Molecules and Cells





Supplementary Fig. S1. Crystal packing of *pm***CA dimers in the asymmetric unit with and without acetazolamide.** (A) Bicarbonatebound *pm*CA (Crystal form 1), water-bound *pm*CA (Crystal form 2), and acetazolamide-bound *pm*CA (Crystal form 3). Crystal packing is shown along crystallographic axes c (left) and b (right). Unit cells are shown as red boxes. Monomers of the *pm*CA dimer are colored magenta and green, respectively.



Supplementary Fig. S2. Structural comparison of *pm*CA with other bacterial α -CAs. Superposition of the *pm*CA monomer with SspCA (A, PDB ID 4G7A), SazCA (B, PDB ID 4X5S), TaCA (C, PDB ID 4COQ), PprCA (D, PDB ID 5HPJ), NgCA (E, PDB ID 1KOP), and LOGACA (F, PDB ID 6EKI).

		Active site Water-mediated interfacial interac Calcium binding site PEG binding site	tion
		$\alpha 1$ $\alpha 2$ $\beta 1$ $\beta 2$ $\beta 3$	
C7	0.0		
PRCA	20	GGGWSIHG-EHGPEHWGDLKDEIIMCKIGKNQSPVDINKIVDA-KLKPIKIEIKAG-AIKV	// E0
nCATI	1	MSHHWGYGK-HNGPEHWHKDFPTAK-GERQSPVDIDTHT-AKYD-PSLKPLSVSYDQATSLR	58
SSPCA	21	EHEWSIEG-EKGPEHWAQLKPEFFWCKL-KNQSPINIDKKI-KVKA-NLPKLNLIIKIAKESEV	80
Jazca	20	HHWSIEG-ENGPENWARLNPEIFWCNL-KNQSPVDISDNI-KVHA-KLEKLHININKAVNPEI	00
DorCh	24	GGGRHWGISG-SIGFEHWGDLSFEILMCKIGKNOSFIDINSAD-AVNA-CLAFVSVIIVSD-AKIV	0Z 77
Nach	24		87
LOCACA	27	HGNHIHWGIIG-HDSPESWGNLSEEFKLCSIGKNOSPUNIEIVBA-FIFKIKVNIKFS-MVDV	79
HDCA	22	CIDELMARKENCEHEMOKIHKDEENCKSCKSCSINIEHIAW-FPEKIKIMI222-2221	67
прен	T	RA R5 R6 R7	07
ртCA	76	LNNGHTIKVSYEP-G-SYIVVDGIKFELKOFHFHAPSEHKLKGOHYPFEAHFVHADKH	133
hCATT	59	TINNGHAFNVEFDDSODKAVLKGGPLDGTYRLTOFHFHWGSLDGOGSEHTVDKKKYAAELHLVHWNTKYG	128
SspCA	81	VNNGHTIOINIKE-D-NTLNYLGEKYOLKOFHFHTPSEHTIEKKSYPLEIHFVHKTED	136
SazCA	89	VNNGHTIQVNVLE-D-FKLNIKGKEYHLKQFHFHAPSEHTVNGKYYPLEMHLVHKDKD	144
TaCA	83	VNNGHTIKVVMGG-R-GYVVVDGKRFYLKQFHFHAPSEHTVNGKHYPFEAHFVHLDKN	138
PprCA	78	VNNGHTIQANLTG-K-NTLTVDGKTFELKQFHFHTPSENYLKGKQYPLEAHFVHATDK	133
NgCA	88	ENNGHTIQVNYPEGG-NTLTVNGRTYTLKQFHFHVPSENQIKGRTFPMEAHFVHLDEN	144
LOGACA	79	TNNGHTIKVSYEP-G-SYIIVDGIRFELKQFHFHAPSEHTIKGKSYPFEAHFVHADKD	134
HpCA	68	FFTHHTLKASFEP-T-NHINYRGHDYVLDNVHFHAPMEFLINNKTRPLSAHFVHKDAK	123
		β8 <u>α3</u> β9 <u>α4</u> β10	
pmCA	134	GNLAVIGVFFKEG-RENPILEKIWKVMPENAGEEVKLAHKINAEDLLPKDRDYYRYSGS	191
hCAII	129	DFGKAVQQPDGLAVLGIFLKVG-SAKPGLQKVVDVLDSIKTKGKS-ADFTNFDPRGLLPESLDYWTYPGS	196
SspCA	137	GKILVVGVMAKLG-KTNKELDKILNVAPAEEGEK-ILDKNLNLNNLIPKDKRYMTYSGS	193
SazCA	145	GNIAVIGVFFKEG-KANPELDKVFKNALKEEGSK-VFDGSININALLPPVKNYYTYSGS	201
TaCA	139	GNITVLGVFFKVG-KENPELEKVWRVMPEEPGQKRHLTARIDPEKLLPENRDYYRYSGS	196
PprCA	134	GELAVVAVMFDFGPRSNNELTTLLASIPSKGQ-TVELKEALNPADLLPRDREYYRFNGS	191
NgCA	145	KQPLVLAVLYEAG-KTNGRLSSIWNVMPMTAG-KVKLNQPFDASTLLPKRLKYYRFAGS	201
LOGACA	135	GNLAVIGVIFKEG-KKNPIIEKIWENLP-E-AGKTIKLAHKINAYDLLPKKKKYYRYSGS	191
НрСА	124	GRLLVLAIGFEEG-KENPNLDPILEGIQKKQNFKEVALDAFLPKSINYYHFNGS	176
		$ \underbrace{\beta 11}_{\alpha 5} \underbrace{\beta 12}_{\beta 12} $	
DmC A	192		43
hCATT	197	LTTPPLLECVTWIVIKEPISVSSEOVIKERKINFNGEGEPEELMV-DNWRPAOPIKNROIKASEK 2	60
SspCA	194	LTTPPCTEGVRWIVLKKPISISKOOLEKLKSVMVN-PNNRPVOEINSRWIIEGF 2	46
SazCA	202	LTTPPCTEGVIWIVI.KOPITASKOOIELEKSIMKH-NNNRPTOPINSRYILESN 2	54
TaCA	197	LTTPPCSEGVRWIVFKEPVEMSREOLEKFRKVMGF-DNNRPVOPI.NARKVMK 2	47
PprCA	192	LTTPPCSEGVRWFVMOEPOTSSKAOTEKLOAVMGNNARPLOPLNARLILE 2	41
NaCA	202	LTTPPCTEGVSWLVLKTYDHIDOAOAEKFTRAVGS-ENNRPVOPLNARVVIE 2	52
LOGACA	192	LTTPPCSEGVRWIVMEEEMELSKEOIEKFRKLMD 2	44
НрСА	177	LTAPPCTEGVAWFVIEEPLEVSAKQLAEIKKRMKNSPNQRPVQPDYNTVIIKSSAETR 2	34

Supplementary Fig. S3. Structure-based sequence alignment of *pm*CA with other α -CAs. *pm*CA, hCAll, SspCA, SazCA, TaCa, PprCA, NgCA, LOGACA, and HpCA amino acid sequences are aligned based on their structures. Conserved catalytic histidine residues, residues involved in water-mediated interactions between monomers, and residues involved in coordination of calcium ions and PEG molecules are colored red, blue, green, and purple, respectively. The positions of α -helices and β -strands are indicated by cylinders and arrows above the sequences, and are numbered accordingly.



Supplementary Fig. S4. Structural differences between *pm***CA and hCAll.** The *pm*CA structure is superimposed with that of hCAll (PDB ID 1CA2). Substantial structural differences include deletion of surface loops in *pm*CA (indicated by arrows). Zinc ions are shown as gray spheres.



Supplementary Fig. S5. Schematic diagram of interfacial interactions in the *pm*CA dimer. Interacting residues in the *pm*CA dimer interface are indicated. Hydrogen bonds are represented by red lines. Ionic and hydrophobic interactions are shown as cyan and green lines, respectively.



Supplementary Fig. S6. Comparison of the structures of the calcium binding site of *pmCA* and hCAII. The side chains of residues that differ significantly are shown. Amino acids in parentheses represent the corresponding residues in hCAII (PDB ID 1CA2).

	Form 1	Form 2	Form 3
	(PDB ID 6IM0)	(PDB ID 6IM1)	(PDB ID 6IM3)
Data collection			
Space group	C2	P2 ₁ 2 ₁ 2	P2 ₁ 2 ₁ 2
Cell dimensions			
a, b, c (Å)	124.8, 109.0, 123.1	118.9, 120.0, 124.0	119.0, 120.8, 123.9
α, β, γ (°)	90.0, 90.0, 90.0	90.0, 90.0, 90.0	90.0, 90.0, 90.0
Resolution (Å)	50.0-2.6 (2.69-2.60)	50.0-2.0 (2.07-2.00)	50.0-2.0 (2.07-2.00)
R _{pim}	0.08 (0.31)	0.02 (0.33)	0.03 (0.44)
Ι/σΙ	9.8 (2.0)	48.1 (3.7)	42.4 (2.1)
CC1/2	0.9 (0.7)	0.9 (0.8)	0.9 (0.7)
Completeness (%)	97.9 (98.7)	99.7 (99.8)	99.2 (98.8)
Redundancy	3.4 (3.3)	9.4 (9.5)	9.2 (9.5)
Refinement			
Resolution (Å)	50.0-2.6	50.0-2.0	50.0-2.0
No. reflections	47055/0470	440444/5070	442005/5000
(work/test)	47255/2478	113414/5670	113905/5968
R _{work} / R _{free}	18.9/22.7	20.3/23.5	20.9/23.9
No. atoms			
Protein	10824	10824	10824
Zinc	6	6	6
Bicarbonate	24	-	-
Acetazolamide	-	-	78
PEG400	65	52	26
Calcium	15	15	16
Water	249	500	472
B factors (Å ²)			
Protein	43.7	48.3	55.3
Zinc	33.3	43.0	44.8
Bicarbonate	46.5	-	-
Acetazolamide	-	-	66.3
PEG	65.0	71.2	77.3
Calcium	50.7	61.5	68.3
Water	30.2	43.9	49.6
R.m.s. deviations			
Bond lengths (Å)	0.013	0.021	0.019
Bond angles (°)	1.814	1.089	2.167
Ramachandran plot (%)			
Most favored	89.5	91.1	92.0
Allowed	10.0	8.4	7.5
Generously allowed	0.5	0.5	0.5
Disallowed	0	0	0

Supplementary Table S1. Data collection and refinement statistics

*Numbers in parentheses were calculated with data in the highest resolution shell.