SUPPORTING INFORMATION

Catalytic Mechanism of Cruzain from *Trypanosoma cruzi* as Determined from Solvent Kinetic Isotope Effects of Steady-State and Pre-Steady-State Kinetics[‡]

Xiang Zhai[§] and Thomas D. Meek*

Department of Biochemistry and Biophysics, Texas A&M University, College Station, TX 77843, USA

[‡]This work was supported by Texas A&M AgriLife Research and National Institutes of Health Grant R21AI127634.

[§]Current address: Discovery Sciences, AstraZeneca R&D Boston, 35 Gatehouse Drive, Waltham, MA 02451

*Author to whom correspondence should be addressed

Phone: (979) 458 9787

Email: tdmeek@tamu.edu

Running Title: Catalytic Mechanism of Cruzain

Table S1-S3 Steady-state Kinetic Data for Wildtype Cruzain-catalyzed Reactions of Z-FR-AMC, Z-RR-AMC and Z-RA-AMC in H_2O and D_2O.

Table S4-S6 Steady-state Kinetic Data for E208A Mutant Cruzain-catalyzed Reactions of Z-FR-AMC, Z-RR-AMC and Z-RA-AMC in H_2O.

Table S7 p*K* Values Determined from Fitting the pH-rate Profiles Data for E208A Mutant of Cruzain According to Eq. 5.

Figure S1-S3 pH-rate Profiles for the E208A mutant of cruzain with Z-FR-AMC, Z-RR-AMC and Z-RA-AMC in H_2O. The dashed line represents data fitted according to eq. 4. The solid line represents data fitted to eq. 5 as described in the text, with the results summarized in Tables S1.

Figure S4-S5 Pre-steady-state Data and the Replots of Pre-steady-state Kinetic Constants for Cruzain-catalyzed Reactions of Z-RR-AMC in H_2O and D_2O.

Table S1. pH-rate Profile and Solvent Isotope Effects of Wildtype Cruzain with Z-FR-AMC.

pH	k_{cat} (s^{-1})	K_m (μM)	k_{cat}/K_m ($M^{-1}s^{-1}$)
3.5	13 ± 1.6	5.2 ± 0.3	$(2.5 \pm 0.4) \times 10^6$
4.1	13 ± 1.3	1.9 ± 0.3	$(6.8 \pm 0.8) \times 10^6$
4.4	14 ± 1.4	2.5 ± 0.2	$(5.6 \pm 0.9) \times 10^6$
5.0	14.1 ± 0.8	1.3 ± 0.1	$(1.1 \pm 0.2) \times 10^7$
5.6	13.6 ± 0.7	0.87 ± 0.1	$(1.6 \pm 0.1) \times 10^7$
6.0	13.5 ± 0.2	0.88 ± 0.1	$(1.5 \pm 0.1) \times 10^7$
6.5	13.5 ± 0.8	0.70 ± 0.04	$(1.9 \pm 0.2) \times 10^7$
7.0	14 ± 1.3	0.68 ± 0.03	$(2.1 \pm 0.3) \times 10^7$
7.5	17 ± 1.7	0.62 ± 0.02	$(2.7 \pm 0.4) \times 10^7$
8.0	16 ± 1.4	0.61 ± 0.04	$(2.6 \pm 0.4) \times 10^7$
8.5	15 ± 0.9	0.56 ± 0.06	$(2.7 \pm 0.5) \times 10^7$

9.0	14 ± 1.7	0.54 ± 0.07	$(2.6 \pm 0.4) \times 10^{7}$
9.5	13 ± 1.0	0.77 ± 0.09	$(1.5 \pm 0.2) \times 10^{7}$

pD	k_{cat} (s^{-1})	K_m (μM)	k_{cat}/K_m ($M^{-1}s^{-1}$)
3.5	5.5 ± 0.3	4.0 ± 0.5	(1.4 ± 0.3) × 10^6
4.0	5.3 ± 0.2	2.9 ± 0.3	(1.8 ± 0.3) × 10^6
4.5	7.6 ± 0.1	2.2 ± 0.1	(3.5 ± 0.2) × 10^6
5.0	8.0 ± 0.2	1.7 ± 0.1	(4.7 ± 0.4) × 10^6
5.5	8.4 ± 0.2	1.3 ± 0.1	(6.5 ± 0.6) × 10^6
6.0	8.3 ± 0.1	0.82 ± 0.03	(1.0 ± 0.1) × 10^7
6.5	8.0 ± 0.1	0.54 ± 0.06	(1.5 ± 0.2) × 10^7
7.0	7.6 ± 0.1	0.44 ± 0.03	(1.7 ± 0.1) × 10^7
7.5	7.5 ± 0.1	0.45 ± 0.01	(1.7 ± 0.1) × 10^7
8.0	7.2 ± 0.1	0.41 ± 0.02	(1.8 ± 0.1) × 10^7
8.5	5.8 ± 0.1	0.32 ± 0.02	(1.8 ± 0.1) × 10^7
9.0	5.2 ± 0.1	0.30 ± 0.02	(1.7 ± 0.2) × 10^7
9.5	4.7 ± 0.1	0.29 ± 0.03	(1.6 ± 0.2) × 10^7

Table S2. pH-rate Profile and Solvent Isotope Effects Data of Wildtype Cruzain with Z-RR-AMC.

pH	k_{cat} (s^{-1})	K_m (μM)	k_{cat}/K_m ($M^{-1}s^{-1}$)
3.5	0.10 ± 0.02	78 ± 4	(2.2 ± 0.1) × 10^3
4.1	0.40 ± 0.03	68 ± 4	(5.9 ± 0.8) × 10^3
4.4	0.70 ± 0.04	56 ± 3	(1.3 ± 0.2) × 10^4
5.0	1.9 ± 0.2	28 ± 3	(7.0 ± 1) × 10^4
5.5	3.7 ± 0.3	17 ± 1	(2.2 ± 0.1) × 10^5
6.0	5.9 ± 0.5	11 ± 1	(5.4 ± 0.4) × 10^5
6.5	6.9 ± 0.1	6.6 ± 0.5	(1.0 ± 0.1) × 10^6
7.0	7.4 ± 0.1	4.6 ± 0.4	(1.6 ± 0.2) × 10^6
7.5	7.2 ± 0.1	3.7 ± 0.4	(1.9 ± 0.2) × 10^6
8.0	8.0 ± 0.2	4.3 ± 0.3	(1.9 ± 0.2) × 10^6
8.5	6.9 ± 0.1	2.9 ± 0.2	(2.4 ± 0.2) × 10^6
9.0	7.1 ± 0.2	3.6 ± 0.3	(2.0 ± 0.2) × 10^6
9.5	5.8 ± 0.2	4.1 ± 0.5	(1.5 ± 0.2) × 10^6
9.9	5.8 ± 0.2	12 ± 1	(4.8 ± 0.1) × 10^5

pD	k_{cat} (s^{-1})	K_m (μM)	k_{cat}/K_m ($M^{-1}s^{-1}$)
3.5	n.d.	n.d.	$(2.3 \pm 0.3) \times 10^2$
4.0	n.d.	n.d.	$(8.4 \pm 0.9) \times 10^2$
4.5	n.d.	n.d.	$(2.6 \pm 0.4) \times 10^3$
5.0	0.30 ± 0.05	36 ± 9	$(8.3 \pm 0.6) \times 10^3$
5.5	1.1 ± 0.1	22 ± 1	$(5.0 \pm 0.7) \times 10^4$
6.0	2.2 ± 0.2	16 ± 1	$(1.4 \pm 0.2) \times 10^5$
6.5	3.5 ± 0.2	13 ± 3	$(2.7 \pm 0.8) \times 10^5$
7.0	4.1 ± 0.1	7.5 ± 0.9	$(5.5 \pm 0.8) \times 10^5$
7.5	4.1 ± 0.3	6.3 ± 0.9	$(6.5 \pm 1.4) \times 10^5$
8.0	4.0 ± 0.2	2.6 ± 0.3	$(1.5 \pm 0.3) \times 10^6$
8.5	3.9 ± 0.1	2.2 ± 0.2	$(1.8 \pm 0.2) \times 10^6$
9.0	3.6 ± 0.1	2.1 ± 0.1	$(1.7 \pm 0.2) \times 10^6$
9.5	3.5 ± 0.1	2.0 ± 0.2	$(1.8 \pm 0.2) \times 10^6$
9.8	2.8 ± 0.1	2.3 ± 0.4	$(1.2 \pm 0.3) \times 10^6$

Table S3. pH-rate Profile and Solvent Isotope Effects Data of Wildtype Cruzain with Z-RA-AMC.

pH	k_{cat} (s^{-1})	K_m (μM)	k_{cat}/K_m ($M^{-1}s^{-1}$)
3.5	0.0030 ± 0.0002	50 ± 9	(6.0 ± 0.9) × 10^1
4.1	0.012 ± 0.002	76 ± 3	(1.6 ± 0.3) × 10^2
4.5	0.023 ± 0.001	37 ± 6	(6.2 ± 0.9) × 10^2
5.0	0.076 ± 0.004	52 ± 1	(1.5 ± 0.4) × 10^3
5.6	0.21 ± 0.04	56 ± 2	(3.8 ± 0.8) × 10^3
6.0	0.44 ± 0.02	52 ± 5	(8.2 ± 0.8) × 10^3
6.5	0.58 ± 0.01	35 ± 1	(1.7 ± 0.1) × 10^4
7.0	0.67 ± 0.02	29 ± 2	(2.3 ± 0.2) × 10^4
7.5	0.89 ± 0.06	38 ± 2	(2.3 ± 0.3) × 10^4
8.0	0.81 ± 0.10	32 ± 2	(2.5 ± 0.2) × 10^4
8.5	0.83 ± 0.13	32 ± 1	(2.5 ± 0.5) × 10^4
9.0	1.0 ± 0.2	58 ± 8	(1.7 ± 0.1) × 10^4
9.5	0.86 ± 0.17	36 ± 1	(2.4 ± 0.4) × 10^4
9.9	0.68 ± 0.03	31 ± 3	(2.2 ± 0.4) × 10^4

pD	k_{cat} (s^{-1})	K_m (μM)	k_{cat}/K_m ($M^{-1}s^{-1}$)
3.5	$(8.5 \pm 0.5) \times 10^{-4}$	16 ± 5	5.3 ± 1.0
4.0	$(1.4 \pm 0.1) \times 10^{-3}$	38 ± 9	$(4.0 \pm 1.0) \times 10^{1}$
4.5	$(2.0 \pm 0.9) \times 10^{-3}$	18 ± 1	$(1.1 \pm 0.6) \times 10^{2}$
5.0	$(1.4 \pm 0.4) \times 10^{-2}$	23 ± 2	$(6.0 \pm 2.0) \times 10^{2}$
5.6	$(4.3 \pm 0.5) \times 10^{-2}$	27 ± 1	$(1.6 \pm 0.2) \times 10^{3}$
6.0	$(1.2 \pm 0.2) \times 10^{-1}$	42 ± 4	$(2.9 \pm 0.8) \times 10^{3}$
6.5	$(3.0 \pm 0.1) \times 10^{-1}$	42 ± 4	$(7.1 \pm 0.9) \times 10^{3}$
7.0	$(4.1 \pm 0.1) \times 10^{-1}$	29 ± 1	$(1.4 \pm 0.1) \times 10^{4}$
7.5	$(4.7 \pm 0.1) \times 10^{-1}$	24 ± 1	$(2.0 \pm 0.1) \times 10^{4}$
7.9	$(4.9 \pm 0.2) \times 10^{-1}$	20 ± 1	$(2.5 \pm 0.2) \times 10^{4}$
8.5	$(4.9 \pm 0.7) \times 10^{-1}$	25 ± 2	$(2.0 \pm 0.4) \times 10^{4}$
9.0	$(4.9 \pm 0.7) \times 10^{-1}$	28 ± 8	$(1.8 \pm 0.8) \times 10^{4}$
9.5	$(4.7 \pm 0.5) \times 10^{-1}$	29 ± 3	$(1.6 \pm 0.3) \times 10^{4}$
9.9	$(3.9 \pm 0.2) \times 10^{-1}$	22 ± 3	$(1.8 \pm 0.3) \times 10^{4}$

Table S4. pH-rate Profile Data of E208A Mutant Cruzain with Z-FR-AMC.

pH	k_{cat} (s^{-1})	K_m (μM)	k_{cat}/K_m ($M^{-1}s^{-1}$)
3.6	18 ± 1	6.1 ± 0.1	(3.0 ± 0.2) × 10^6
3.9	19 ± 1	5.0 ± 0.1	(3.8 ± 0.2) × 10^6
4.3	22 ± 1	5.0 ± 0.2	(4.4 ± 1.0) × 10^6
4.5	23 ± 1	3.6 ± 0.1	(6.4 ± 0.3) × 10^7
4.8	23 ± 1	2.8 ± 0.1	(8.2 ± 0.5) × 10^7
5.1	24 ± 1	2.5 ± 0.1	(9.6 ± 0.6) × 10^7
5.4	23 ± 1	2.0 ± 0.1	(12 ± 0.8) × 10^7
5.7	23 ± 1	1.7 ± 0.1	(14 ± 1.0) × 10^7
6.0	23 ± 1	1.6 ± 0.1	(14 ± 1.0) × 10^7
6.3	23 ± 1	1.5 ± 0.1	(15 ± 1.2) × 10^7
6.6	22 ± 1	1.6 ± 0.1	(14 ± 1.1) × 10^7
6.9	22 ± 1	1.6 ± 0.1	(14 ± 1.1) × 10^7
7.2	23 ± 1	1.9 ± 0.1	(12 ± 0.8) × 10^7
7.5	21 ± 1	1.6 ± 0.1	(13 ± 1.0) × 10^7
8.0	21 ± 1	1.3 ± 0.1	(16 ± 1.4) × 10^7
8.5	19 ± 1	1.3 ± 0.1	(15 ± 1.4) × 10^7
9.0	20 ± 1	1.5 ± 0.1	(13 ± 1.1) × 10^7
9.5	19 ± 1	1.9 ± 0.1	(10 ± 0.7) × 10^7
10.0	21 ± 1	3.6 ± 0.3	(5.8 ± 1.4) × 10^7

Table S5. pH-rate Profile Data of E208A Mutant Cruzain with Z-RR-AMC.

pH	k_{cat} (s^{-1})	K_m (μM)	k_{cat}/K_m ($M^{-1}s^{-1}$)
4.2	0.085 ± 0.002	105 ± 7	$(8.1 \pm 0.5) \times 10^2$
4.5	0.10 ± 0.01	83 ± 11	$(1.2 \pm 0.1) \times 10^3$
4.8	0.12 ± 0.01	67 ± 7	$(1.8 \pm 0.1) \times 10^3$
5.1	0.14 ± 0.01	58 ± 5	$(2.4 \pm 0.1) \times 10^3$
5.4	0.15 ± 0.01	47 ± 3	$(3.2 \pm 0.1) \times 10^3$
5.7	0.18 ± 0.01	47 ± 3	$(3.8 \pm 0.1) \times 10^3$
6.0	0.22 ± 0.01	48 ± 2	$(4.6 \pm 0.1) \times 10^3$
6.3	0.26 ± 0.02	52 ± 5	$(5.0 \pm 0.2) \times 10^3$
6.6	0.27 ± 0.01	49 ± 4	$(5.5 \pm 0.4) \times 10^3$
6.9	0.27 ± 0.01	47 ± 5	$(5.8 \pm 0.6) \times 10^3$
7.2	0.27 ± 0.01	47 ± 2	$(5.8 \pm 0.1) \times 10^3$
7.5	0.27 ± 0.01	44 ± 2	$(6.2 \pm 0.1) \times 10^3$
8.0	0.24 ± 0.01	44 ± 3	$(5.5 \pm 0.3) \times 10^3$
8.5	0.23 ± 0.01	37 ± 2	$(6.2 \pm 0.3) \times 10^3$
9.0	0.23 ± 0.01	49 ± 3	$(4.7 \pm 0.2) \times 10^3$
9.5	0.18 ± 0.01	50 ± 3	$(3.6 \pm 0.1) \times 10^3$
10.0	0.14 ± 0.01	74 ± 7	$(1.9 \pm 0.1) \times 10^3$

Table S6. pH-rate Profile Data of E208A Mutant Cruzain with Z-RA-AMC.

pH	k_{cat} (s^{-1})	K_m (μM)	k_{cat}/K_m ($M^{-1}s^{-1}$)
4.2	$(1.6 \pm 0.1) \times 10^{-3}$	149 ± 13	11 ± 1
4.5	$(2.4 \pm 0.1) \times 10^{-3}$	152 ± 18	16 ± 2
4.8	$(4.0 \pm 0.1) \times 10^{-3}$	123 ± 4	33 ± 1
5.1	$(6.0 \pm 0.1) \times 10^{-3}$	162 ± 7	37 ± 2
5.4	$(7.6 \pm 0.1) \times 10^{-3}$	155 ± 4	49 ± 2
5.7	$(1.0 \pm 0.1) \times 10^{-2}$	166 ± 3	60 ± 6
6.0	$(1.2 \pm 0.2) \times 10^{-2}$	162 ± 5	74 ± 13
6.3	$(1.4 \pm 0.1) \times 10^{-2}$	160 ± 8	88 ± 8
6.6	$(1.4 \pm 0.1) \times 10^{-2}$	159 ± 17	88 ± 11
6.9	$(1.3 \pm 0.1) \times 10^{-2}$	136 ± 7	96 ± 9
7.2	$(1.6 \pm 0.1) \times 10^{-2}$	166 ± 9	96 ± 8
7.5	$(1.7 \pm 0.1) \times 10^{-2}$	163 ± 3	104 ± 6
8.0	$(9.5 \pm 0.1) \times 10^{-3}$	113 ± 9	84 ± 7
8.5	$(9.9 \pm 0.1) \times 10^{-3}$	132 ± 5	75 ± 3
9.0	$(8.8 \pm 0.1) \times 10^{-3}$	145 ± 8	61 ± 3
9.5	$(7.6 \pm 0.1) \times 10^{-3}$	146 ± 20	52 ± 7
10.0	$(5.3 \pm 0.5) \times 10^{-3}$	195 ± 25	27 ± 4

Table S7. pH Rate Profile Data for E208A Cruzain Mutant-Catalyzed Reactions.

	k_{cat}				k_{cat}/K_m			
Substrate	pK_a	pK_1	pK_2	c (s^{-1})	pK_a	pK_1	pK_2	c ($M^{-1}s^{-1}$)
Z-FR-AMC	n.d.	n.d.	n.d.	21.8 ± 0.4	4.4 ± 0.4	5.0 ± 0.2	9.9 ± 0.1	(1.5 ± 0.1) × 10^7
Z-RR-AMC	4.4 ± 0.4	4.9 ± 0.2	10.0 ± 0.1	0.25 ± 0.01	4.4 ± 0.8	5.5 ± 0.2	9.7 ± 0.1	(5.7 ± 0.2) × 10^3
Z-RA-AMC	4.3 ± 0.6	5.4 ± 0.1	10.1 ± 0.1	(1.5 ± 0.1) × 10^{-2}	4.6 ± 0.8	5.4 ± 0.3	9.5 ± 0.1	(8.9 ± 0.3) × 10^1

Figure S1. pH-rate Profile of E208A Cruzain-catalyzed Reaction of Z-FR-AMC. The dashed line represents data fitted according to eq. 4. The solid line represents data fitted to eq. 5.

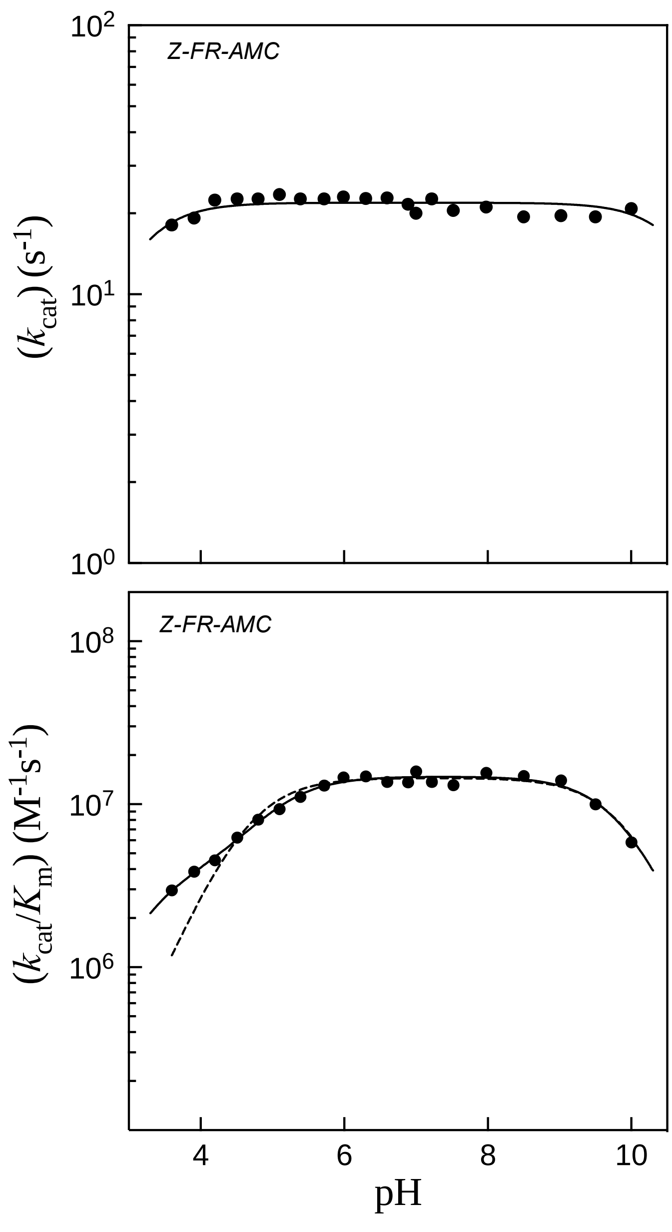

Figure S2. pH-rate Profile of E208A Cruzain-catalyzed Reaction of Z-RR-AMC. The dashed line represents data fitted according to eq. 4. The solid line represents data fitted to eq. 5.

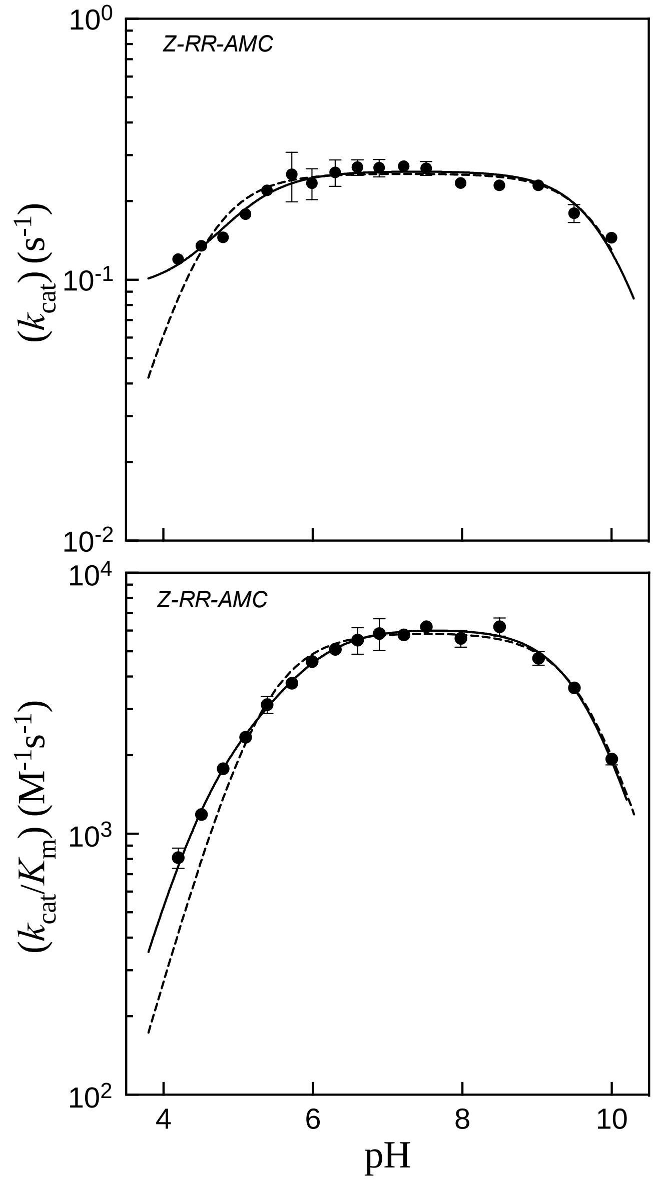

Figure S3. pH-rate Profile of E208A Cruzain-catalyzed Reaction of Z-RA-AMC. The dashed line represents data fitted according to eq. 4. The solid line represents data fitted to eq. 5.

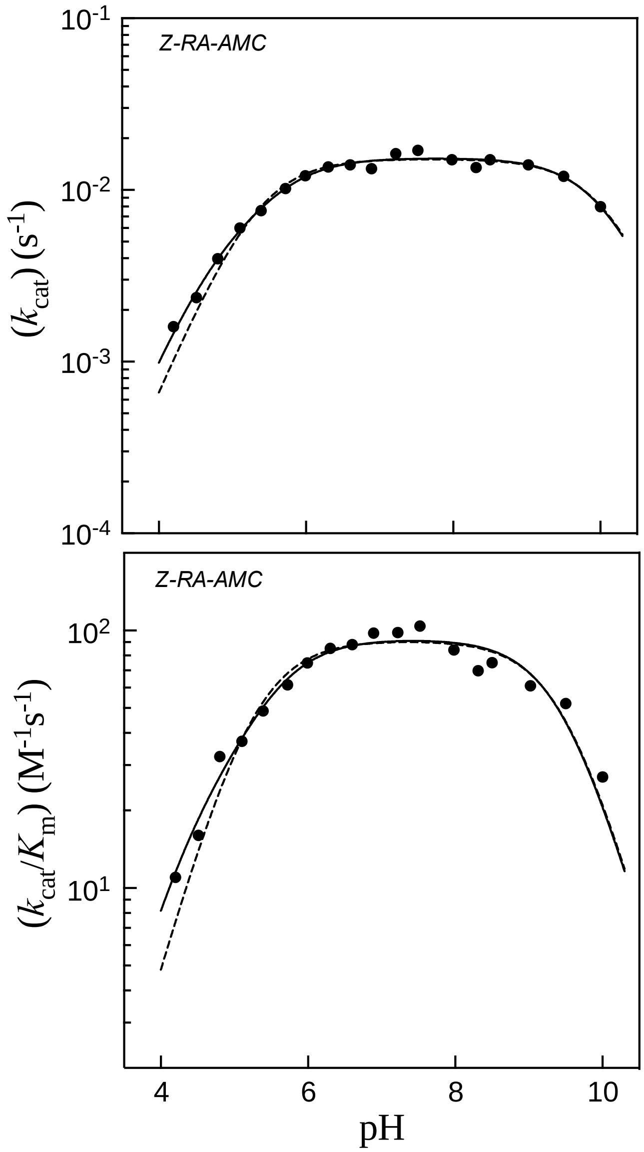

Figure S4. Pre-steady-state Data for Wildtype Cruzain-catalyzed Reaction of Z-RR-AMC in H_2O and D_2O.

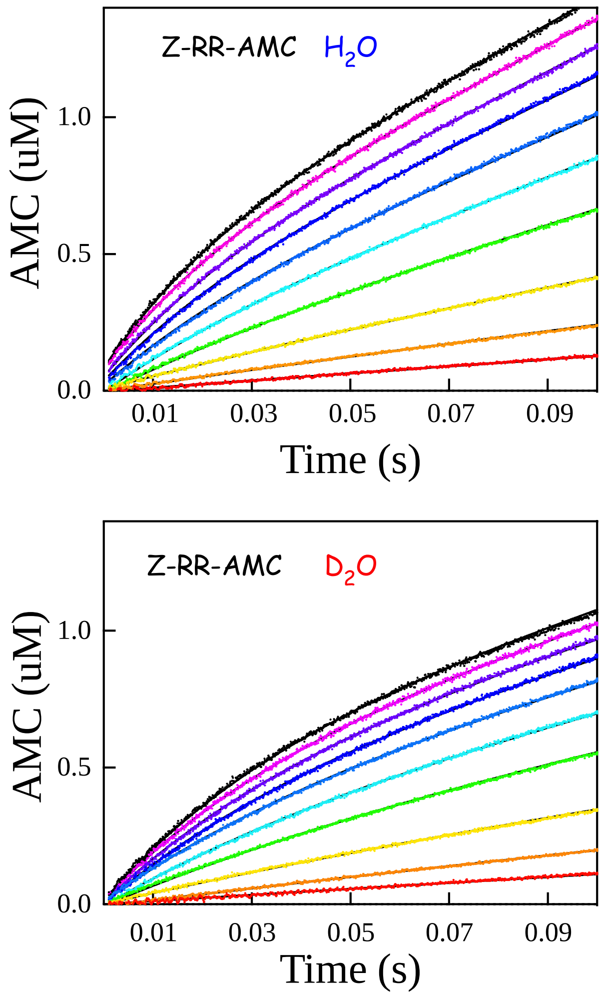

Figure S5. Replots of the Pre-steady-state Kinetic Parameters for Wildtype Cruzain-catalyzed Reactions of Z-RR-AMC in H_2O and D_2O. Data were fitted to eq. 1, 9 and 10 for v_{ss}, β and λ, respectively.