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Supporting information for article:

**Structure of highly acidic  $\beta$ -lactamase from moderate halophile *Chromohalobacter* sp. 560 and the discovery of a  $\text{Cs}^+$  selective binding site**

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**Table S1** Metal ions observed in NQ-HaBLA crystals.

Crystal	Site	Metal	No.	Distance (Å)	Ligands			
					Atom	Residue	No.	Chain
Without soaking	Site-1	Ca	1	2.6	Oδ1	Asp	56	A
Na <sup>+</sup> / Cs <sup>+</sup> = 100 mM/0 mM				2.6	Oε1	Glu	170	C
Ca <sup>2+</sup> / Sr <sup>2+</sup> = 200 mM/0 mM				2.5	O	HOH	543	S
				2.6	O	HOH	689	S
				2.4	O	HOH	690	S
	Site-2	Ca	2	2.3	Oδ1	Asp	58	C
				2.4	Oδ1	Asp	128	A*
				2.3	O	HOH	29	S
				2.5	O	HOH	33	S
				2.4	O	HOH	314	S
				2.6	O	HOH	402	S*
				2.3	O	HOH	608	S
	Site-3	Ca	3	2.4	Oδ1	Asp	85	A
				2.5	Oδ1	Asp	87	A
				2.3	Oδ1	Asp	187	B
				2.4	O	HOH	109	S
				2.4	O	HOH	110	S
	Site-4	Ca	4	2.1	Oδ1	Asp	124	A
				2.4	O	HOH	46	S
				2.7	O	HOH	234	S
				2.7	O	HOH	274	S
				2.4	O	HOH	364	S
				2.6	O	HOH	622	S
	Site-5	Ca	5	2.5	Oδ1	Asp	199	B
				2.4	O	HOH	211	S
				2.6	O	HOH	515	S
				2.8	O	HOH	747	S
	Site-6	Ca	6	2.5	Oδ1	Asp	219	A
				2.4	Oδ1	Asp	220	A
				2.5	O	HOH	64	S
				2.4	O	HOH	113	S
				2.4	O	HOH	276	S
				2.3	O	HOH	317	S
				2.4	O	HOH	318	S
		Ca	7	2.6	Oδ1	Asp	219	B
				2.4	Oδ1	Asp	220	B
				2.6	O	HOH	65	S
				2.2	O	HOH	114	S
				2.4	O	HOH	115	S
				2.3	O	HOH	116	S
				2.5	O	HOH	294	S

	Ca	8	2.7	O $\delta$ 1	Asp	219	C		
			2.5	O	HOH	135	S		
			2.1	O	HOH	330	S		
			2.4	O	HOH	702	S		
			2.4	O	HOH	729	S		
Site-7	Ca	9	2.3	O $\delta$ 1	Asp	291	A*		
			2.3	O $\epsilon$ 1	Glu	295	A*		
			2.5	O $\epsilon$ 1	Glu	352	A		
			2.1	O	HOH	93	S*		
			2.4	O	HOH	94	S		
			2.5	O	HOH	107	S		
			2.8	O	HOH	108	S		
Ca		10	2.4	O $\delta$ 1	Asp	291	B		
			2.3	O $\epsilon$ 1	Glu	295	B		
			2.5	O $\epsilon$ 1	Glu	352	B*		
			2.3	O	HOH	78	S		
			2.4	O	HOH	104	S		
			2.2	O	HOH	284	S*		
			2.6	O $\delta$ 1	Asp	291	C*		
Ca		11	2.4	O $\epsilon$ 1	Glu	295	C*		
			2.6	O $\epsilon$ 1	Glu	352	C		
			2.3	O	HOH	91	S*		
			2.2	O	HOH	92	S*		
			2.4	O	HOH	118	S		
Condition-1A		Site-2	Ca	1	2.2	O $\delta$ 1	Asp	58	C
Na <sup>+</sup> / Cs <sup>+</sup> = 0 mM/100 mM					2.3	O $\delta$ 1	Asp	128	A*
Ca <sup>2+</sup> / Sr <sup>2+</sup> = 200 mM/0 mM					2.2	O	HOH	30	S
Site-3					2.1	O	HOH	456	S*
					2.4	O	HOH	526	S
			Ca	2	2.5	O $\delta$ 1	Asp	85	A
					2.5	O $\delta$ 1	Asp	87	A
					2.3	O $\delta$ 1	Asp	187	B
					2.1	O	HOH	461	S
					2.3	O	HOH	462	S
Site-5	Ca	3	2.4	O $\delta$ 1	Asp	199	A		
					2.6	O	HOH	378	S
					2.4	O	HOH	538	S
	Ca	4	2.4	O $\delta$ 1	Asp	199	B		
					2.5	O	HOH	399	S
					2.4	O	HOH	491	S
	Ca	5	2.6	O $\delta$ 1	Asp	219	A		
Site-6					2.5	O $\delta$ 1	Asp	220	A
					2.5	O	HOH	57	S
					2.5	O	HOH	188	S
					2.2	O	HOH	471	S

	Ca	6	2.6	Oδ1	Asp	219	B	
			2.6	Oδ1	Asp	220	B	
			2.7	O	HOH	58	S	
			2.3	O	HOH	201	S	
			2.5	O	HOH	527	S	
	Ca	7	2.7	Oδ1	Asp	219	C	
			2.6	Oδ1	Asp	220	C	
			2.7	O	HOH	103	S	
			2.8	O	HOH	335	S	
			2.4	O	HOH	566	S	
Site-7	Ca	8	2.4	Oδ1	Asp	291	A*	
			2.6	Oε1	Glu	295	A*	
			2.5	Oε1	Glu	352	A	
			2.1	O	HOH	80	S*	
			2.4	O	HOH	81	S	
			2.4	O	HOH	92	S	
	Ca	9	2.4	Oδ1	Asp	291	B	
			2.5	Oε1	Glu	295	B	
			2.6	Oε1	Glu	352	B*	
			2.6	O	HOH	90	S	
			2.3	O	HOH	195	S*	
			2.3	O	HOH	473	S	
	Ca	10	2.6	Oε1	Glu	295	C*	
			2.5	Oε1	Glu	352	C	
			2.0	O	HOH	79	S*	
			2.3	O	HOH	509	S*	
			2.6	O	HOH	530	S	
			2.5	O	HOH	549	S*	
Site-8	Cs	1	3.1	O	Gln	186	A	
			3.7	O	Thr	188	A	
			3.9	Cδ2	Trp	189	A	
			3.7	Cε2	Trp	189	A	
			3.8	Cε3	Trp	189	A	
			3.5	Cζ2	Trp	189	A	
			3.5	Cζ3	Trp	189	A	
			3.3	Cη2	Trp	189	A	
	Cs	2	3.3	O	Gln	186	C	
			3.3	O	Thr	188	C	
			3.7	Cδ2	Trp	189	C	
			3.6	Cε2	Trp	189	C	
			3.3	Cε3	Trp	189	C	
			3.5	Cζ2	Trp	189	C	
			3.4	Cζ3	Trp	189	C	
			3.4	Cη2	Trp	189	C	
Condition-1B	Site-2	Ca	1	2.4	Oδ1	Asp	58	C
Na <sup>+</sup> / Cs <sup>+</sup> = 75 mM				2.2	Oδ1	Asp	128	A*

Ca <sup>2+</sup> / Sr <sup>2+</sup> = 200 mM/0 mM							
			2.4	O	HOH	110	S
			2.5	O	HOH	441	S
			1.7	O	HOH	587	S
			2.2	O	HOH	588	S
Site-3	Ca	2	2.5	Oδ1	Asp	85	A
			2.7	Oδ1	Asp	87	A
			2.4	Oδ1	Asp	187	B
			2.4	O	HOH	92	S
Site-4	Ca	3	2.1	Oδ1	Asp	124	A
			2.4	O	HOH	233	S
			2.6	O	HOH	443	S
			2.6	O	HOH	572	S*
			2.2	O	HOH	592	S
Site-5	Ca	4	2.6	Oδ1	Asp	199	B
			2.4	O	HOH	327	S
			2.4	O	HOH	350	S
			2.4	O	HOH	482	S
			2.5	Oδ1	Asp	219	A
Site-6	Ca	5	2.4	Oδ1	Asp	220	A
			2.5	O	HOH	57	S
			2.5	O	HOH	85	S
			2.5	O	HOH	150	S
			2.4	O	HOH	187	S
			2.4	O	HOH	210	S
			2.4	Oδ1	Asp	219	B
Ca		6	2.4	Oδ1	Asp	220	B
			2.5	O	HOH	322	S
			2.4	O	HOH	394	S
			2.6	Oδ1	Asp	219	C
Ca		7	2.7	Oδ1	Asp	220	C
			2.8	O	HOH	171	S
			2.3	O	HOH	329	S
			2.5	O	HOH	457	S
			2.3	Oδ1	Asp	291	A*
Site-7	Ca	8	2.6	Oε1	Glu	295	A*
			2.5	Oε1	Glu	352	A
			2.1	O	HOH	114	S
			2.4	O	HOH	123	S
			2.3	O	HOH	139	S
			2.4	Oδ1	Asp	291	B
Ca		9	2.7	Oε1	Glu	295	B
			2.5	Oε1	Glu	352	B*
			2.2	O	HOH	221	S
			2.6	O	HOH	374	S
			2.7	Oδ1	Asp	291	C*
Ca		10	2.4	Oε1	Glu	295	C*

				2.4	Oε1	Glu	352	C		
				2.5	O	HOH	337	S		
				1.6	O	HOH	591	S		
Site-8	Cs	1	3.2	O	Gln	186	A			
			3.5	O	Thr	188	A			
			4.0	Cδ2	Trp	189	A			
			3.8	Cε2	Trp	189	A			
			3.9	Cε3	Trp	189	A			
			3.4	Cζ2	Trp	189	A			
			3.9	Cζ3	Trp	189	A			
			3.2	Cη2	Trp	189	A			
			3.4	O	HOH	68	S			
			3.7	O	HOH	571	S			
Condition-1C	Cs	2	3.4	O	Gln	186	C			
			3.2	O	Thr	188	C			
			3.8	Cδ2	Trp	189	C			
			3.9	Cε2	Trp	189	C			
			3.6	Cε3	Trp	189	C			
			3.7	Cζ2	Trp	189	C			
			3.3	Cζ3	Trp	189	C			
			3.4	Cη2	Trp	189	C			
Na <sup>+</sup> / Cs <sup>+</sup> = 90 mM/10 mM		Site-2	Ca	1	2.2	Oδ1	Asp	58	C	
Ca <sup>2+</sup> / Sr <sup>2+</sup> = 200 mM/0 mM					2.4	Oδ1	Asp	128	A*	
Site-3	Ca	2			2.6	O	HOH	110	S	
					2.7	O	HOH	389	S	
					2.4	O	HOH	493	S	
					2.6	Oδ1	Asp	85	A	
					2.4	Oδ1	Asp	87	A	
					2.5	Oδ1	Asp	187	B	
					2.5	O	HOH	92	S	
					2.7	O	HOH	531	S	
					2.2	Oδ1	Asp	124	A	
					2.6	O	HOH	229	S	
Site-4	Ca	3			2.4	O	HOH	391	S	
					2.7	O	HOH	485	S*	
					2.3	Oδ1	Asp	199	B	
					2.2	O	HOH	311	S	
Site-5	Ca	4			2.3	O	HOH	331	S	
					2.6	Oδ1	Asp	219	A	
					2.5	Oδ1	Asp	220	A	
					2.8	O	HOH	57	S	
					2.4	O	HOH	85	S	
Site-6	Ca	5			2.2	O	HOH	150	S	
					2.7	O	HOH	186	S	
					2.3	O	HOH	208	S	
					2.6	Oδ1	Asp	219	B	

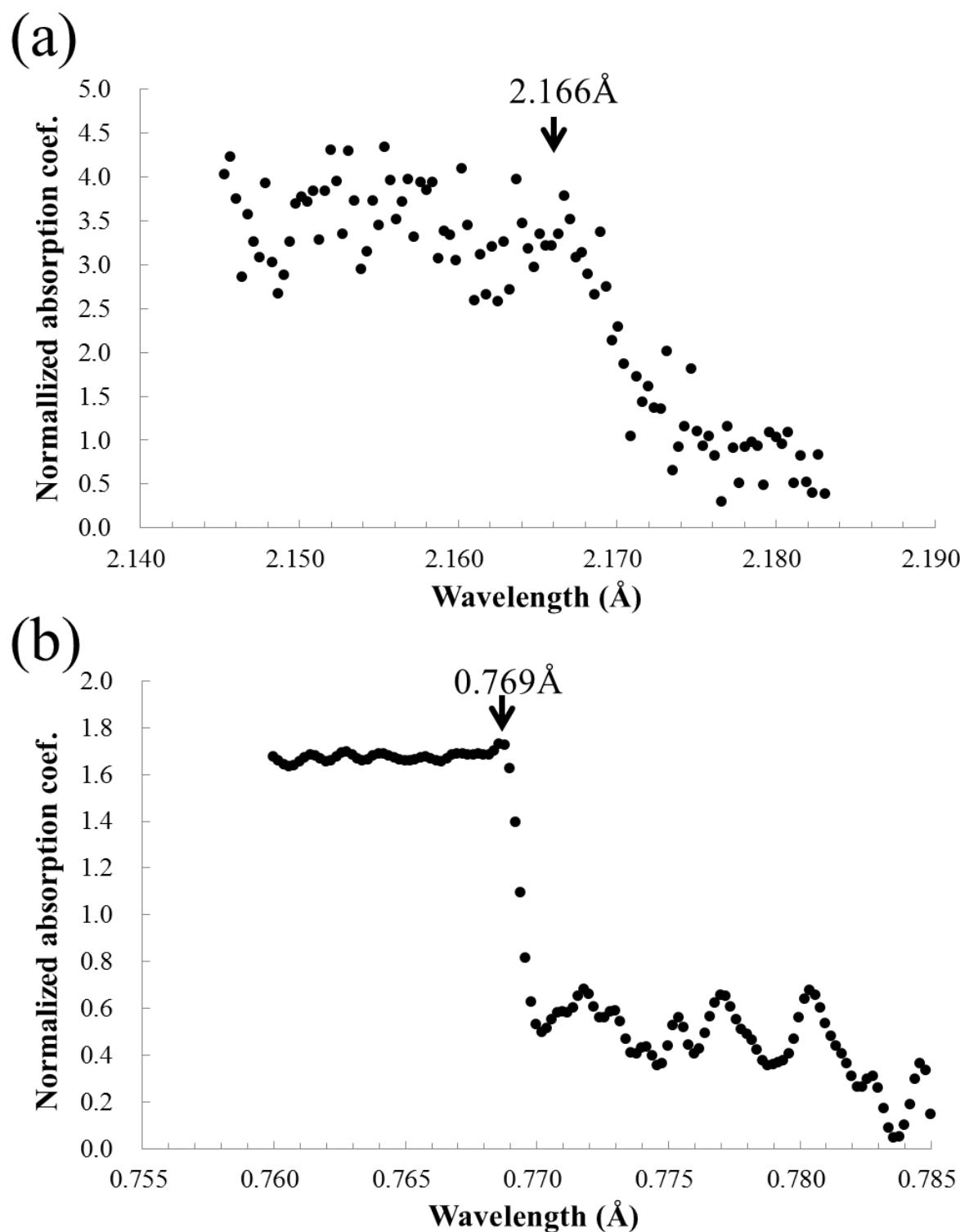
				2.3	Oδ1	Asp	220	B
				2.4	O	HOH	358	S
	Ca	7		2.6	Oδ1	Asp	219	C
				2.6	Oδ1	Asp	220	C
				2.7	O	HOH	170	S
				2.5	O	HOH	312	S
				2.8	O	HOH	404	S
Site-7	Ca	8		2.4	Oδ1	Asp	291	A*
				2.4	Oε1	Glu	295	A*
				2.5	Oε1	Glu	352	A
				2.2	O	HOH	114	S
				2.4	O	HOH	123	S
				2.4	O	HOH	139	S
	Ca	9		2.4	Oδ1	Asp	291	B
				2.6	Oε1	Glu	295	B
				2.6	Oε1	Glu	352	B*
				2.2	O	HOH	218	S
	Ca	10		2.4	Oε1	Glu	295	C*
				2.5	Oε1	Glu	352	C
				2.6	O	HOH	319	S
				1.9	O	HOH	496	S
Site-8	Cs	1		3.6	O	Gln	186	A
				3.7	O	Thr	188	A
				4.0	Cδ2	Trp	189	A
				3.9	Cε2	Trp	189	A
				3.7	Cε3	Trp	189	A
				3.6	Cζ2	Trp	189	A
				3.4	Cζ3	Trp	189	A
				3.4	Cη2	Trp	189	A
Condition-2A	Site-2	Sr	1	2.4	Oδ1	Asp	58	B
Na <sup>+</sup> / Cs <sup>+</sup> = 100 mM/0 mM				2.5	O	HOH	82	S
Ca <sup>2+</sup> / Sr <sup>2+</sup> = 0 mM/200 mM				2.7	O	HOH	438	S
				2.5	O	HOH	454	S
	Sr	2		2.4	Oδ1	Asp	58	C
				2.4	Oδ1	Asp	128	A*
				2.7	O	HOH	257	S
				2.7	O	HOH	386	S*
				2.7	O	HOH	444	S
Site-6	Sr	3		2.6	Oδ1	Asp	219	A
				2.6	Oδ1	Asp	220	A
				2.6	O	HOH	48	S
				2.4	O	HOH	182	S
				2.5	O	HOH	211	S
				2.5	O	HOH	502	S
	Sr	4		2.6	Oδ1	Asp	219	B
				2.6	Oδ1	Asp	220	B

				2.6	O	HOH	49	S
				2.6	O	HOH	84	S
				2.6	O	HOH	194	S
				2.7	O	HOH	439	S
	Sr	5		2.7	O $\delta$ 1	Asp	219	C
				2.6	O $\delta$ 1	Asp	220	C
				2.5	O	HOH	96	S
				2.7	O	HOH	369	S
				2.8	O	HOH	310	S
Site-7	Sr	6		2.6	O $\delta$ 1	Asp	291	A*
				2.7	O $\epsilon$ 1	Glu	295	A*
				2.6	O $\epsilon$ 1	Glu	352	A
				2.5	O	HOH	72	S*
	Sr	7		2.6	O $\delta$ 1	Asp	291	B
				2.7	O $\epsilon$ 1	Glu	295	B
				2.6	O $\epsilon$ 1	Glu	352	B*
				2.7	O	HOH	404	S
				2.3	O	HOH	449	S
	Sr	8		2.7	O $\delta$ 1	Asp	291	C*
				2.7	O $\epsilon$ 1	Glu	295	C*
				2.7	O $\epsilon$ 1	Glu	352	C
				2.8	O	HOH	70	S*
				2.6	O	HOH	71	S*
				2.6	O	HOH	85	S
				2.6	O	HOH	86	S
Condition-2B	Site-6	Sr	1	2.9	O $\delta$ 1	Asp	219	A
Na <sup>+</sup> / Cs <sup>+</sup> = 100 mM/0 mM				2.9	O $\delta$ 1	Asp	220	A
Ca <sup>2+</sup> / Sr <sup>2+</sup> = 100 mM/100 mM				2.3	O	HOH	15	S
				2.3	O	HOH	90	S
Site-7	Ca	1		2.5	O $\delta$ 1	Asp	291	B
				2.7	O $\epsilon$ 1	Glu	295	B
				2.5	O $\epsilon$ 1	Glu	352	B*

\* The neighboring chain of an asymmetric unit that is generated by a symmetric operation.

**Table S2** Kinetic parameters ( $k_{cat}$ ,  $K_M$  and  $k_{cat} / K_M$ ) of penicillin G hydrolysis with HaBLA obtained by ITC at 25 °C.

Metal Chloride	$k_{cat}$ (S <sup>-1</sup> )	$K_M$ (μM)	$k_{cat} / K_M$ (S <sup>-1</sup> μM <sup>-1</sup> )
none	4.25 ± 0.04	0.133 ± 0.003	32.1 ± 0.9
0.1 M NaCl	4.19 ± 0.06	0.077 ± 0.008	54.8 ± 6.4
0.5 M NaCl	3.67 ± 0.03	0.035 ± 0.004	107.7 ± 13.7
1.0 M NaCl	3.30 ± 0.06	0.034 ± 0.008	101.5 ± 20.8
2.0 M NaCl	2.96 ± 0.06	0.023 ± 0.007	142.8 ± 20.4
4.0 M NaCl	2.72 ± 0.05	0.043 ± 0.014	71.0 ± 25.6
1.0 M NaCl	3.30 ± 0.06	0.034 ± 0.008	101.5 ± 20.8
1.0 M CsCl	3.06 ± 0.03	0.028 ± 0.001	108.9 ± 1.1
1.0 M NaCl / 40 mM MgCl <sub>2</sub>	3.27 ± 0.01	0.037 ± 0.004	89.3 ± 8.0
1.0 M NaCl / 40 mM CaCl <sub>2</sub>	3.11 ± 0.05	0.043 ± 0.001	72.8 ± 1.1
1.0 M NaCl / 40 mM SrCl <sub>2</sub>	3.12 ± 0.02	0.041 ± 0.010	76.7 ± 2.0



**Figure S1** Examples of the X-ray absorption spectra of NQ-HaBLA crystals soaked in solutions containing (a) 100 mM  $\text{Cs}^+$  (condition-1A) and (b) 200 mM  $\text{Sr}^{2+}$  (condition-2A). These spectra (a) and (b) were obtained using BL7 at SAGA-LS and NW12A at PF, respectively. Arrows indicate the wavelength used for the X-ray anomalous diffraction data collection.