Supporting Information

McGowan et al. 10.1073/pnas.0911813107

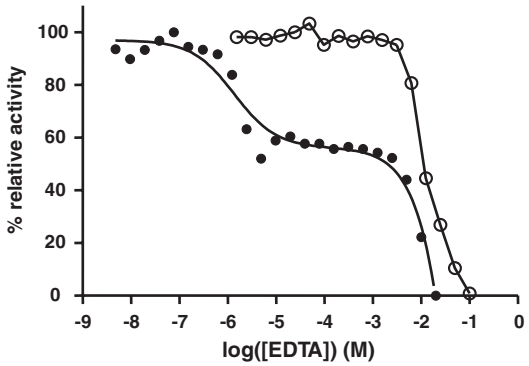

Fig. S1. Metal binding of *Pfl*A-M1 (*Open Circle*) versus *Pf*A-M17 (*Closed Circle*) (1). Graph shows relative enzymatic activity (%) against increasing EDTA concentration (M).

1 Maric S, et al. (2009) The M17 leucine aminopeptidase of the malaria parasite *Plasmodium falciparum*: Importance of active site metal ions in the binding of substrates and inhibitors. *Biochemistry* 48(23):5435–5439

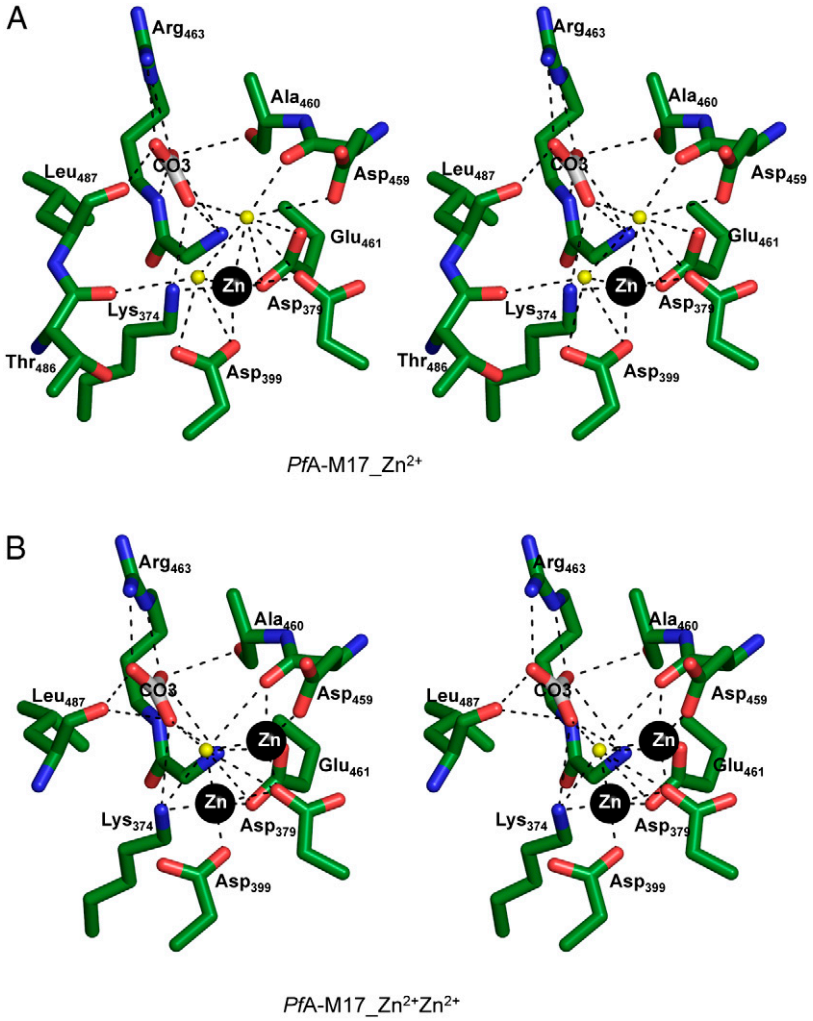

Fig. S2. Stereo diagrams of the active sites of 2.0 Å *Pf*A-M17_Zn^{2+} (*A*) and 2.4 Å *Pf*A-M17_$Zn^{2+}Zn^{2+}$ (*B*). X-ray crystal structures show the change in active site metals and metal coordination. Carbon atoms of residues are colored by green. Water molecules are shown as yellow spheres. Hydrogen and metal bonds are indicated (*Dashed Lines*). Zinc (Zn) is shown as black spheres, and carbon atom of carbonate ion (CO3) is colored grey.

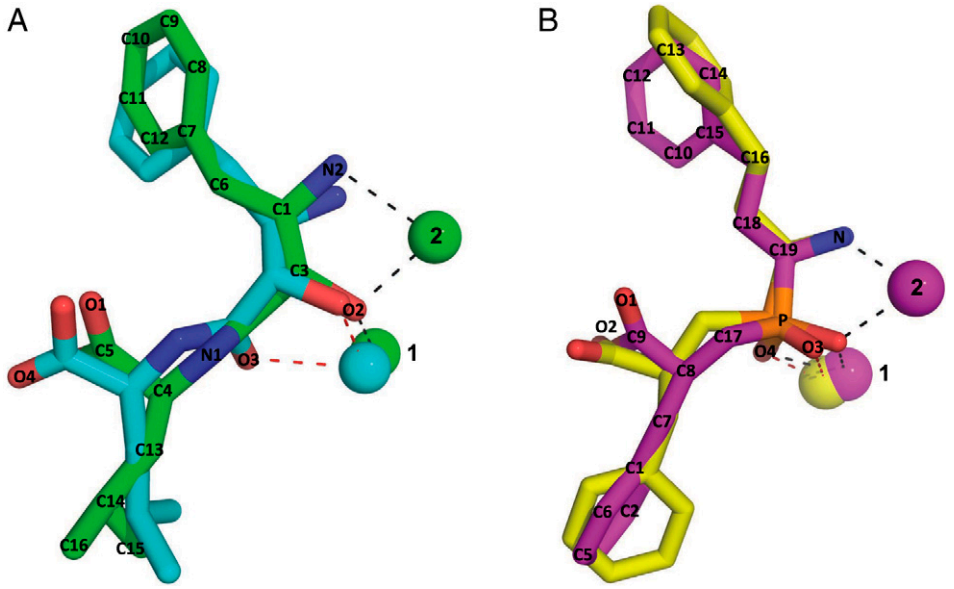

Fig. S3. Sidechain rotamers of P1/P1′ positions of neutral aminopeptidase inhibitors. Carbon and metal atoms of bestatin (*A*) are colored in cyan (*Pf*A-M1) and green (*Pf*A-M17), and carbon and metal atoms of Co4 (*B*) are shown in yellow (*Pf*A-M1) and magenta (*Pf*A-M17). Black dashed lines indicate protein-metallo bonds for *Pf*A-M17 and red for *Pf*A-M1.

Table S1. Data Collection and refinement statistics

Data collection	*Pf*A-M17_Zn^{2+}	*Pf*A-M17_$Zn^{2+}Zn^{2+}$	*Pf*A-M17-BES	*Pf*A-M17-Co4
Space Group	$P2_12_12_1$	$P2_12_12_1$	$P2_12_12_1$	$P2_12_12_1$
Cell dimensions (Å)	a=173.8, b=176.3, c=222.9,	a=171.7, b=173.7, c=220.3,	a=173.6, b=178.1, c=230.5	a=172.1, b=174.2, c=227.7
Resolution (Å)	57.3 – 2.0 (2.11 – 2.00)	56.1 – 2.4 (2.53 – 2.4)	62.1 – 2.0 (2.11 – 2.0)	78.9 – 2.6 (2.74 – 2.60)
Total number of observations	11156121	3662103	6476290	2662653
Number of unique observations	455194	256048	469417	217885
Multiplicity	24.5 (24.5)	14.3 (14.6)	13.8 (11.8)	12.2 (12.4)
Data Completeness (%)	100.0 (100.0)	100.0 (100.0)	98.5 (96.6)	99.9 (100.0)
<I/ _I>	11.1 (2.6)	5.8 (2.4)	11.3 (2.5)	4.1 (2.1)
R_{pim} (%)[b]	7.5 (31.0)	10.1 (33.9)	6.9 (29.1)	16.4 (38.4)
Pseudo-translation (vector)	42.0% (0.011, 0.000, 0.500)	40.3% (0.012, 0.000, 0.500)	51.1% (0.015, 0.000, 0.500)	39.1% (0.017, 0.000, 0.500)
Structure refinement				
Non hydrogen atoms				
Protein	47964	47946	48146	48161
Solvent (HOH/PEG/SO4)	4744	3585	5747	3323
Metal	12	24	24	24
Ligand	-	-	252	28.8
R_{free} (%)	23.1	24.2	24.2	27.7
R_{cryst} (%)	18.3	17.6	19.2	22.0
Rms deviations from ideality				
Bond lengths (Å)	0.018	0.017	0.018	0.013
Bond angles (°)	1.56	1.71	1.63	1.46
Ramachandran plot				
Favoured (%)	98.3	97.3	98.2	96.7
Allowed (%)	99.9	99.9	99.9	99.8
B factors (Å²)				
Mean main chain	20.3	24.1	16.3	29.0
Mean side chain	23.3	26.4	18.4	30.9
Mean ligand	-	-	28.3	40.5
Mean water molecule	26.1	29.8	24.7	21.5
r.m.s.d. bonded Bs				
Main chain	1.07	1.19	0.66	1.08
Side chain	3.53	4.06	1.98	2.99

[a]Values in parentheses refer to the highest resolution shell.
[b]Agreement between intensities of repeated measurements of the same reflections and can be defined as: $\sum(I_{h,i} - \langle I_h \rangle)/\sum I_{h,i}$, where $I_{h,i}$ are individual values and $\langle I_h \rangle$ is the mean value of the intensity of reflection h.

Table S2. *Pf*A-M17-ligand interactions

Metal Core: Metal position	Bestatin (Mg^{2+}/Zn^{2+})	Co4 (Zn^{2+}/Zn^{2+})	Type of bond
Loose (1)	Asp_{379} O_D (r*Pf*A-M17)	Asp_{379} O_D (r*Pf*A-M17)	metal-protein
Loose (1)	Asp_{459} O_D (r*Pf*A-M17)	Asp_{459} O_D (r*Pf*A-M17)	metal-protein
Loose (1)	Asp_{459} O (r*Pf*A-M17)	Asp_{459} O (r*Pf*A-M17)	metal-protein
Loose (1)	Glu_{461} Oε (r*Pf*A-M17)	Glu_{461} Oε (r*Pf*A-M17)	metal-protein
Loose (1)	BES O_2	Co4 O_3	metal-ligand
Loose (1)	—	Co4 O_4	metal-ligand
Tight (2)	Lys_{374} N_z(r*Pf*A-M17)	Lys_{374} N_z (r*Pf*A-M17)	metal-protein
Tight (2)	Asp_{379} O_D (r*Pf*A-M17)	Asp_{379} O_D (r*Pf*A-M17)	metal-protein
Tight (2)	Asp_{399} O_D (r*Pf*A-M17)	Asp_{399} O_D (r*Pf*A-M17)	metal-protein
Tight (2)	Glu_{461} Oε (r*Pf*A-M17)	Glu_{461} Oε (r*Pf*A-M17)	metal-protein
Tight (2)	BES O_2	Co4 O_4	metal-ligand
Tight (2)	BES N_2	Co4 N	metal-ligand
Protein-ligand			
*Pf*A-M17	Bestatin	Co4	Type of bond
Lys_{374} N_z	BES N_2/O_2	Co4 N/O_4	H-bond
Lys_{386} N_z	BES O_3	Co4 O_3	H-bond
Asp_{379} O_D	BES O_2	—	H-bond
Asp_{399} O_D	BES N_2	Co4 N	H-bond
Asp_{459} O_D/O	BES O_2/O_3	Co4 O_3/O_4	H-bond
Glu_{461} Oε	BES O_2	Co4 O_4	H-bond
Thr_{486} O	BES N_2	—	H-bond
Leu_{487} O	BES N_1	—	H-bond
Gly_{489} N	BES O_4	Co4 O_1	H-bond
$CO3_{1002}$ O2	BES O_2	Co4 O_4	H-bond
$CO3_{1002}$ O3	BES N	—	H-bond
WAT HOH	BES O_4	Co4 O_1	H-bond
WAT HOH	BES O_1	—	H-bond
Met_{392}	BES P1 (Phe)	Co4 P1	vdw
Met_{396}	BES P1 (Phe)	Co4 P1	vdw
Phe_{398}	BES P1 (Phe)	Co4 P1	vdw
Asn_{457}	BES P1′ (Leu)	Co4 P1′	vdw
Leu_{487}	BES C_6	Co4 C_{17}	vdw
Thr_{488}	—	Co4 C_{17}	vdw
Gly_{489}	BES P1 (Phe)	Co4 P1	vdw
Leu_{492}	—	Co4 P1	vdw
Ile_{547}	BES P1′ (Leu)	—	vdw
Ala_{577}	BES P1 (Phe)	Co4 P1	vdw

Table S3. Kinetic parameters for the hydrolysis of fluorogenic peptide substrates for *Pf*A-M1 (2) and *Pf*A-M17 (3)

	k_{cat} x 10^3 (s^{-1})		K_m (mM)		k_{cat}/K_m($M^{-1}s^{-1}$)	
Enzyme	*Pf*A-M1	*Pf*A-M17	*Pf*A-M1	*Pf*A-M17	*Pf*A-M1	*Pf*A-M17
H-Leu-NHMec	1.52	39	329.9	12.12	4607	3218
H-Phe-NHMec	0.18	1.45	194.8	7.92	924	183.1
H-Pro-NHMec	0.0032	0.59	734.4	234.91	4	2.51
H-Ala-NHMec	2.04	0.2	888.9	52.9	2295	3.78
H-Arg-NHMec	1.07	—	717.4	NC	1491	—
H-Gly-NHMec	0.116	—	348.6	NC	333	—
H-Val-NHMec	0.036	—	1068.1	NC	34	—
H-Ile-NHMec	0.04	—	1706	NC	23	—
H-Asp-NHMec	—	—	NC	NC	—	—
H-Glu-NHMec	—	—	NC	NC	—	—

NC equals Amino acids not cleaved (even at substrate concentrations of greater than 200 uM).

2 McGowan S et al. (2009) Structural basis for the inhibition of the essential Plasmodium falciparum M1 neutral aminopeptidase. *Proc Natl Acad Sci USA* 106(8):2537–2542

3 Stack CM et al. (2007) Characterization of the Plasmodium falciparum M17 leucyl aminopeptidase. A protease involved in amino acid regulation with potential for antimalarial drug development. *J Biol Chem* 282(3):2069–2080.

Table S4. Comparison of inhibitor interactions with *Pf*A-M1 (2) and *Pf*A-M17

	Bestatin		Co4	
Enzyme	*Pf*A-M17	*Pf*A-M1 (2)	*Pf*A-M17	*Pf*A-M1 (2)
K_i (nM)	25 (4)	478.2	13 (4)	79
Metallo-bonds	11	5	12	5
H-bonds	12	7	8	5
vdw interactions	9	7	8	10
BSA (Å^2)	44.2	23.8	195.1	62.2

2 McGowan S et al. (2009) Structural basis for the inhibition of the essential Plasmodium falciparum M1 neutral aminopeptidase. *Proc Natl Acad Sci USA* 106(8):2537–2542
4 Skinner-Adams TS et al. (2007) Identification of phosphinate dipeptide analog inhibitors directed against the *Plasmodium falciparum* M17 leucine aminopeptidase as lead antimalarial compounds. *J Med Chem* 50(24):6024–6031.