**Supplementary file 3. Changes in enthalpy, entropy, free energy, and dissociation constants for PKI5-24 binding to apo and ATPγN - saturated PKA-CWT and PKA-CF100A.** All errors were derived from triplicate measurements. The error for the cooperativity coefficient (σ) was propagated from the errors in *K*d. Values for PKA-CWT were originally published in Walker *et al.* 22.

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| PKI5-24 to apo kinase | | | | | |
|  | *K*d (μM) | ΔG (kcal/mol) | ΔH (kcal/mol) | -TΔS (kcal/mol) | σ |
| PKA-CWT | 17 ± 2 | -6.57 ± 0.08 | -10.8 ± 0.5 | 4.2 ± 0.5 | N/A |
| PKA-CF100A | 5 ± 1 | -7.7 ± 0.1 | -17 ± 5 | 7 ± 3 | N/A |

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| PKI5-24 to ATPγN-saturated kinase | | | | | |
|  | *K*d (μM) | ΔG (kcal/mol) | ΔH (kcal/mol) | -TΔS (kcal/mol) | σ |
| PKA-CWT | 0.16 ± 0.02 | -9.33 ± 0.07 | -13.9 ± 0.5 | 4.6 ± 0.4 | 106 ± 18 |
| PKA-CF100A | 2 ± 1 | -7.9 ± 0.3 | -17 ± 1 | 9 ± 1 | 3 ± 1 |