Supporting Information

Table 1. Reaction constants of designed substrates and substrates reported in the literature

Peptide	**K_M or K_M^{app} (μM)**	**k_{cat}^{app} (min^{-1})**	**k_{cat}^{app}/K_M^{app} (μM^{-1} min^{-1})**	**Reference**
ERKTIDE (ATGPLSPGPFGRR)	450± 230	120± 8	0.27	35
ERKTIDE (ATGPLSPGPFGRR)	≥1500	≥336	0.24	[a]Prowse
ERKSub (TGPLSPGPF)	127 ± 17	250 ± 20	1.9	
STE7ERK	0.6 ± 0.4	170 ± 40	280	

[a]Prowse, C. N., Hagopian, J. C., Cobb, M. H., Ahn, N. G., and Lew, J. (2000) Catalytic reaction pathway for the mitogen-activated protein kinase ERK2. *Biochemistry 39*, 6258-6266.

Table 2. Reaction constants of designed substrates and protein substrates

Substrate	K_M^{app} (μM)	k_{cat}^{app} (min^{-1})	k_{cat}^{app}/K_M^{app} (μM^{-1} min^{-1})	Reference
Myelin Basic Protein	3.3 ± 0.5	63 ± 1	19	21
	55 ± 25	80 ± 19	1.5	35
	4.2 ± 0.8	600 ± 120	142	Prowse[a]
	10 ± 1.3	390 ± 25.8	39	Zhou[b]
GST-ELK1 (307-428)	1.5 ± 0.5	100 ± 20	66	21
	1.95 ± 0.2	612 ± 32.4	313	Zhou[a]
GST-AOP (480-732)	0.7 ± 0.3	80 ± 20	114	21
GST-LIN1 (281-441)	0.8 ± 0.05	40 ± 4	50	21
ELKERK	4.4 ± 3.1	140 ± 20	32	
MEK1ERK	3.7 ± 3.3	120 ± 40	32	
MEK2ERK	5.6 ± 4.9	140 ± 60	25	
STE7ERK	0.6 ± 0.4	170 ± 40	280	

Reference:

[a]Prowse, C. N., Hagopian, J. C., Cobb, M. H., Ahn, N. G., and Lew, J. (2000) Catalytic reaction pathway for the mitogen-activated protein kinase ERK2. *Biochemistry 39*, 6258-6266.

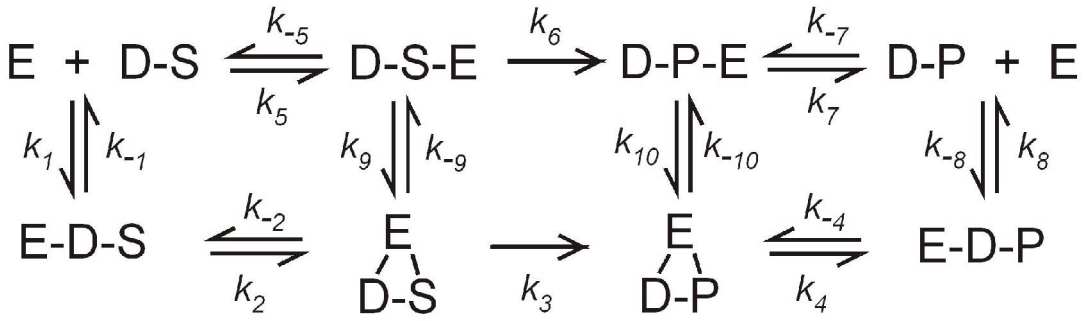
[b]Zhou, B. and Zhang, Z.Y. (2002) The Specificity of Extracellular Signal-regulated Kinase 2 Dephosphorylation by Protein Phosphatases *J. Biol. Chem.*, *27,* 13889-13899.

Validation of the IMAP Assay Using Capillary Electrophoresis (CE). A custom-designed CE instrument which consisted of an electrophoresis system equipped with a fluorescence detection system was used (Li et al, 2001). Separation of phosphorylated and nonphosphorylated peptide was performed in a 40 cm long, 50 μm inner diameter (i.d.) and 360 μm outer diameter (o.d.) fused silica capillary. An optical window in the polyamide coating of the capillary was created 23 cm from the inlet. The separation buffer was 100 mM Tris, 100 mM Tricine (pH 8). The outlet was held at a negative potential of 12 kV, and the inlet reservoir was held at ground potential. Under these conditions, the current through the capillary was typically 20 μA. The samples were loaded into the capillary by gravitational fluid flow. Prior to its use, the capillary was conditioned by sequentially washing with 0.1 M NaOH (12 h), water (4 h), 0.1 M HCl (12 h), and water (12 h). The capillary was rinsed between electrophoretic runs by washing sequentially with 0.1M NaOH, water, and separation buffer.

Reference:

Li, H., Wu, H. Y., Wang, Y., Sims, C. E., and Allbritton, N. L. (2001) Improved capillary electrophoresis conditions for the separation of kinase substrates by the laser micropipet system. *Journal of Chromatography B: Biomedical Sciences and Applications 757*, 79-88.

Scheme 1.



The abbreviations are: E, ERK1; D-S, docking peptide-substrate; E-D-S, ERK1 bound to D-S via the docking peptide only; D-S-E, ERK1 bound to D-S via the substrate only; D-P, docking peptide-phosphorylated substrate; D-P-E, ERK1 bound to D-P via the phosphorylated substrate only; E-D-P, ERK1 bound to D-P via the docking peptide only;

$\begin{matrix} E \\ / \ \backslash \\ D\text{-}S \end{matrix}$, ERK1 bound to D-S at both the docking peptide and substrate;

$\begin{matrix} E \\ / \ \backslash \\ D\text{-}P \end{matrix}$, ERK1 bound to D-P at both the docking peptide and phosphorylated substrate.

Derivation of the equation $v = \dfrac{V_{max}{}^{app}[D-S]}{1 + K_M{}^{app} * [D-S]}$ **using steady state kinetics based on Scheme1.**

Scheme 1 is a representation of the steps involved in the phosphorylation of the designed modular docking peptide.

Assumptions

1. Concentration of the modular peptide (D-S) is much greater than the total enzyme concentration (Et),i.e.[D-S]>> [Et]
2. No product is present initially.$[D\text{-}P]_{t=0}=0$
3. An added assumption is that the rate of formation of the intermediates is zero. A steady state concentration of each intermediate is achieved.
4. Since initial rates are measured when less than 10% of product is formed, it is assumed that the rates of the reverse reactions (k_{-8} and k_{-7}) are small compared to the rates of the forward reactions (k_8 and k_7) and hence can be neglected.

$$\frac{d[E-D-S]}{dt} \cong 0 = k_1[E][D-S] + k_{-2}[\Delta EDS] - k_{-1}[E-D-S] - k_2[E-D-S] \qquad (1)$$

for [ΔEDS] representing the concentration of the enzyme with both the substrate and docking peptide simultaneously bound.

$$\frac{d[\Delta EDS]}{dt} \cong 0 = k_2[E-D-S] - k_{-2}[\Delta\text{EDS}] + k_9[D-S-E] - k_{-9}[\Delta\text{EDS}] - k_3[\Delta\text{EDS}] \qquad (2)$$

$$\frac{d[D-S-E]}{dt} \cong 0 = k_5[E][D-S] - k_{-5}[D-S-E] - k_6[D-S-E] - k_9[D-S-E] \qquad (3)$$

$$\frac{d[D-P-E]}{dt} \cong 0 = k_6[D-S-E] - k_7[D-P-E] - k_{10}[D-P-E] + k_{-10}[\Delta EDP] \qquad (4)$$

$$\frac{d[\Delta EDP]}{dt} \cong 0 = k_3[\Delta EDS] + k_{-4}[E-D-P] - k_4[\Delta EDP] - k_{-10}[\Delta EDP] + k_{10}[D-P-E] \qquad (5)$$

for [ΔEDP] representing the concentration of the enzyme with the phosphorylated substrate and docking peptide simultaneously bound.

$$\frac{d[E-D-P]}{dt} \cong 0 = k_4[\Delta EDP] - k_{-4}[E-D-P] - k_8[E-D-P] \qquad (6)$$

Solving equations 1, 2, 3, 4, 5 and 6

$$[E-D-P] = \frac{k_4}{(k_8 + k_{-4})}[\Delta EDP]$$

$$[D-S-E] = \frac{k_5}{(k_6 + k_9 + k_{-5})}[E][D-S] + \frac{k_{-9}}{(k_6 + k_9 + k_{-5})}[\Delta EDS]$$

$$[D-P-E] = \frac{k_6 * k_5}{(k_7 + k_{10})(k_6 + k_9 + k_{-5})}[E][D-S] + \frac{k_{-9} * k_6}{(k_7 + k_{10})(k_6 + k_9 + k_{-5})}[\Delta EDS] + \frac{k_{-10}}{(k_7 + k_{10})}[\Delta EDP]$$

$$[E-D-S]=\frac{k_1}{(k_{-1}+k_2)}[E][D-S]+\frac{k_{-2}}{(k_{-1}+k_2)}[\Delta EDS]$$

$$[\Delta EDS]=\frac{\{\frac{k_2*k_1}{(k_{-1}+k_2)}+\frac{k_9*k_5}{(k_6+k_9+k_{-5})}\}}{\{(k_3+k_{-9}+k_{-2})-\frac{k_2*k_{-2}}{(k_{-1}+k_2)}-\frac{k_9*k_{-9}}{(k_6+k_9+k_{-5})}\}}[E][D-S]$$

$$[\Delta EDS]=\mathrm{A}[E][D-S] \text{ where } \mathrm{A}=\frac{\{\frac{k_2*k_1}{(k_{-1}+k_2)}+\frac{k_9*k_5}{(k_6+k_9+k_{-5})}\}}{\{(k_3+k_{-9}+k_{-2})-\frac{k_2*k_{-2}}{(k_{-1}+k_2)}-\frac{k_9*k_{-9}}{(k_6+k_9+k_{-5})}\}}$$

$$[E-D-P]=\mathrm{B}[\Delta EDP] \text{ where } \mathrm{B}=\frac{k_4}{(k_8+k_{-4})}$$

$$[D-S-E]=\mathrm{C}[E][D-S] \text{ where } \mathrm{C}=[\frac{k_5+\mathrm{A}*k_{-9}}{(k_6+k_9+k_{-5})}]$$

$$[D-P-E]=\mathrm{F}[E][D-S]+\mathrm{G}[\Delta EDP] \text{ where } \mathrm{F}=[\frac{k_6*k_5+\mathrm{A}*k_{-9}*k_6}{(k_7+k_{10})(k_6+k_9+k_{-5})}]$$

$$\text{and } \mathrm{G}=\frac{k_{-10}}{(k_7+k_{10})}$$

$$[E-D-S]=\mathrm{H}[E][D-S] \text{ where } \mathrm{H}=\{\frac{k_1+\mathrm{A}*k_{-2}}{(k_{-1}+k_2)}\}$$

$$[\Delta EDP]=\mathrm{J}[E][D-S] \text{ where } \mathrm{J}=\frac{\mathrm{A}*k_3+\mathrm{F}*k_{10}}{(k_4+k_{-10}-\mathrm{B}*k_{-4}-\mathrm{G}*k_{10})}$$

Rate of formation of product D-P = v

$$v = k_7[D-P-E] + k_8[E-D-P]$$

$$v = (k_7 * \mathrm{F} + k_7 * \mathrm{G} * \mathrm{J} + k_8 * \mathrm{B} * \mathrm{J})[E][D-S]$$

$$v = \mathrm{L}[E][D-S] \text{ where } \mathrm{L} = (k_7 * \mathrm{F} + k_7 * \mathrm{G} * \mathrm{J} + k_8 * \mathrm{B} * \mathrm{J})$$

Total enzyme concentration = $[Et]$

$$[E] = [Et] - [D-S-E] - [D-P-E] - [E-D-P] - [E-D-S] - [\Delta EDS] - [\Delta EDP]$$

$$[E] = [Et] - [D-S-E] - [D-P-E] - [E-D-P] - [E-D-S] - [\Delta EDS] - [\Delta EDP]$$

$$[E] = [Et] - \mathrm{C}[E][D-S] - \mathrm{F}[E][D-S] + \mathrm{G} * \mathrm{J}[E][D-S] - \mathrm{B} * \mathrm{J}[E][D-S] - \mathrm{H}[E][D-S]$$
$$- \mathrm{J}[E][D-S] - \mathrm{A}[E][D-S]$$

$$[Et] = [E]\{1 + (\mathrm{C} + \mathrm{F} + \mathrm{G} * \mathrm{J} + \mathrm{B} * \mathrm{J} + \mathrm{H} + \mathrm{J} + \mathrm{A})[D-S]\}$$

$$\frac{v}{[Et]} = \frac{L[E][D-S]\_}{[E]\{1 + K_M{}^{app}[D-S]\}}$$

$$v = \frac{\mathrm{V_{max}}^{\mathrm{app}}[D-S]}{1 + \mathrm{K_M}^{\mathrm{app}}[D-S]} \quad \text{where } \mathrm{K_M}^{\mathrm{app}} = (\mathrm{C} + \mathrm{F} + \mathrm{G} * \mathrm{J} + \mathrm{H} + \mathrm{J} + \mathrm{A})$$

$$\mathrm{V_{max}}^{\mathrm{app}} = \mathrm{L}[Et]$$