Supplementary Information for:

Entropy of Stapled Peptide Inhibitors in Free State is the Major Contributor to the Improvement of Binding Affinity with GK domain

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Supplementary text:

1. Glutamic-acid replacement of phosphoserine residue in linear peptide diminishes binding to GK domain

The natural ligand of GK domain, phosphor-SAPAP1 N-terminal (p-SAPAP1, ARRE(pS)YLKATQ) contains a phosphoserine group (pS)**.** The phosphor group is not suitable for peptide delivery *in vivo*, as it is easily degraded by phosphatases in cells and its highly negative charge hinders cell penetration. Therefore, we initiate our design of PPI inhibitor by replacing the phosphoserine residue with the glutamic acid (E), so that the glutamic acid could mimic the interactions of the phosphoserine group with the binding pocket (Figure S1A). Not surprisingly, this linear peptide ARREEYLRAIQ did not display sufficient binding affinity to the GK domain according to ITC measurements (Figure S1B).

2. Optimization of linear peptide sequence

We performed optimizations on the linear peptide sequence (ARREEYLRATQ) based on the binding surface of GK based on previously solved crystal structures (1–3): Firstly, we substituted A(-4) at the N-terminal with I(-4) to target the hydrophobic surface of GK (I627 and L552). Then, we added R(-5) to the N-terminal to form salt bridge with the negatively charged residues of GK interacting surface (D545, D549, and D629); (C). Lastly, we substituted T(5) with I(5) to enhance the hydrophobic interaction with Y604 and Y580 of GK domain. As a result, we obtained the optimized linear peptide sequence (RIRREEYLKAIQ) with a binding affinity of 34 μM that serves as the template to introduce our staple at i~i+4 positions.

Supplementary Figures:

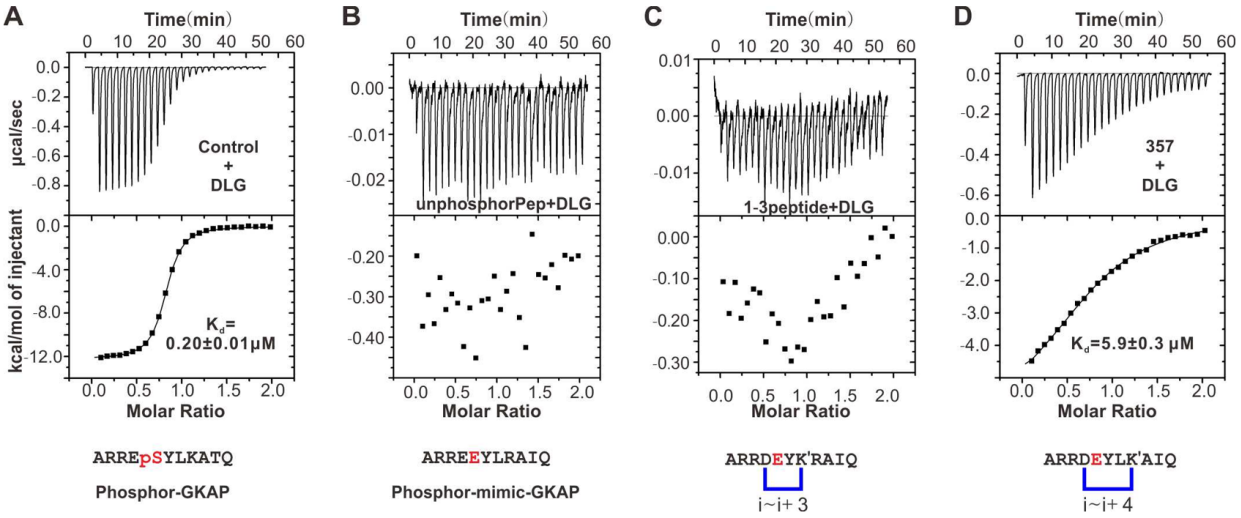

Figure S1. ITC measurements of the bindings between PSD95 GK and (A) phosphor-, (B) linear, (C) i~i+3 stapled-, (D) i~i+4 stapled peptide, respectively.

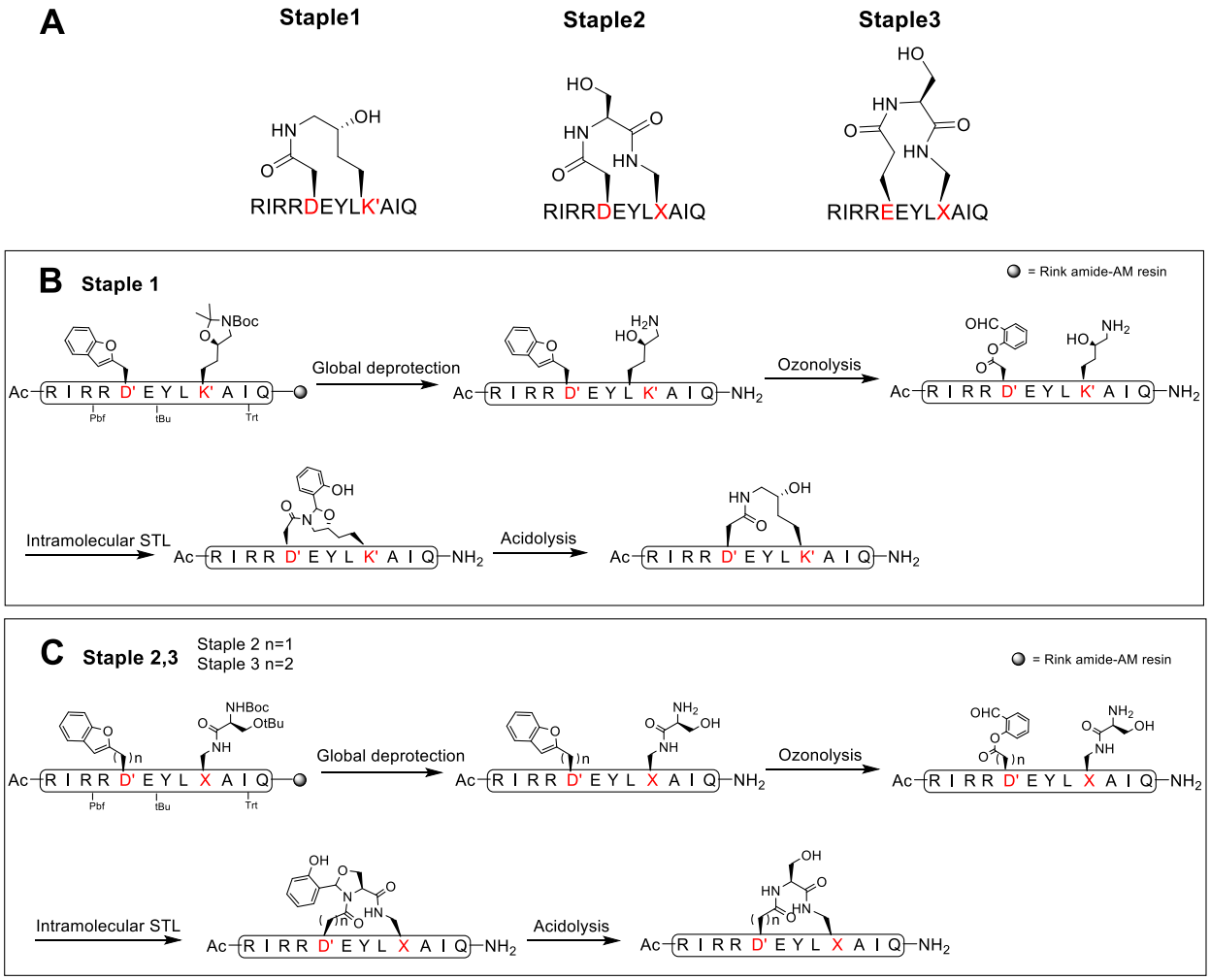

Figure S2. Molecular structure and synthetic pathway of Staple 1, Staple 2, and Staple 3.

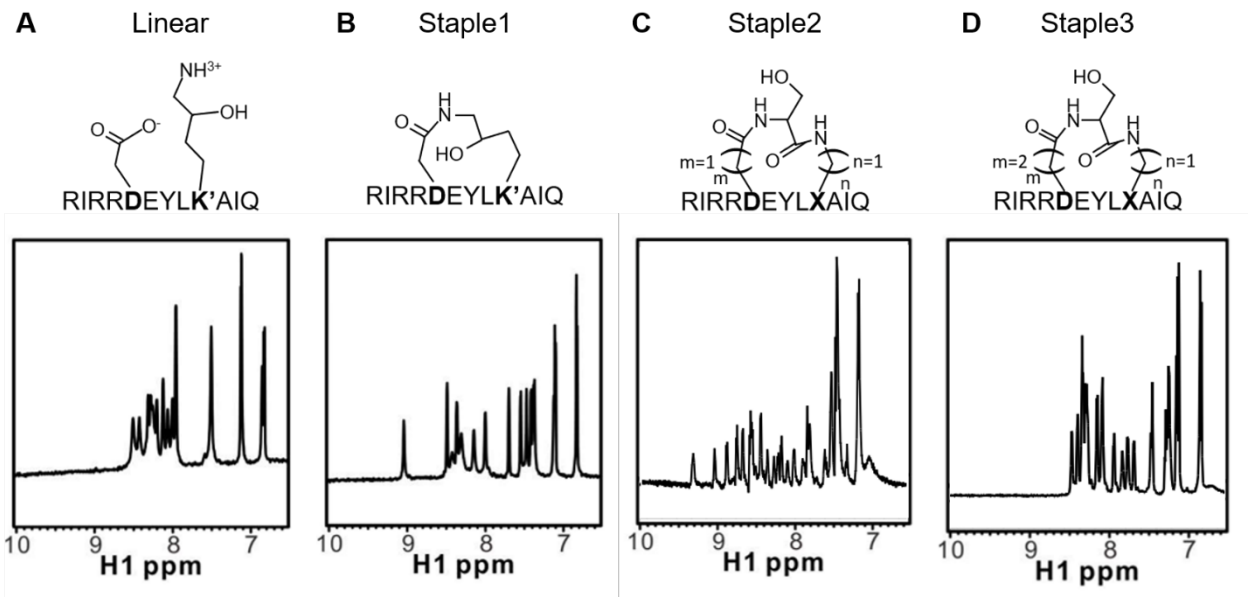

Figure S3. 1D ^{1}H NMR spectra of a) Linear, b) Staple1, c) Staple2, d) Staple3 peptides at 6.5-10.1 ppm.

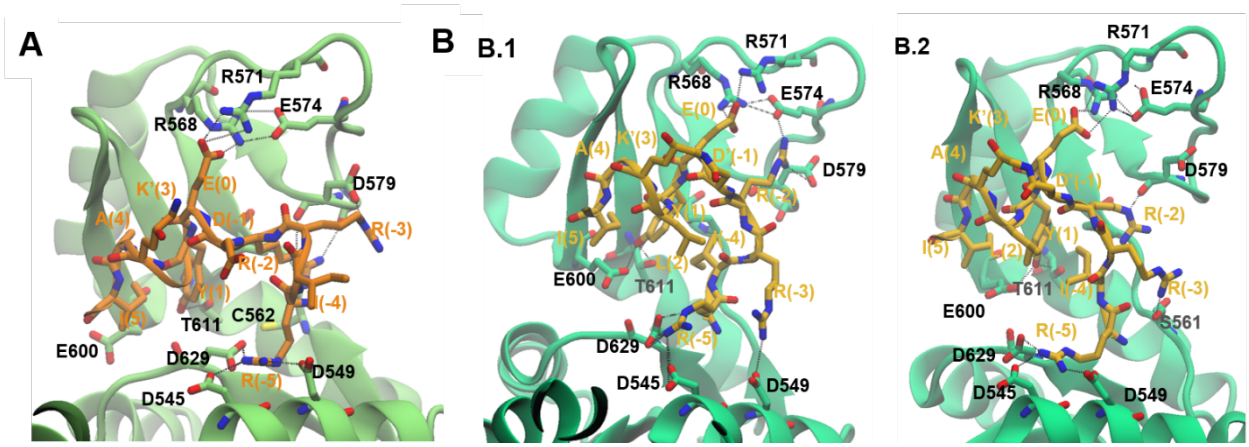

Figure S4. Detailed structure of GK in complex with a) Linear and b) Staple1 peptides. Staple 1 peptide crystalize in two slightly different conformations at the N terminal (b.1. and b.2.).

Experimental Thermodynamics Contributions

$\Delta G_{binding} = \Delta H - T\Delta S$

Peptide Ligands	$\Delta G_{binding}$ (kcal/mol)	ΔH (kcal/mol)	$\Delta\Delta H$[1]	$-T\Delta S$ (kcal/mol)	$-T\Delta\Delta S$[2]
Linear	-6.10±0.15	-16.60	-	10.50	-
Staple1	-8.00±0.02	-14.00	2.60	6.00	-4.50
Staple2	-7.07±0.02	-9.70	6.90	2.63	-7.87
Staple3	-7.77±0.01	-9.30	7.30	1.53	-8.97

[1] $\Delta\Delta H = \Delta H_{stapled} - \Delta H_{Linear}$
[2] $-T\Delta\Delta S = -T(\Delta S_{stapled} - \Delta S_{Linear})$

Table S1. Thermodynamics contribution to the binding affinity, $\Delta G_{binding}$ measured by ITC experiment. ΔH correspond to the enthalpy change and ΔS corresponds to the entropy change.

Computational Thermodynamics Contributions

$$\Delta G_{binding} = \Delta G_{solvation} + \Delta E_{MM} - T\Delta S$$

Peptide Ligands	$\Delta G_{binding}$ (kcal/mol)	ΔH (kcal/mol)	$-T\Delta S$ (kcal/mol)
Linear	-75.24±11.28	-284.41±3.11	209.17±10.85
Staple1	-177.55±11.62	-301.20±3.19	123.65±11.17
Staple2	-170.41±9.55	-281.04±3.08	110.63±9.04
Staple3	-176.35±13.30	-262.29±3.13	85.94±12.92

Table S2. Thermodynamics contribution to the binding affinity, $\Delta G_{binding}$, calculated by computational methods. Computational ΔH corresponds to the potential energy change in protein and ligand (ΔE_{MM}) upon binding and solvation free energy change upon binding ($\Delta G_{solvation}$). ΔS corresponds to the configurational entropy change in protein and ligand upon binding. ΔH was calculated by MM/PBSA method and GROMACS. ΔS was calculated by quasi-harmonic approximation.

Computational Solute Potential Energy Contributions

$$\Delta E_{MM} = \Delta E_{ligand-intra} + \Delta E_{GK-intra} + \Delta E_{intraction}$$

Peptide Ligands	ΔE_{MM} (kcal/mol)	$\Delta E_{ligand-intra}$ (kcal/mol)	$\Delta E_{GK-intra}$ (kcal/mol)	$\Delta E_{Intraction}$ (kcal/mol)
Linear	-541.86±5.85	90.82±1.52	-42.39±5.82	-590.26±1.60
Staple1	-523.44±5.73	66.17±1.25	-84.32±5.86	-505.29±1.87
Staple2	-514.74±5.68	73.77±1.25	-34.14±5.91	-554.51±1.71
Staple3	-484.05±5.78	54.98±1.21	-2.89±5.71	-536.25±1.71

Table S3. Thermodynamics contributions to the potential energy change of GK-peptide ligand complex, ΔE_{MM} calculated from MD simulation structures by GROMACS. $\Delta E_{ligand-intra}$ is the potential energy change within the ligand upon binding, $\Delta E_{GK-intra}$ is the potential energy change within the protein (GK domain) upon binding, and the $\Delta E_{interaction}$ is the interaction energy between GK and ligand in the complex (bound state).

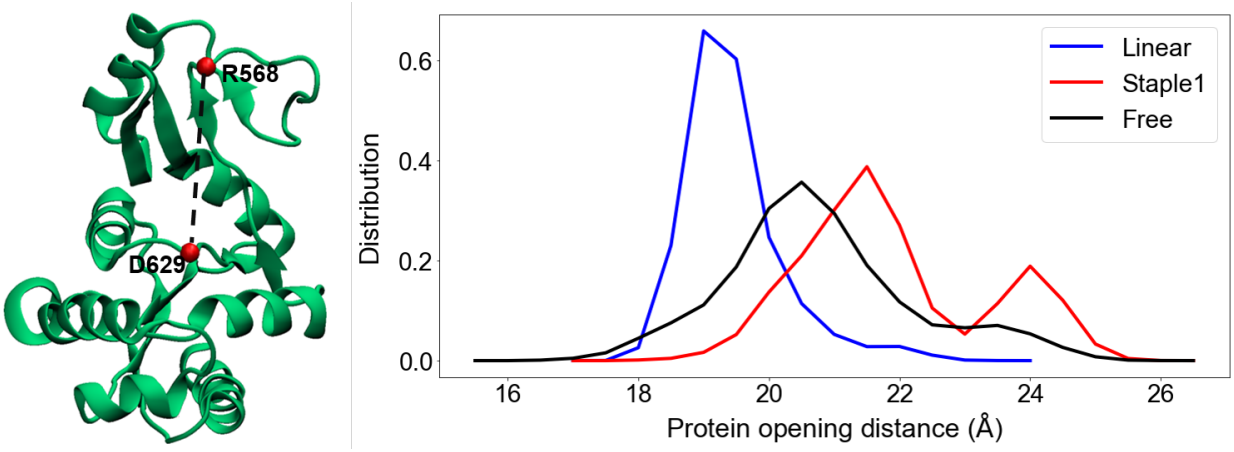

Figure S5. Definition of opening distance of GK domain. Distance between C-α atom of R568 and D629 (Left). Distribution of GK opening distance when GK is bound with Linear (blue), Staple 1 (red) and GK in free (black) states (Right).

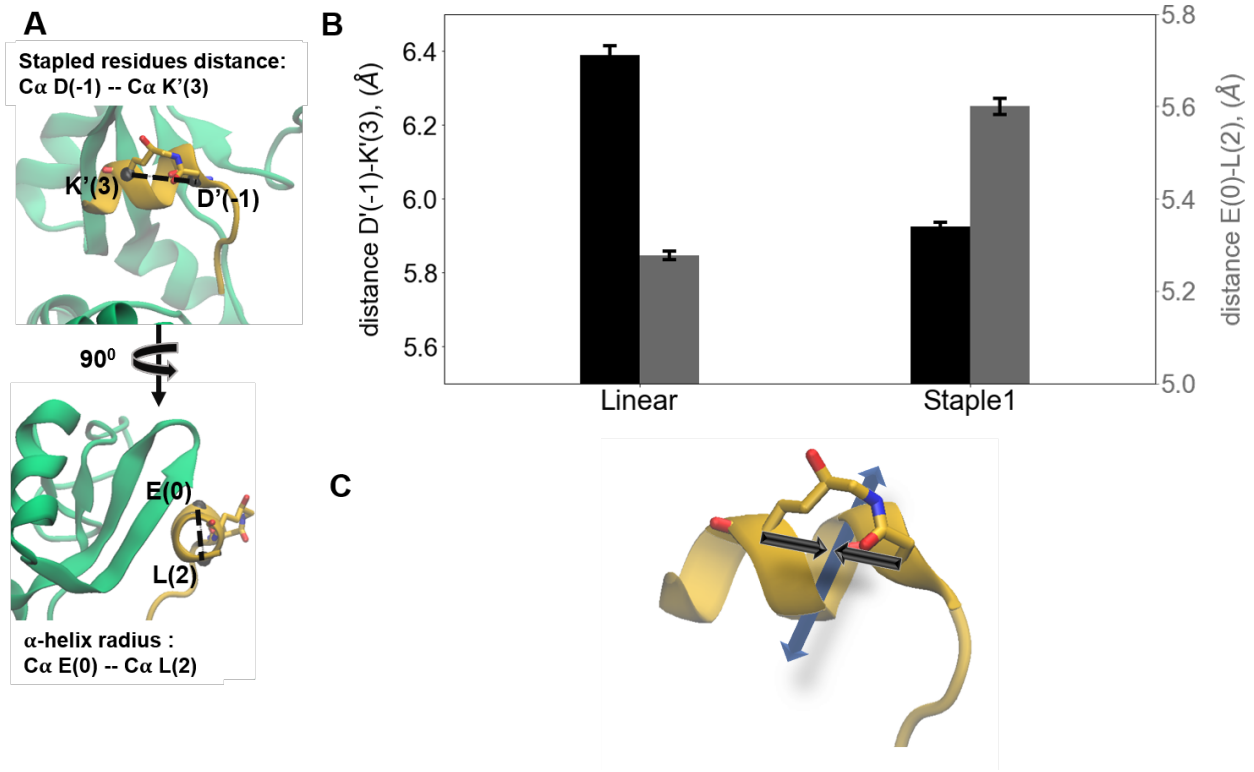

Figure S6. a) Definition of distance between stapled residues and α-helix radius, b) Average distance between stapled residues and α-helix radius, c) illustration showing the constrain between stapled residues increases the alpha helix radius.

$$\Delta S_{total} = \Delta S_{ligand} + \Delta S_{protein}$$

$$\Delta S_{ligand} = (S_{bound} - S_{free})_{ligand} \qquad \Delta S_{protein} = (S_{bound} - S_{free})_{protein}$$

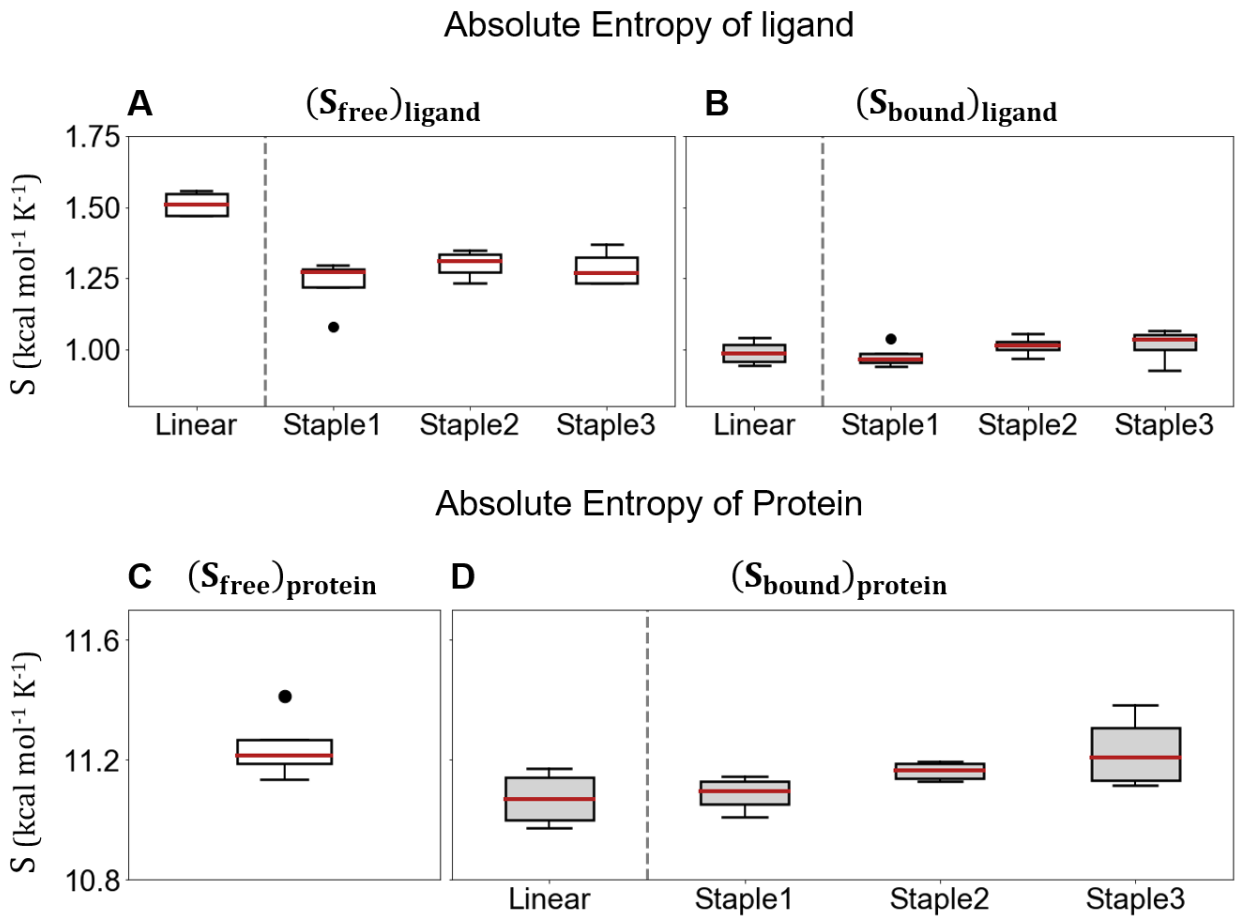

Figure S7. Absolute entropy calculation by quasi-harmonic approximation of a) free state, b) bound state of Linear, Staple 1, Staple 2, and Staple 3 peptides, c) free state of protein, d) protein when bound with Linear, Staple 1, Staple 2, and Staple 3 peptides. Equation for calculation of computational ΔS_{total} are shown (Top).

References

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2. Zhu J, et al. (2011) Guanylate kinase domains of the MAGUK family scaffold proteins as specific phospho-protein-binding modules. *EMBO J* 30(24):4986–4997.
3. Zhu J, et al. (2014) Phosphorylation-dependent interaction between tumor suppressors Dlg and Lgl. *Cell Res* 24(4):451–63.