Supporting Information for:

A Fluorescent Probe for Monitoring PTP-PEST Enzymatic Activity

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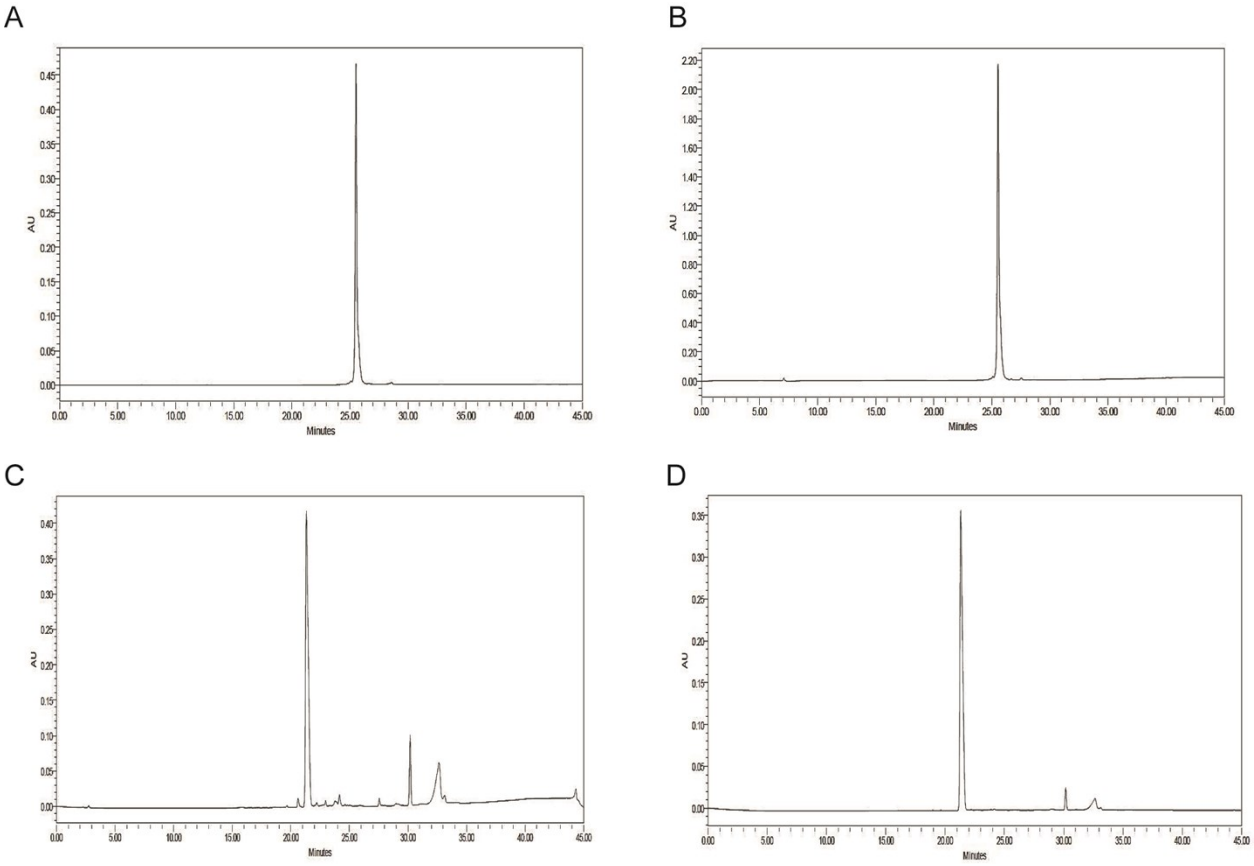
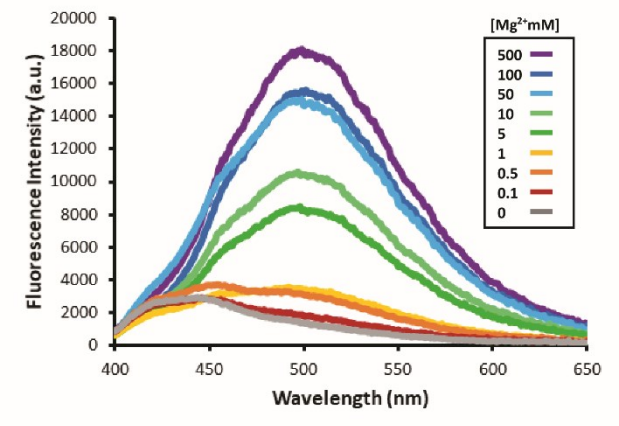
Figure S1



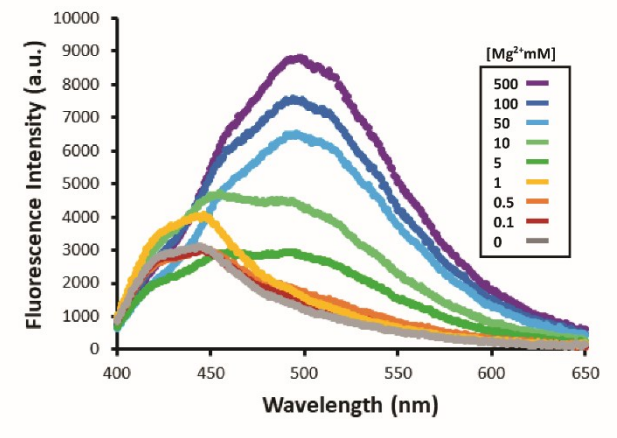
HPLC chromatograms for PTP-PEST peptides. pPEST1tide (A,B) and PEST1tide (C,D). Samples were monitored at 228 nm (A,C) and 316 nm (B,D). Buffer A is nanopure H_2O with 0.1% (v/v) TFA and buffer B is acetonitrile with 0.1% (v/v) TFA. Peptides were separated using a linear gradient of buffer B from 5 to 95% (v/v) between 5 and 35 minutes with a flow rate of 1 mL/min on a C_{18} analytical column (YMC, AA12S05-2546WT).

Figure S2



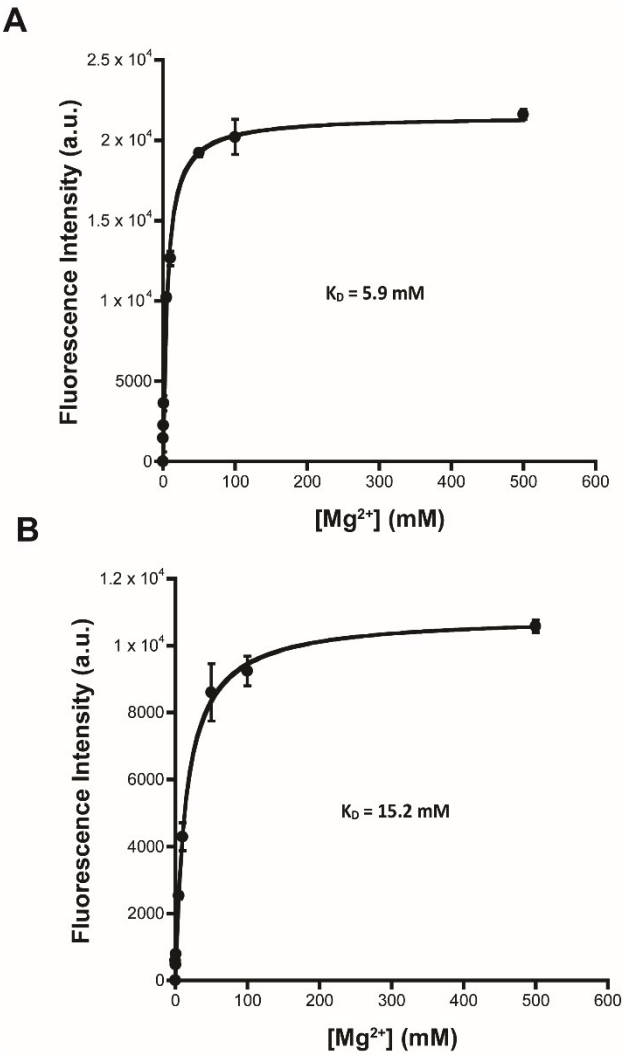
Emission spectra for pPEST1tide (1 μM) in the presence of increasing concentrations of Mg^{2+}.

Figure S3



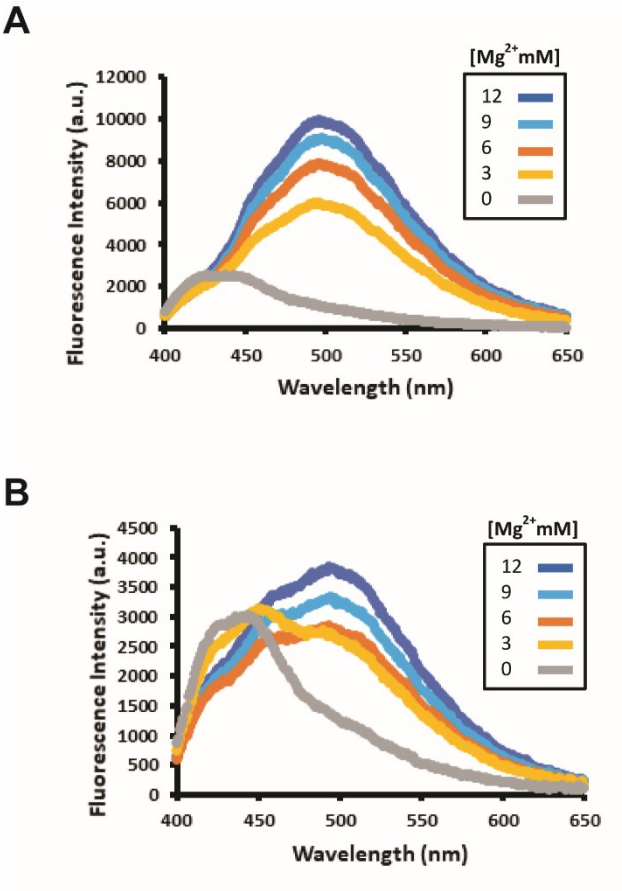
Emission spectra for PEST1tide (1 μM) in the presence of increasing concentrations of Mg^{2+}.

Figure S4



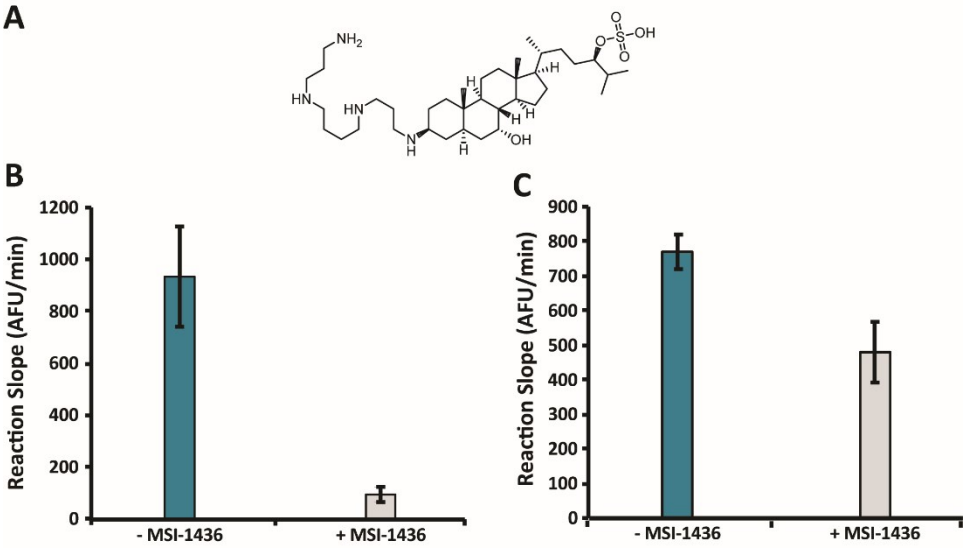
Mg^{2+} K_D determination for pPEST1tide (1 μM, A) and PEST1tide (1 μM, B). Error bars represent the standard deviation of triplicate experiments.

Figure S5



Emission spectra with increasing concentrations of Mg^{2+} for pPEST1tide (A) and PEST1tide (B). Peptides (10 μM) were incubated with the indicated concentrations of Mg^{2+}.

Figure S6



MSI-1436 selectivity inhibits PTP1B activity. (A) The structure of MSI-1436. Recombinant PTP1B (2.5 nM, B) or PTP-PEST (2.5 nM, C) in the absence or presence of MSI-1436 (10 μM) assayed with 100 μM DiFMUP. Error bars represent the standard deviation of triplicate experiments.

Table S1

ESI-MS data for PTP-PEST peptides.

Peptide	Expected Mass $(M+H)^+$	Observed Mass $(M+H)^+$
pPEST1tide	1731.7	1731.2
PEST1tide	1651.7	1652.4

Table S2

Fold fluorescence increases of pPEST1tide at varying Mg^{2+} concentrations.

$[Mg^{2+}]$ (mM)[a]	Fold Fluorescence Increase[b]
12	3.6
9	4.0
6	4.4
3	3.6
0	-

[a]Concentrations of Mg^{2+} represent 0.5, 1.0, 1.5, and 2.0 times the Mg^{2+} K_D for the substrate peptide. [b]The fold fluorescence increase is defined as the average fluorescence of the substrate peptide divided by the average fluorescence of the non-phosphorylated peptide assayed at the indicated concentration of Mg^{2+}.