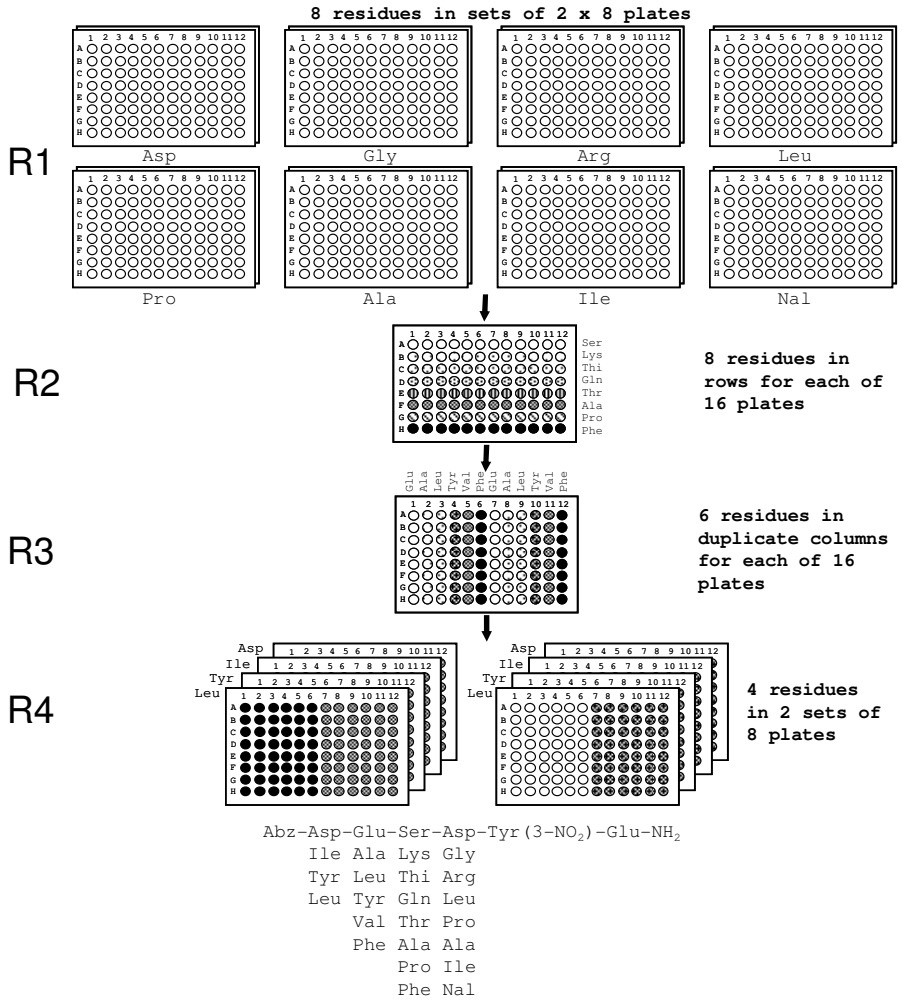
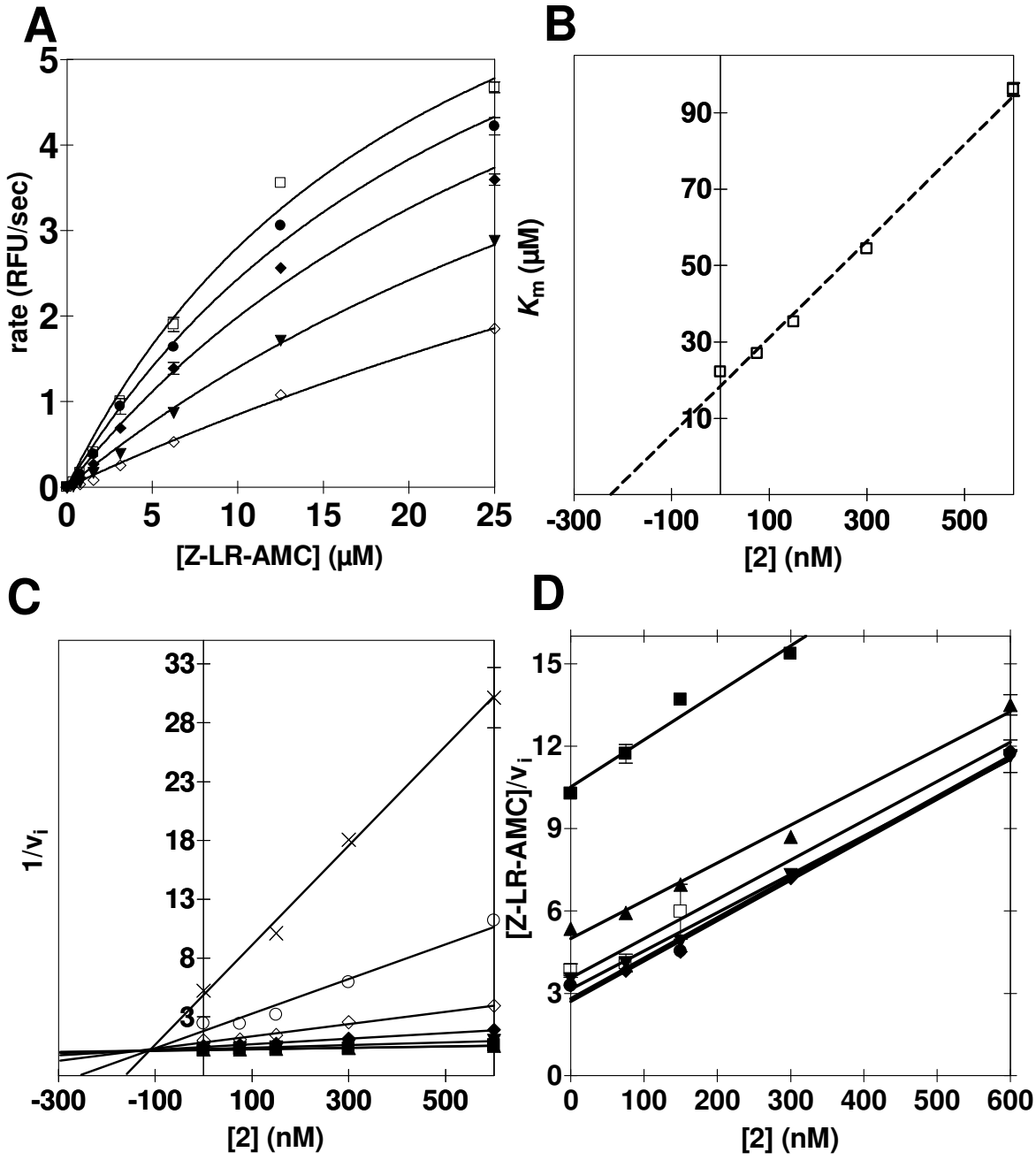
SUPPLEMENTARY INFORMATION



Supplementary Figure 1 Synthesis of the spatially addressed of 1536-membered FRET substrate library

Gears were subjected to four rounds of divergent combinatorial expansion to yield a 1536 member library distributed in sixteen 96-well microtitre plates. Synthesis was based on the 96-well microtiter plate format, the first round was composed of sixteen lots of ninety six gears elaborated by two sets with eight residues which corresponded to R1 (*i.e.* Asp, Gly, Arg, Leu, Pro, Ala, Ile and Nal). The next round of synthesis expanded the synthesis by eight residues arranged along each row of each plate and corresponded to R2 (*i.e.* Ser, Lys, Thi, Gln, Thr, Ala, Pro and Phe). The third round of synthesis expanded the synthesis by six residues arranged in two sets (columns 1-6 and 7-12) down each column of each plate and corresponding to R3 (*i.e.* Glu, Ala, Leu, Tyr, Val and Phe). The fourth round of synthesis was based on four residues corresponding to R4 (*i.e.* Asp, Ile, Tyr and Leu). The final library synthesis step involved capping with Boc-2-Abz-OH (Neosystems) to yield a library of a general sequence, after cleavage and de-protection, of Abz-$R4_{1-4}$-$R3_{1-6}$-$R2_{1-8}$-$R1_{1-8}$-Tyr(3-NO_2)-Glu-NH_2.



Supplementary Figure 2 Demonstration of competitive inhibition of FP-2 by compound 2

A: a plot of the dependence of Z-LR-AMC turnover by FP-2 in the presence of various concentrations of compound **2**, the solid lines represent the non-linear regression analysis of the data using the equation $v_i=(V_{max}.[S_o])/([S_o]+(K_m))$ (see EXPERIMENTAL; [30]). **B**: a plot of the K_m values, determined at various inhibitor concentrations, versus compound **2** concentrations. The dotted line represents a linear regression analysis of the data. **C**: a plot of the reciprocal of the initial velocity (v_i) versus compound **2** concentration at various substrate concentrations [30]. **D**: a plot of the substrate concentration divided by initial velocity (v_i) versus compound **2** concentration at various substrate concentrations [30].

	Relative activity	Synthesis round R4	R3	R2	R1
All	-	Xaa	Xaa	Pro	Xaa
	-	Asp/Ile	Xaa	Xaa	Asp/Pro
FP-2					
	+++++	**Leu**	**Glu**	**Thi**/Thr/Ala/**Phe**	**Pro**
	++	Tyr	Glu	Thi/Thr/Ala/Phe	Pro
	-	Leu/Tyr	Val	Thi/Thr/Ala/Phe	Pro
	++++	Ile	Leu	Lys	Gly
	-	Xaa	Val	Lys	Xaa
	+++	**Leu**/Tyr	**Glu**	Ser/Lys/**Thi**/Ala/Phe	**Gly**/**Ala**
	-	Leu/Tyr	Val	Ser/Lys/Thi/Ala/Phe	Gly/Ala
	-	Asp	Glu	Ser/Lys/Thi/Ala/Phe	Gly/Ala
	+++	**Leu**/Tyr	**Glu**	Ser/**Lys**/Thi/Ala/Phe	Arg/**Ile**
	-	Leu/Tyr	Val	Ser/Lys/Thi/Ala/Phe	Arg/Ile
	+++	**Leu**/Tyr	**Glu**	Ser/**Lys**/Gln/Thr/Ala	Leu/**Nal**
	-	Leu/Tyr	Val	Ser/Lys/Gln/Thr/Ala	Leu/Nal
FP-3					
	++++	**Leu**/Tyr	Leu/**Tyr**	**Lys**/(**Thi**, Thr)	**Ala**/Gly
	-	Leu/Tyr	Leu/Tyr	Lys	Asp/Pro
	-	Leu/Tyr	Glu/Ala/Val	Lys	Ala/Gly
	++	Leu/Tyr	Leu/Tyr	Lys/Gln	Arg
	-	Leu/Tyr	Leu/Tyr	Lys	Ile
	-	Leu/Tyr	Glu/Ala/Val	Lys	Arg
	++++	Asp/**Ile**/Tyr/**Leu**	Leu/**Tyr**	**Lys**/(Thr)	**Leu**
	-	Asp/Ile/Tyr/Leu	Leu/Tyr	Lys/(Thr)	Nal
BP-2					
	++++	**Asp**/**Ile**/**Tyr**/Leu	Leu/**Val**	**Thi**/**Thr**	**Gly**
	-	Asp/Ile/Tyr/Leu	Leu/Val	Thi/Thr	Asp/Pro/Ile/Nal
	+++++	**Ile**	Leu/(**Val**)	**Lys**	*Ala*
	-	Asp/Tyr/Leu	Leu/(Val)	Lys	Ala
	++	Ile	Leu	Lys/Gln/Ala	Arg
	+++	Leu	Leu	Thi	Arg
	-	Leu	Leu	Thi	Ile
	+++	Ile	Leu	Lys	Leu

Supplementary Table 1 Global assessment of the general FRET substrate preferences of FP-2, FP-3 and BP-2

The relative peptide cleavage activity was expressed in arbitrary terms on a sliding scale ranging from no activity (-) to varying degrees of increasing activity (+) based on the rates observed in the FRET substrate screen. Xaa equated to a general tolerance for more than one residue represented in the library (Supplementary Figure 1). Residue combinations producing selective substrates for FP-2 (bold), FP-3 (white on black background) and BP-2 (bold underlined) have been highlighted. R1, R2, R3 and R4 denote the four rounds of split-and-mix library synthesis (Supplementary Figure 1).