Supplemental Material

Analysis of tBoc-LysGMP-MCA Synthesis

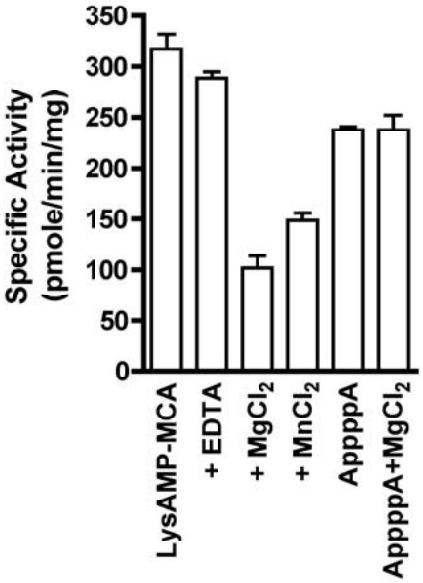
^{1}NMR (500mHz, D_2O, 40^{o}C) δ 7.83 (d, *J*=2.0 Hz, 1H), 7.95 (s, 1H), 7.77 (d, *J*=8.6 Hz, 1H), 7.53 (d, *J*=8.8 Hz, 1H), 7.29 (d, *J*=7.5 Hz, 1H), 6.26 (d, *J*=1.3 Hz, 1H), 5.78 (d, *J*=4.9 Hz, 1H), 4.57-4.61 (m, 1H), 4.34-4.38 (m, 1H), 4.15-4.19 (m, 1H), 3.85-4.01 (m, 4H), 2.61-2.73 (m, 2H), 1.78 (s, 3H), 1.58-1.69 (m, 2H), 1.51-1.55 (m, 2H), 1.35-1.45 (m, 9H), ^{31}P NMR (300mHz, DMSO-d_6) δ =-0.062

Negative polarity MALDI: peak at 749.01, calculated peak 750.

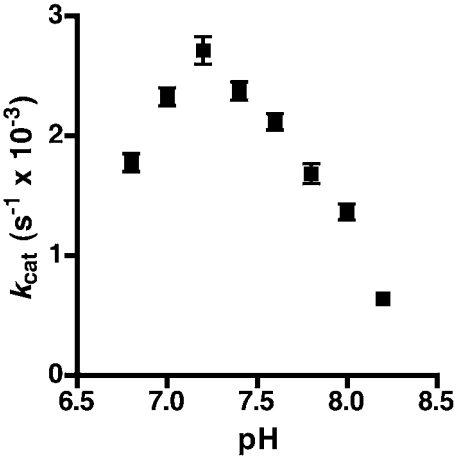
Supplementary Table 1. Primers used in this study.

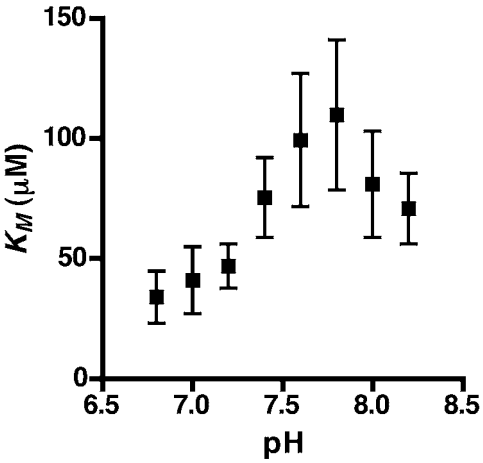
Primer Name	Primer Sequence (Primers are 5'-3')	Mutation
7024	GCGCGGCGTAGCATGGCGCAACGCATGGCTATTTGAA AAAGGAAAATGTCTGTAGGCTGGAGCTGCTTC	
7025	ATGATCAGACGATAGCCATCTTCGGCAATACCTTCTTG CTCAGCAATTTTGAATATCCTCCTTAGTTCC	
7026	GGATGTGGTAAACCTCTTGTC	
7027	CTCGACCATACTCTATACTCG	
7779	CAAGGCTTGAAGATTTCATATGCAGGACCCCAAA	NdeI site
0720	GATTCATATGCAGGACCC	Aptx-168
0721	GGGCTCGAGTCACTGTG	Aptx-168
0730	GATAAATACCCACAGGCCCG	K197Q
0731	CGGGCCTGTGGGTATTTATC	K197Q
0722	CCCAAAGGTCCGTTACC	A198V
0723	GGTAACGGACCTTTGGG	A189V
0732	CAAAGGCCCATTACCATTGGC	R199H
0733	GCCAATGGTAATGGGCCTTTG	R199H
7780	CATTGGCTGGTCTTACTGTGGACCTCCATTTCC	P206L
7773	AGTATGAGCCATGTAGCTCTTCATGTGATCAGC	H260A
0734	CATCTTCATGGGATCAGCCAG	V263G
0735	CTGGCTGATCCCATGAAGATG	V263G
0728	CAGCCAGGGTTTTGATTC	D267G
0729	GAATCAAAACCCTGGCTG	D267G
7781	AAAAACAAAAAACATAGGAATTCTTTCAATACA	W279R
7782	AAAAACAAAAAACATTAGAATTCTTTCAATACA	W279X
0724	GCACACTGTTGGGGGAAAAG	689insT
0725	CTTTTCCCCCAACAGTGTGC	689insT
0726	CATTGGAATCTTTCAATAC	840delT
0726	GTATTGAAAGATTCCAATG	840delT

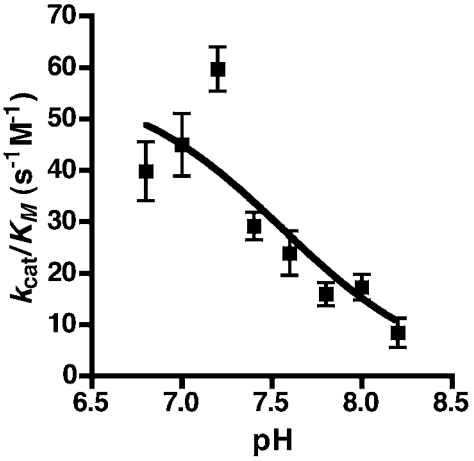
Supplemental Figure 1. Effects of metals on Aprataxin with tBoc-LysAMP-MCA and AppppA. tBoc-LysAMP-MCA hydrolysis was assayed in 25 µl volume containing 12 nmol of enzyme, 10 mM HEPES pH 7.2, 100 mM NaCl and 3mM EDTA, 1 mM $MgCl_2$ or 1 mM $MnCl_2$ for 30 min at room temperature. Fluorescence was counted following a 10 min trypsin digestion. AppppA hydrolysis was assayed using 60 nmol of wild-type enzyme with 5-200 µM substrate with or without 0.5 mM $MgCl_2$ in a 50 µL volume. Assays were incubated at room temperature for 30 min and stopped by addition of 50 µL Na_2CO_3, pH 11.6. 50 µL of the reaction was analyzed by HPLC.



Supplemental Figure 2. pH profiles of Aprataxin with tBoc-LysAMP-MCA. a) k_{cat}; b) K_M and c) k_{cat}/K_M. tBoc-LysAMP-MCA activity was assayed in a 25 µl volume containing 12 nmol of enzyme, 10 mM HEPES pH 6.8-8.2, 100 mM NaCl, for 30-60 min at room temperature. Kinetic values were determined from Michaelis-Menten plots using non-linear regression.


3
2
1
0
k_{cat} (s^{-1} x 10^{-3})
6.5
7.0
7.5
8.0
8.5
pH


150
100
50
0
K_M (μM)
6.5
7.0
7.5
8.0
8.5
pH


70
60
50
40
30
20
10
0
k_{cat}/K_M ($s^{-1}M^{-1}$)
6.5
7.0
7.5
8.0
8.5
pH