The Charged Linker Modulates the Conformations and Molecular Interactions of Hsp90

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Figure S1. Comparison of NMR spectra of Hsp90 NTD constructs. The absence of significant CSP and intensity changes on the NTD between NTD_252 and NTD_217 indicates that only C-linker is responsible for interactions with N-linker and NTD.
Figure S2. The β2-β3 turn stabilizes the N-linker region. A) CSPs of NTD_{217} WT vs. E71Q shows strong perturbations on N-linker residues 209-213, indicating a charge-mediated interaction. B) Mapping of perturbations derived from E71Q mutation (green) on crystal structure of NTD bound to ATP (PDB 1AM1 [1]). Side chains of affected residues 209-215 are shown as sticks and spheres.
| Construct   | $K_d$ (μM) | Stoichiometry | $\Delta H$ (kJ/mol) | $\Delta G$ (kJ/mol) | $-T^*\Delta S$ (kJ/mol) |
|-------------|------------|---------------|---------------------|---------------------|--------------------------|
| NTD_217 ADP | 20.7 ± 1.91| 1.04 ± 0.017  | -60.2 ± 2.21        | -26.8               | 33.5                     |
| NTD_274 ADP | 14.9 ± 0.82| 0.912 ± 0.009 | -54.8 ± 1.05        | -27.6               | 27.3                     |

**Figure S3**: Summary of isothermal titration calorimetry experiments for nucleotide binding to NTD-linker constructs.

**Supplementary References**

[1] C. Prodromou, S. M. Roe, R. O’Brien, J. E. Ladbury, P. W. Piper, L. H. Pearl, *Cell* **1997**, *90*, 65-75.