Supporting Information for:

Interdomain linkers regulate histidine kinase activity by controlling subunit interactions

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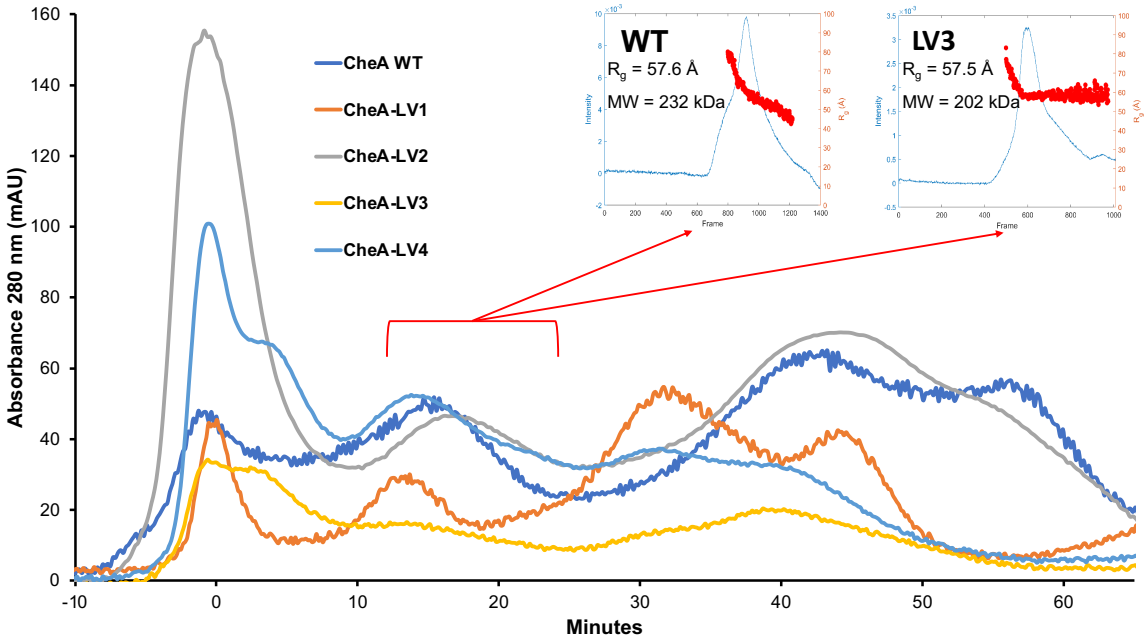
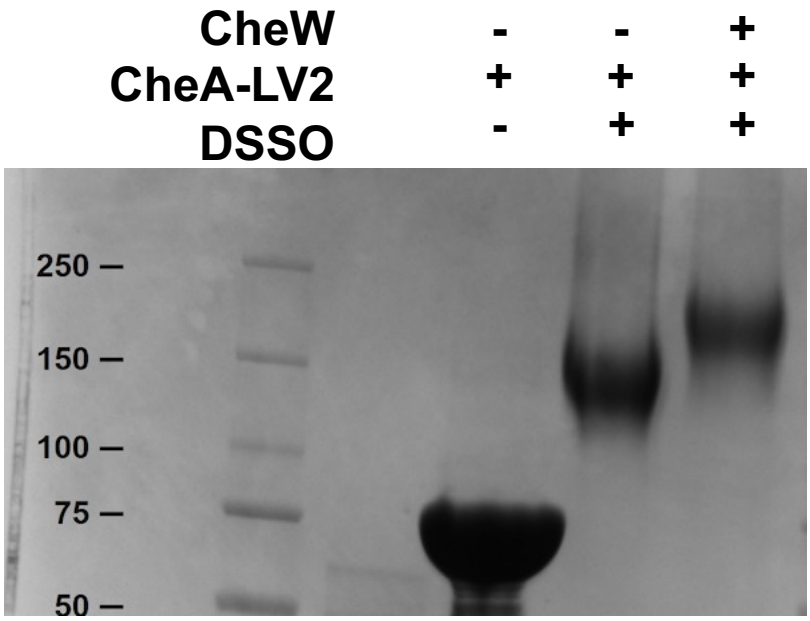
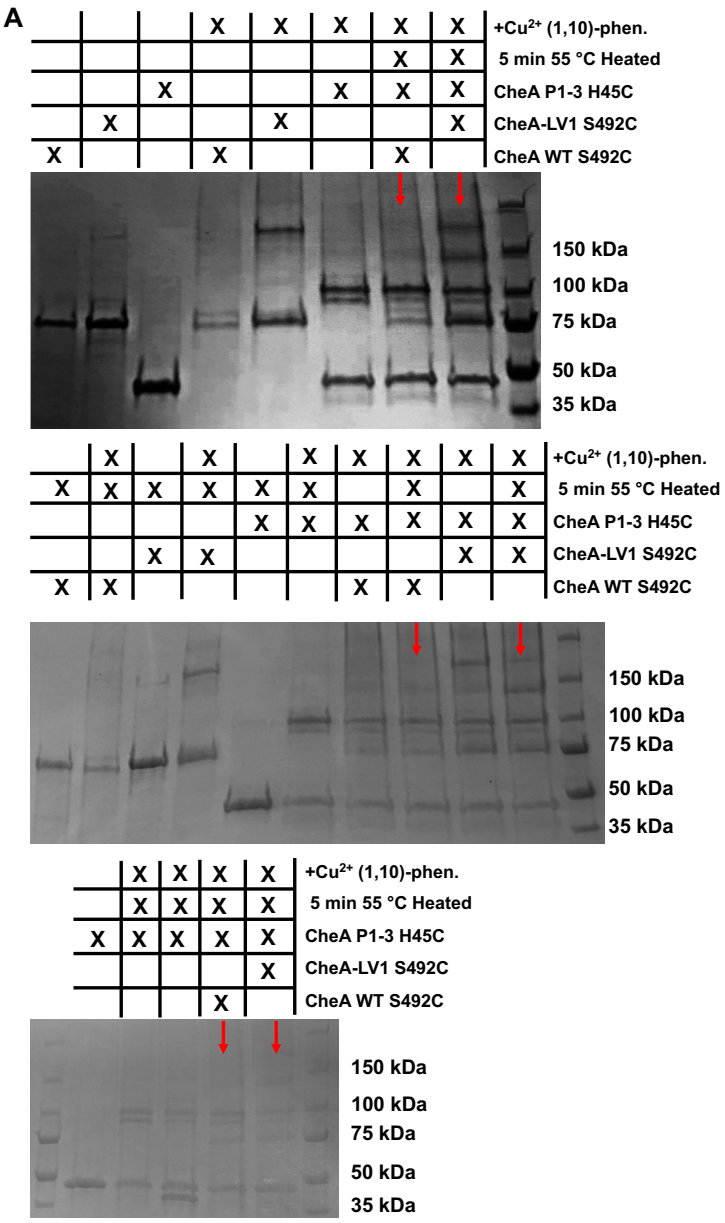
The PDF file includes:



Supplemental Figure 1. Size-exclusion chromatography traces of CheA WT and linker variants. Each of the proteins was purified on a Preparative Superdex 200 column. The void peaks were aligned at 0 min to account for variations in column performance. The red bracket indicates the SEC peak collected for the dimer species. These proteins were then used in SEC-SAXS, representative data for which is displayed in insets. MW estimations from the Porod volume, as well as radius of gyration (Rg) measuerments indicate that the CheA WT and linker variants are dimeric.



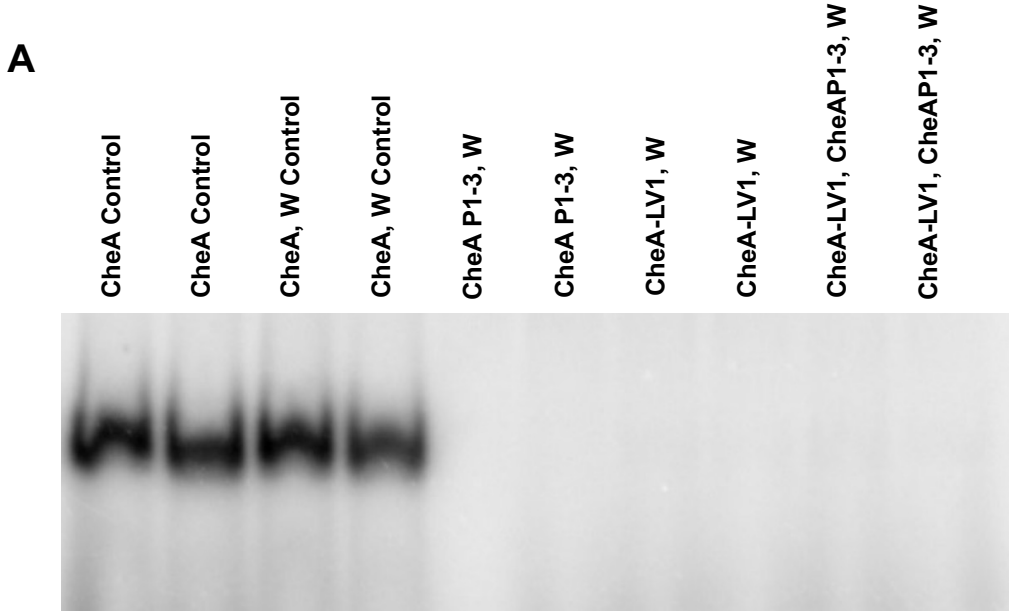
Supplementary Figure 2 DSSO crosslinking of CheA-LV2 (75 kDa) to CheW (17 kDa). The molecular weight markers are indicated in kilodaltons (kDa) to the left.

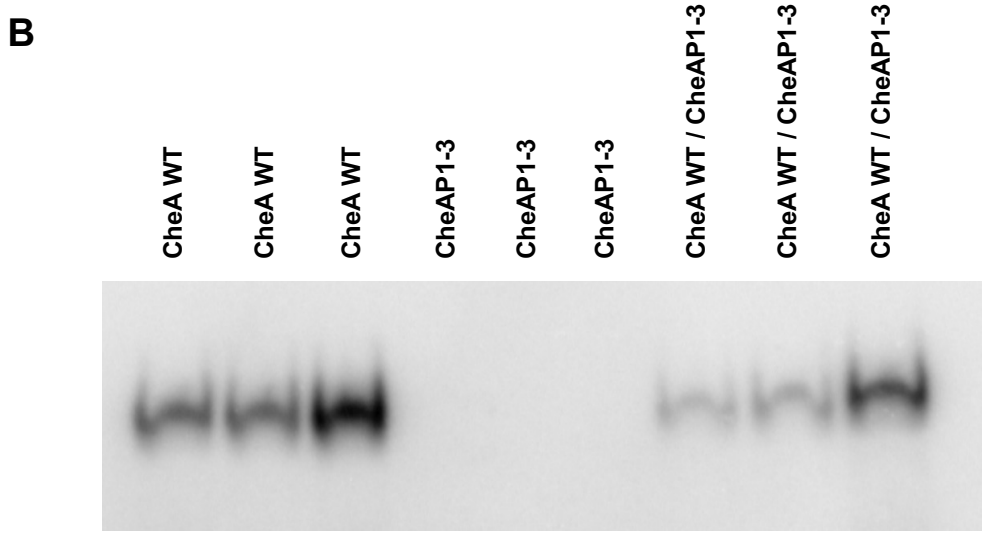


Supplemental Figure 3.

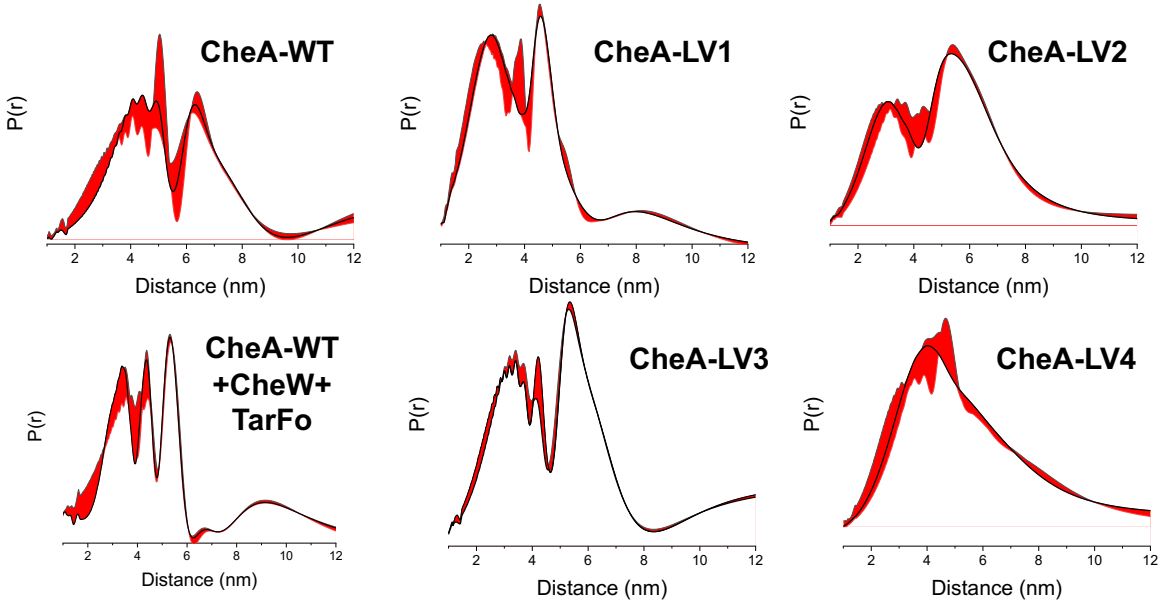
Quantification of CheA FL / CheA P1-3 Heterodimer Formation **(A)** SDS-PAGE gels of disulfide crosslinking experiments used to quantify CheA FL bands. Red arrows indicate the lanes used in that calculation. The chart above each gel specifies the contents of each sample by lane, cysteine-cysteine crosslinking was initiated by the addition of Cu^{2+} (1,10)-phenanthroline. Subunit exchange was provided by heating samples at 55 °C for 5 mins. **(B)** Detail of calculations shown in (C). First, the band intensities of crosslinking products measured in ImageJ are corrected "a" for their molecular weights. Next, the proportions "b" of each CheA FL crosslinking species are calculated. The percent increases "c" of the heterodimer band for LV1

relative to WT is calculated, and their average "D" is reported with its standard deviation. **(C)** Raw data and outcome of calculations detailed in (B). The summary shown at the bottom left reveals that the percentage of CheA FL / CheA P1-3 heterodimer in CheA-LV1 increases 12 ± 8 % relative to CheA WT.





Supplemental Figure 4. Autophosphorylation of CheA WT and CheA-LV1 after CheAP1-3 subunit exchange. **(A)** Autoradiography of WT and CheA-LV1 autophosphorylation with and without CheAP1-3 after subunit exchange. CheA-LV1 registers at very low autophosphorylation. CheAP1-3 bands are absent. **(B)** Autoradiography of CheA WT autophosphorylation with and without CheAP1-3 after subunit exchange. Phosphorylated CheAP1-3 bands are absent in all cases and CheAP1-3 reduces WT CheA autophosphorylation after subunit exchange.



Supplemental Figure 5.

Distance distribution reconstructions by WavPDS with error bounds (red) for ADP-NO bound WT CheA, CheA WT bound to CheW and Tar_{FO} and CheA linker variants[58].