Supporting Information

Quaternary structure of α-amino-β-carboxymuconate-ε-semialdehyde decarboxylase (ACMSD) controls its activity

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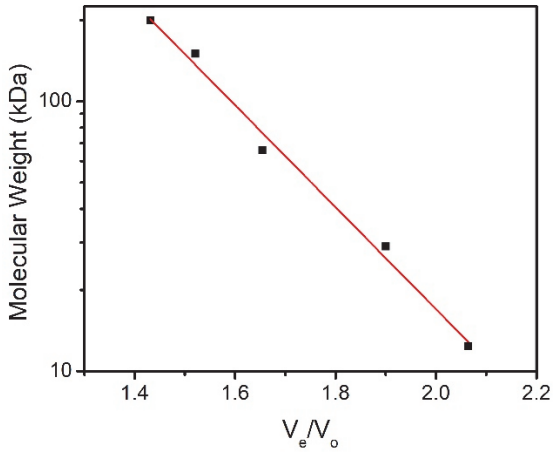

Figure S1. Calibration curve of standard proteins in MWGF200 Kit on Superdex 200. The standard proteins in the plot from top to bottom are β-amylase (200 kDa), alcohol dehydrogenase (150 kDa), albumin (66 kDa), carbonic anhydrase (29 kDa) and cytochrome C (12.4 kDa), respectively.

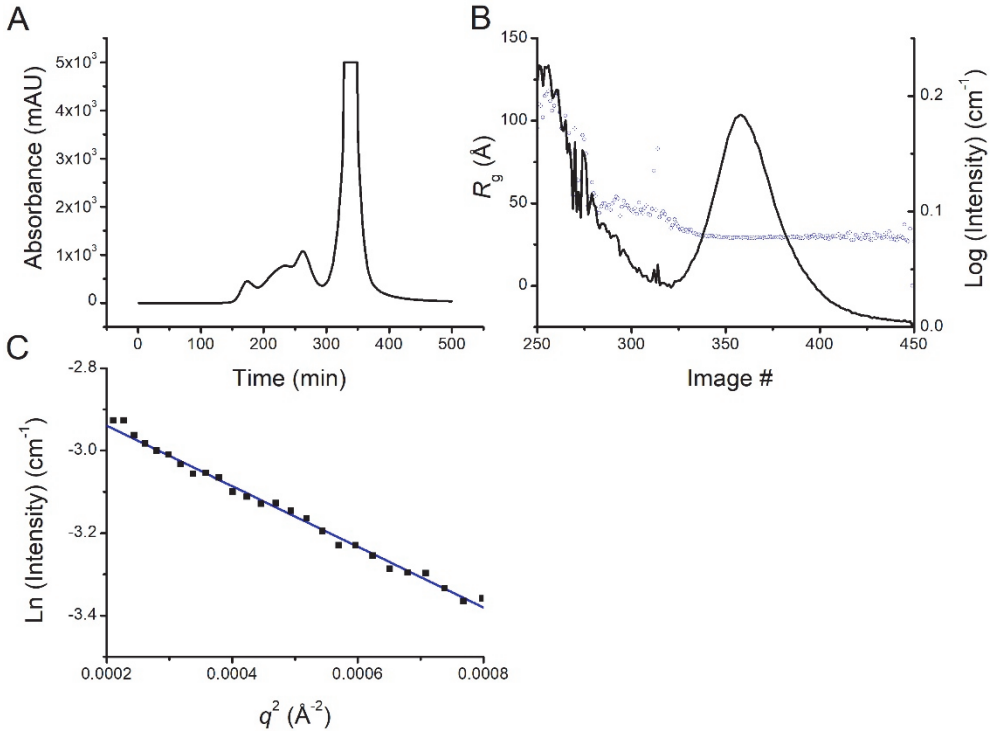

Figure S2. SEC-SAXS analysis of ACMSD. (**A**) The gel-filtration profile from an analytical Superdex column (Superdex 200 Increase PC 3.2/300). (**B**) *Guinier* R_g-$I(0)$ plot. R_g values (blue circles) and the $I(0)$ forward scattering intensities (black line) were obtained by *Guinier* analysis on each SEC fraction. (**C**) *Guinier* plot of the high-ordered oligomer portion.

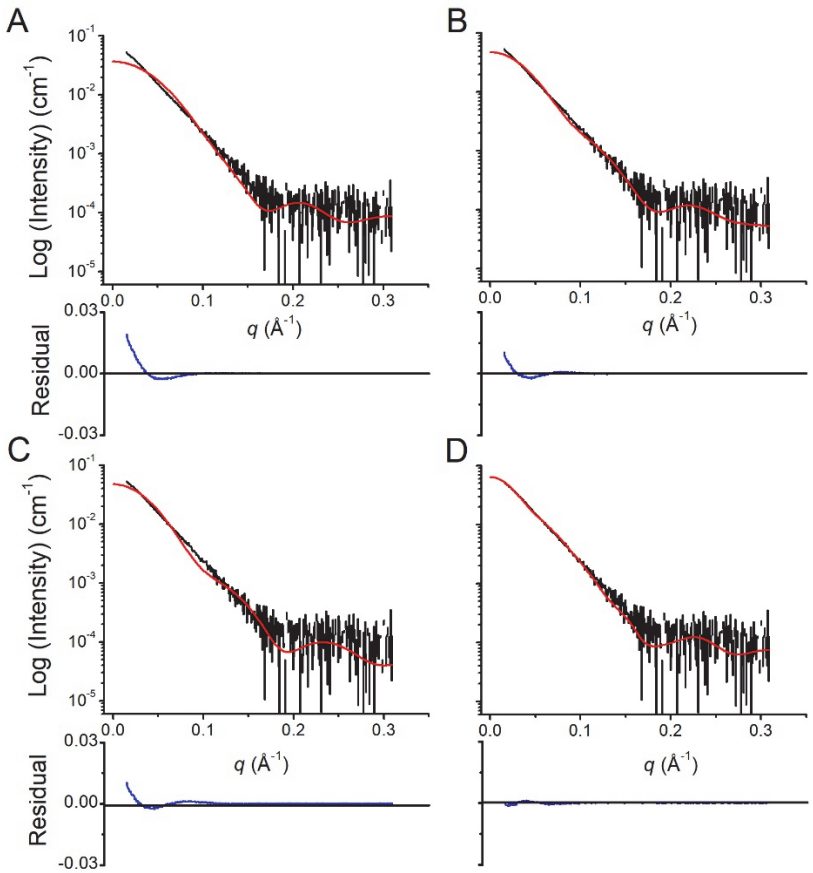

Figure S3. The back calculated scattering curves fitted with experimental SAXS curve. The black lines are the experimental curves, whereas the red lines are the theoretical SAXS curves generated based on dimer (**A**), trimer (**B**), two parallel-dimeric tetramer (**C**) and two tandem-dimeric tetramer (**D**).

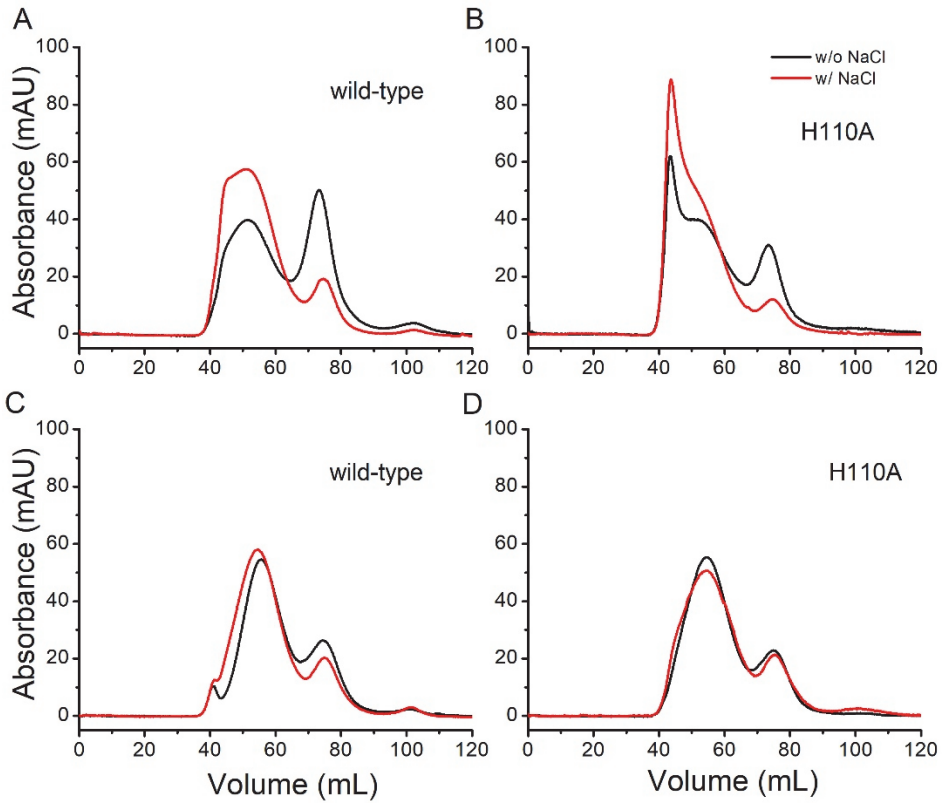

Figure S4. SEC separation profiles of wtACMSD and H110A under pH 6.5 (**A**, **B**), and pH 8.5 (**C**, **D**). The separation profiles with addition of NaCl and without addition of NaCl are shown as red and black lines, respectively.

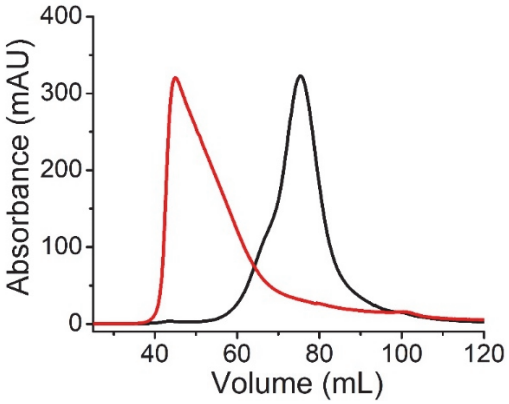

Figure S5. apoACMSD is found to be mostly dimer. The buffer contains 25 mM HEPES pH 7.0 and 150 mM NaCl. Black line, apoACMSD; red line, holo ACMSD.

Table S1. Kinetic constants of wtACMSD and H110A with ACMS

Protein	Condition	Ionic strength (mM)	k_{cat} (s^{-1})	K_m (μM)
wtACMSD	HEPES	5	15.8 ± 1.4	22.9 ± 5.9
	HEPES add NaF	39	23.6 ± 1.0	35.8 ± 2.2
H110A	HEPES	5	9.95 ± 0.1	26.1 ± 2.0
	HEPES add NaF	39	27.8 ± 3.5	54.7 ± 9.4

Table S2. Percent of higher-ordered oligomer and tetramer of ACMSD and the H110A mutant in various buffer conditions

	Percent	pH 6.0	pH 6.5	pH 7.0	pH 7.5	pH 8.0	pH 8.5	pH 9.0
wtACMSD	High-ordered	0	18	46 (0)*	24	0	3	3
	Tetramer	80	66	47 (33)*	63	80	77	78
H110A	High-ordered	38	43	41 (48)*	42	0	0	0
	Tetramer	50	49	45 (31)*	49	78	78	77

* The distribution measured under low ionic strength.

Table S3. Crystallization data collection and refinement statistics

	wtACMSD	H110A
PDB code	**6MGS**	**6MGT**
Data collection		
Space group	$C222_1$	$P4_22_12$
Cell dimensions: a, b, c (Å)	102.483, 154.171, 154.560	90.934, 90.934, 170.107
Molecules per asymmetry unit	3	2
Resolution	50 – 3.12 (3.17 – 3.12)[a]	50 – 2.77 (2.82 – 2.77)
No. of observed reflections	21665 (1059)	17420 (867)
Redundancy	4.4 (3.8)	5.0 (5.1)
Completeness (%)	98.5 (97.0)	92.0 (94.5)
I/σ(I)	10.8 (1.2)	14.3 (1.1)
R_{merge} (%)[b]	95.1 (14.2)	90.8 (11.4)
$CC_{1/2}$[c]	99.3 (78.2)	99.4 (59.3)
Refinement[d]		
R_{work}	22.9	19.6
R_{free}	30.1	25.8
RMSD bond length (Å)[e]	0.010	0.009
RMSD bond angles (°)	1.221	1.119
Ramachandran statistics[f]		
Preferred (%)	89.1	94.4
Allowed (%)	10.0	5.3
Outliers (%)	0.8	0.3
Average B-factor (Å²)		
Chain A/B/C	45.5/66.5/61.8	72.0/86.9/NA

[a] Values in parentheses are for the highest resolution shell.

[b] $R_{merge} = \Sigma_{hkl}\Sigma i\ |I_i(hkl) - \langle I(hkl)\rangle\ | / \Sigma_{hkl}\Sigma_i I_i(hkl)$, in which the sum is over all the *i* measured reflections with equivalent miller indices *hkl*; $\langle I(hkl)\rangle$ is the averaged intensity of these *i* reflections, and the grand sum is over all measured reflections in the data set.

[c] According to Karplus and Diederichs (2).

[d] All positive reflections were used in the refinement.

[e] According to Engh and Huber (3).

[f] Calculated by using MolProbity (4).

Table S4. Averaged *B*-factor values of each monomer of ACMSD and mutant structures

Protein (PDB entry)	Space group	Resolution (Å)	*B*-factor (Å^2)		Ratio of chain B /chain A
			Chain A	Chain B	
ACMSD (2HBV)	*C*121	1.65	32.6	49.8	1.53
H228Y (4ERI)	*C*121	2.00	36.7	54.3	1.47
R51A/R239A (4IG2)	*C*121	1.80	37.2	60.7	1.63
R51A/R239A (4IFK)	*C*121	2.00	32.1	47.3	1.47
R51A (4IFO)	$P4_22_12$	2.50	47.6	61.0	1.28
R239A (4IFR)	$P4_22_12$	2.40	72.5	84.0	1.16
H228Y (4ERG)	$P4_22_12$	2.80	80.5	91.8	1.14
H228Y (4ERA)	$P4_22_12$	2.40	54.4	65.0	1.19
H228G (4EPK)	$P4_22_12$	2.60	61.3	76.2	1.24
ACMSD (2HBX)	$P4_22_12$	2.50	61.8	76.6	1.24

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