Electronic Supplementary Information For:

Identification of Key Active Residues and Solution Conditions that Affect Peptide-Catalyzed Ester Hydrolysis

Kyle B. Meerbott[a] *and Marc R. Knecht,*[a,b,]*

[a]Department of Chemistry, University of Miami, 1301 Memorial Drive, Coral Gables, Florida 33146, United States,

[b]Dr. J.T. Macdonald Foundation Biomedical Nanotechnology Institute, University of Miami, UM Life Science Technology Building, 1951 NW 7th Ave, Suite 475, Miami, Florida, 33136, United States

*To whom correspondence should be addressed: MRK: Phone: (305) 284-9351, email: knecht@miami.edu

pH	CPN3 (× 10^{-3})	AuBP1(× 10^{-3})	No-Peptide (× 10^{-3})
6.5	0.22 ± 0.20	0.26 ± 0.24	0.04 ± 0.12
7	0.68 ± 0.14	0.22 ± 0.09	0.26 ± 0.09
7.42	1.5 ± 0.007	0.7 ± 0.2	0.6 ± 0.2
8	3.1 ± 0.2	2.0 ± 0.3	2.0 ± 0.3

Buffer	Peptide (× 10^{-3})	Control (× 10^{-3})	Net (× 10^{-3})
Phosphate	3.1 ± 0.2	2.0 ± 0.3	1.1 ± 0.4
Tris	2.5 ± 0.3	1.4 ± 0.1	1.1 ± 0.3
HEPES	2.8 ± 0.1	1.8 ± 0.1	1.0 ± 0.1
MOPS	2.7 ± 0.2	1.7 ± 0.2	1.0 ± 0.3

Substrate	*k* (× 10^{-3})	Control (× 10^{-3})	Net *k* (× 10^{-3})
4-pNPA	3.6 ± 0.1	2.4 ± 0.2	1.2 ± 0.2
4-pNPB	7.5 ± 0.4	5.7 ± 0.5	1.8 ± 0.6
Indoxyl Acetate	2.3 ± 0.6	2.4 ± 0.1	-0.1 ± 0.6
4-pNPA_DMF	1.5 ± 0.04	0.89 ± 0.07	0.57 ± 0.08
4-pNPP_DMF	0.8 ± 0.3	0.42 ± 0.06	0.4 ± 0.3

Peptide	*k* (× 10^{-3})
CPN3	3.7 ± 0.1
CPN3-S4A	2.5 ± 0.1
CPN3-T6A	3.5 ± 0.1
CPN3-S4A,T6A	3.1 ± 0.1
CPN3-DTNB	1.7 ± 0.2
Control-DTNB	1.8 ± 0.1
Control	2.2 ± 0.3

Figure S1. Various *k* values reported for the indicated experiment.