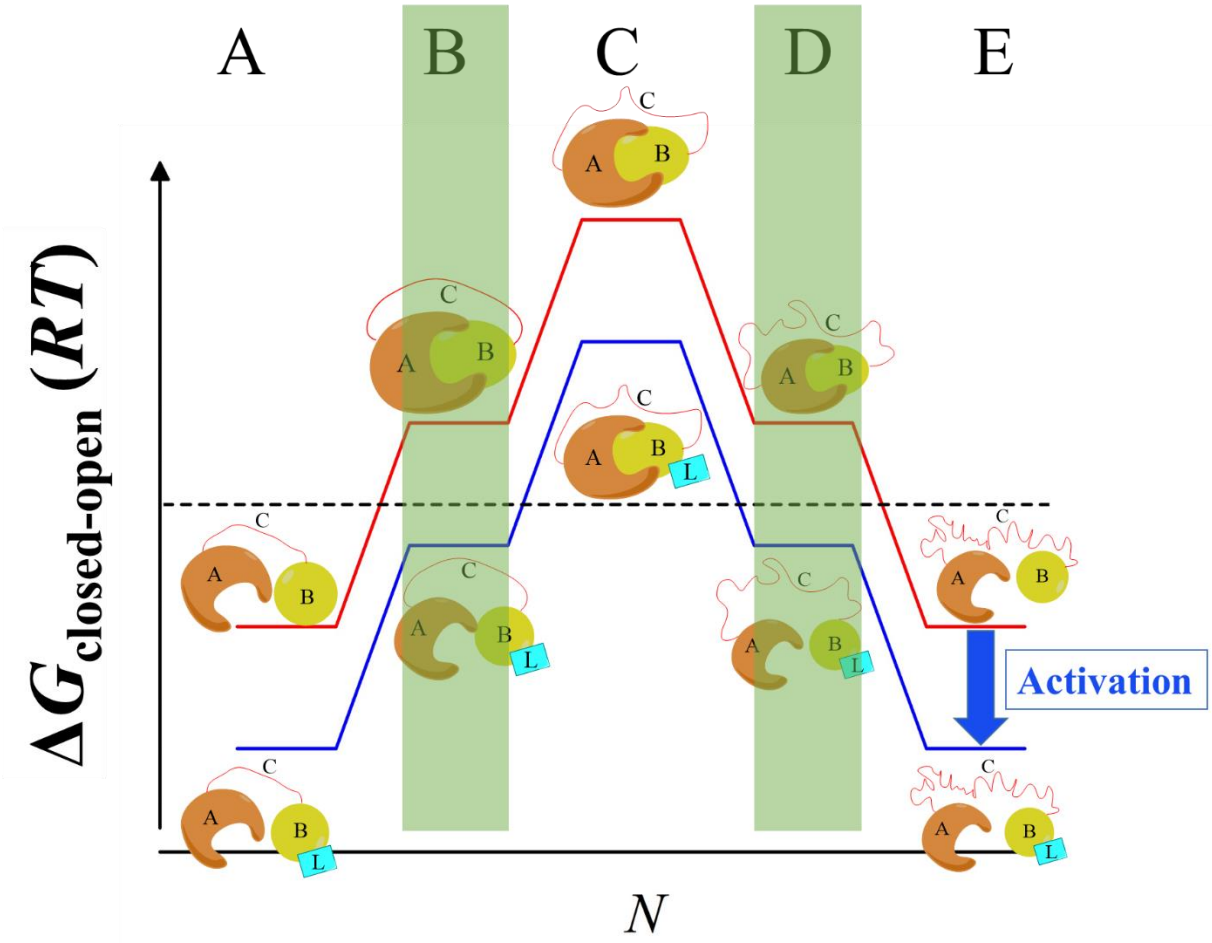

Figure S1. Entropic effect definition as a function of linker length. From left to right, the disordered linker length increases through five proteins. The red line is for the initial ensemble, and the blue line is for the final ensemble. The allosteric binding of an activated ligand is represented by a blue arrow. Allosteric pathways exist in the green background conditions B and D, while the other conditions are defined as weak binding.

2. Coarse-grained molecular dynamics (CG-MD)

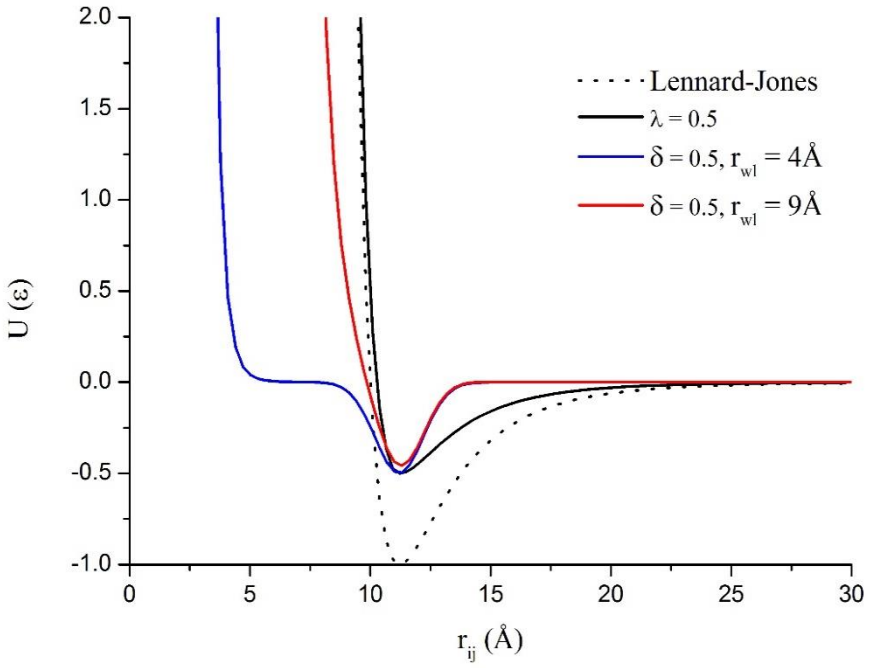

Figure S2. Non-bonded interactions in CG-MD. Non-bonded interactions are only considered when two beads, i and j, are separated sequentially by at least three residues as in common Gō Potential [Eq. (2)]. The non-bonded interaction adopted in this study is represented as a red solid line with non-bonded strength δ = 0.5. A 12-6 Lennard-Jones potential was plotted as a dashed line, while the potential used in a previous study is represented as a black solid line with non-bonded strength λ = 0.5.

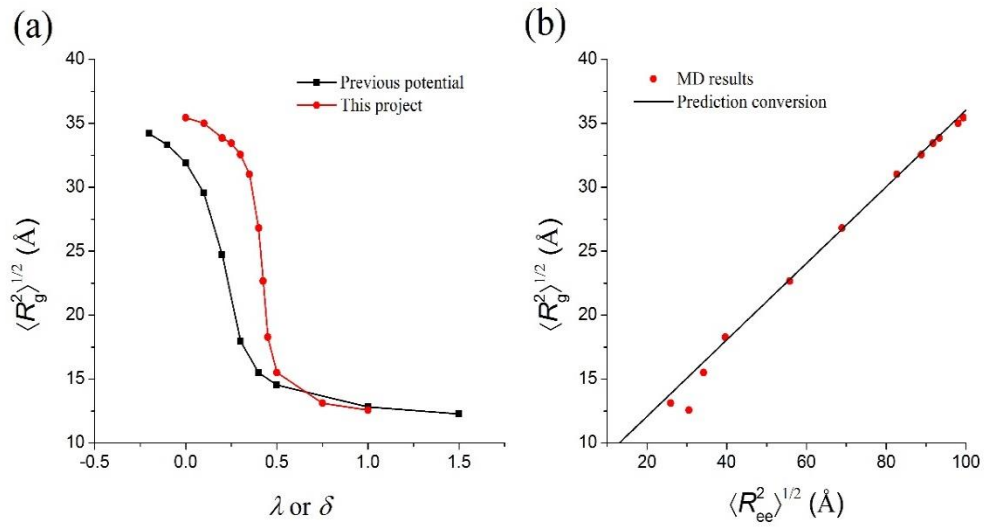

Figure S3. Dimension properties of CG-MD of an isolated linker, using N = 60 as an example. (a) Determination of non-bonded strength parameter δ, plotted as a red line. The coil-globule transition curve of λ was plotted as a black line as reference. (b) $<R_g^2>^{1/2}$ depends linearly on $<R_{ee}^2>^{1/2}$ with different δ value, plotted as red points. The former prediction conversion formula[1] $\left\langle R_g^2 \right\rangle^{1/2} = (0.047 \ln N + 0.107)\left\langle R_{ee}^2 \right\rangle^{1/2} + (0.301 - 0.047 \ln N)(0.557N + 22.5)$ is represented as a black line.

3. The evidence of convergence of simulation

In our Gō-like simulation, chains were converged quickly from the initial conformation to the equilibrium ensemble, see Fig. S4.

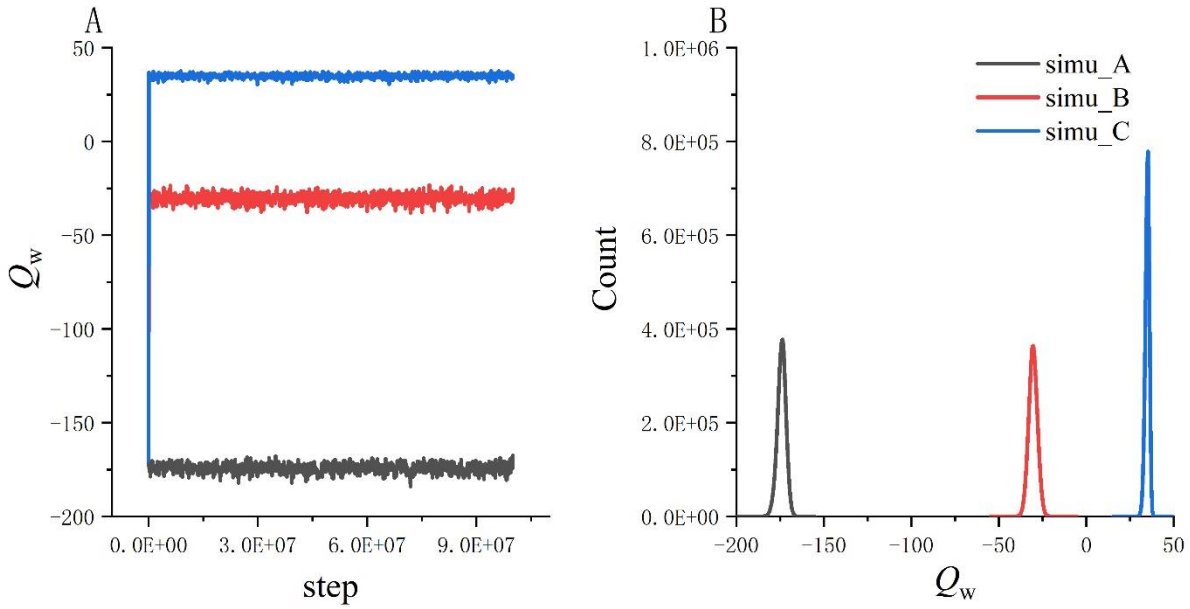

Figure S4. The evidence of convergence of simulation, taking 1BRS as example system. Trajectories under different bias ($Q_{W,bias}$ = −180, −30, 40) were plotted with different color.

4. The free energy difference $\Delta G_{\text{Closed-Open}}$ of the initial ensemble

The entropic effect of the disordered linker is mainly decided by the $\Delta G_{\text{Closed-Open}}$ of the initial ensemble. That is, the function of an allosteric protein is programmed once it is expressed. We plotted a two-dimensional $\Delta G_{\text{Closed-Open}}$ map of the linker length and the flexibility property of the initial ensemble (Fig. S5). The initial ensemble can be divided into two main stages: red for flexible linkers and yellow for rigid linkers. A ~ $4.5RT$ decrease in $\Delta G_{\text{Closed-Open}}$ resulted in ~100-folds in the concentration of the open state.

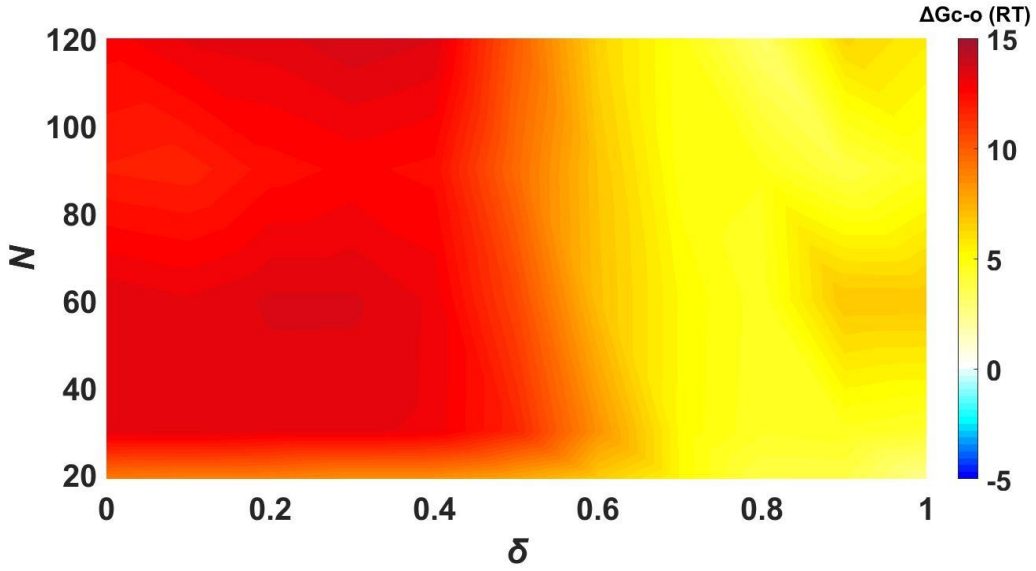

Figure S5. The free energy difference $\Delta G_{\text{Closed-Open}}$ of the initial ensemble, β = 1.35, color bar as in Fig. 3.

5. The chain entropic incorporation caused by excluded-volume term

In our Gō-like model, the chain entropic contribution will be affected by the excluded-volume interaction between linker and domains. Here, we tuned r_{rep} from 4.0 Å to 5.0/6.0 Å, and the obtained results were shown in Fig. S6. The free energy profile changed a lot when r_{rep} = 6.0 Å, and resulted in a decreasing of $[B]_0$ to 27.0 μM. But for r_{rep} increasing from 4.0 Å to 5.0 Å, it caused only a slight decreasing of 0.03 mM in $[B]_0$.

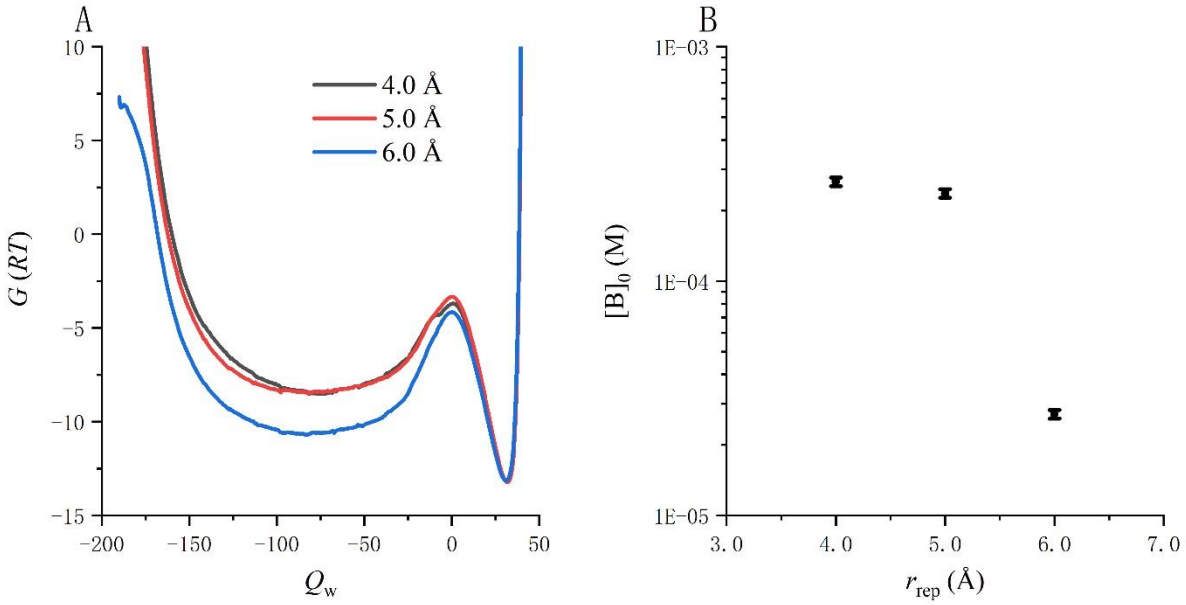

Figure S6. The chain entropic incorporation caused by excluded-volume term, taking 1BRS as example system. Tuned r_{rep} from 4.0 Å to 5.0/6.0 Å. (A) The free energy profiles as a function of the binding reaction coordinate, Q_w (positive for the bound state and negative for the unbound state with $-Q_w$ approximately proportional to the domain separation distance), at β = 1.0. (B) Characteristic concentration $[B]_0$ of disordered linker in 1BRS as a function of r_{rep}.

REFERENCES

1. Li MD; Sun TL; Jin F; Yu DQ; Liu ZR (2016) Dimension conversion and scaling of disordered protein chains. Molecular Biosystems *12*:2932-2940.