**Supplementary file 1. Kinetic parameters of Kemptide phosphorylation by PKA-CWT and PKA-CF100A obtained from coupled assays.** The *KM* and *Vmax* values were obtained from a nonlinear least squares analysis of the concentration-dependent initial phosphorylation rates. Errors in the *kcat*/*KM* ratios were propagated from the individual errors in *KM* and *kcat*.

|  |  |  |
| --- | --- | --- |
|  | **PKA-CWT** | **PKA-CF100A** |
| *Vmax* | 0.322 ± 0.005 | 0.379 ± 0.009 |
| *KM* | 30 ± 1 | 42 ±3 |
| *kcat* | 15 ± 1 | 17 ± 1 |
| *kcat*/*KM* | 0.50 ± 0.04 | 0.41 ± 0.08 |