Supporting Information

Molecular recognition of protein surfaces: High affinity ligands for the CBP KIX domain

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Table S1: Binding and washing conditions for selections 1 & 3[a]

Round	Target protein	Temperature (°C)	Number of washes	Wash length (min)	Buffer
1	GST-KIX	4	10	1	TBST
2	GST-KIX	4	10	5	TBST
3	GST-KIX	25	10	2	TBST
4	GST-KIX	25	10	5	TBST
5	GST-KIX	25	10	5	TBST
6	GST-KIX	25	10[b], 20[c]	5	TBST
7	GST-KIX	25	10[b], 20[c]	5	TBST + 5 mM DTT
8	GST-KIX	25	10[b], 20[c]	5	TBST + 5 mM DTT
9[b]	GST-KIX	25	10	5	TSBT + 5 mM DTT

a. Selection 1 included a PKA-catalyzed phosphorylation step in each round. In all rounds, GST-KIX (30 nM) was immobilized on glutathione-coated microtiter wells. *b*. Selection 3 only. *c*. Selection 1 only.

Table S2: Binding and washing conditions for selections 2 & 4[a]

Round	Target protein	Temperature (°C)	Number of washes	Wash length (min)	Buffer
1	GST-KIX	4	10	1	TBST
2	HisKIX	4	10	5	PBST
3	GST-KIX	4	10	5	TBST + 5 mM DTT
4	HisKIX	25	10	2	PBST
5	GST-KIX	25	10	2	TBST + 5 mM DTT

6	HisKIX	25	10	5	PBST
7	GST-KIX	25	10	5	TBST + 5 mM DTT
8	HisKIX	25	10	5	PBST
9	GST-KIX	25	10	5	TSBT + 5 mM DTT

a. Selection 2 included a PKA-catalyzed phosphorylation step in each round. GST-KIX (30 nM) was immobilized on glutathione-coated microtiter wells, and HisKIX (100 nM) was immobilized on Ni-NTA-coated microtiter wells.

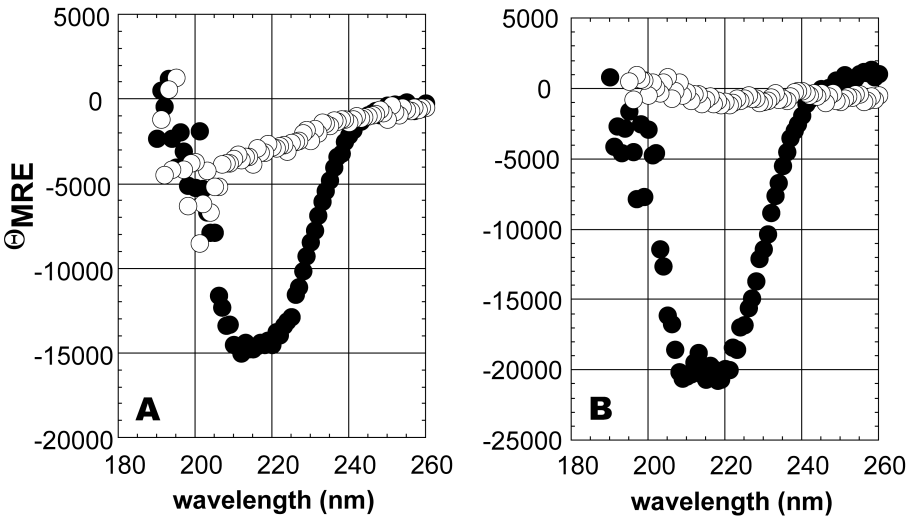

Figure S1. Circular dichroism spectra showing the mean residue ellipticity ($[\theta]_{MRE}$) of (A) PPKID4^{P} and (B) PPKID6^{U} at 10 μM concentration in buffer supplemented with 0% (open symbols) or 50% (closed symbols) trifluoroethanol. CD spectra were acquired using an AVIV Model 202 spectrometer at 4 ^{o}C in 10 mM potassium phosphate buffer (pH 7) using a 1 cm pathlength CD cell (Hellma). Spectra represent the average of 3 scans (2 s time constant, 1 nm bandwidth) and were background-corrected but not smoothed.

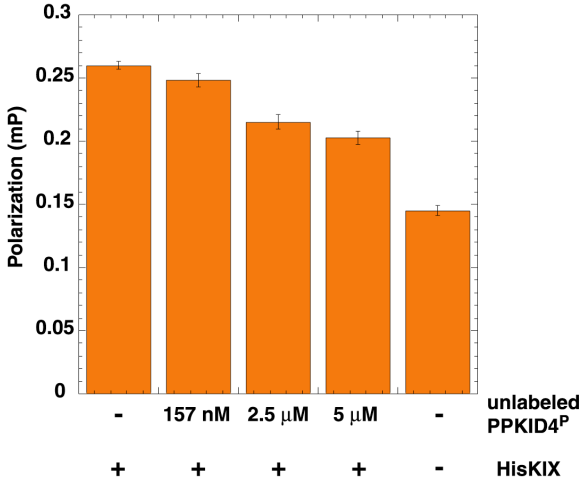

Figure S2. Figure S2. Competition between HisKIX-bound PPKID4^{P}Flu and unlabeled PPKID4^{P} monitored by fluorescence polarization. Error bars denote the standard deviation. As expected, the polarization of fluorescently labeled PPKID4^{P} increases as the concentration of unlabeled competitor decreases. Polarization of fluorescently labeled PPKID4^{P} in the absence of competitor (where 74% of PPKID4^{P} is bound by HisKIX) and in the absence of competitor and HisKIX (unbound PPKID4^{P}) are shown for reference. Although PPKID4^{P} aggregates in the absence

of CBP KIX at concentrations above 10 μM, and the change in polarization resulting from this aggregation prevents us from calculating an IC_{50} value, the data at PPKID4^{P} concentrations below 10 μM (at 5 μM and 2.5 μM) clearly show that unlabeled PPKID4^{P} competes effectively with PPKID4^{P}.