Supporting Information

Alvizo et al. 10.1073/pnas.1411461111

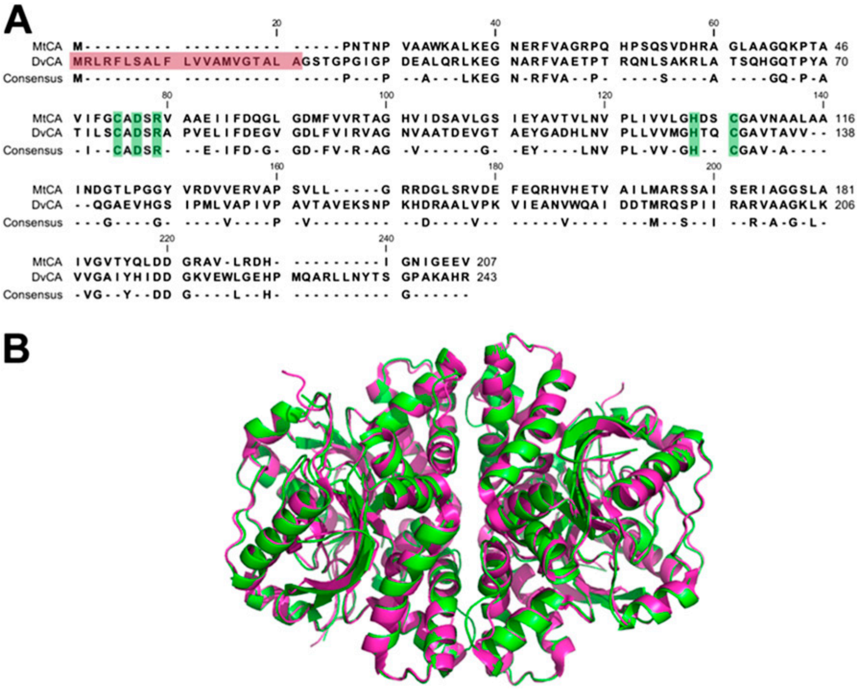

Fig. S1. (*A*) Sequence alignment between the β-carbonic anhydrase from *Mycobacterium tuberculosis* (MtCA) and *Desulfovibrio vulgaris*. The signal peptide from DvCA is highlighted in red and crucial catalytic and metal coordinating residues are highlighted in green. (*B*) The homology model of DvCA shown in green overlaid with the crystal structure of MtCA (Protein Data Bank ID code 2A5V) shown in pink. The rmsd between the template and the model was calculated to be 0.65 Å.

A)

Round 2 BB	Position	Mutation	
T	4	F	
T	30	Q	R
R	31	P	
L	34	H	
A	40	L	
A	84	Q	
Q	119	M	
G	120	R	
T	139	M	
K	147	E	T

B)

Round 2 BB	Position	Mutation			
R	16	S			
R	31	P			
A	40	W			
A	95	V			
A	121	K	L	V	W
V	131	F			
T	139	K	Q		
N	145	F	L	W	
K	147	E	T		
E	159	H	V		
N	213	E			
A	221	C			

C)

Round 2 BB	Position	Mutation		
T	4	F		
Q	32	R		
K	37	R		
Q	43	M		
A	60	C		
D	96	K	E	A
T	139	M		
E	142	L		
S	144	L		
H	148	T		
M	170	F		
E	200	R		
H	222	C		
R	223	C		

D)

Round 2 BB	Position	Mutation	
S	35	A	
S	42	A	
T	47	R	
E	68	A	
V	70	I	
A	95	V	
H	97	F	
H	124	G	R
V	138	L	
S	144	L	
V	157	A	
D	168	E	
A	219	T	
A	221	C	

Fig. S2. Round 2 libraries incorporated diversity from the first round of saturation mutagenesis. Library *A* is the elite library with a theoretical size of 2,304. *B*, *C*, and *D* are lower-tiered libraries with theoretical size of 69,120; 32,768; and 24,576, respectively. The accumulated size of the entire set was calculated at 128,768 total variants.

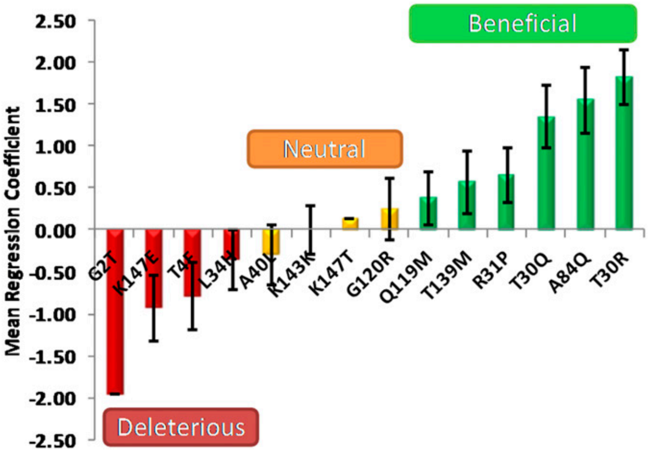

Fig. S3. Elite round 2 combinatorial library ProSAR analysis results. The A84Q mutation was predicted to be one of the most beneficial changes from this library. The G2T mutation was a random mutation not initially included in the library and the K143K mutations represent a silent codon change at that position.

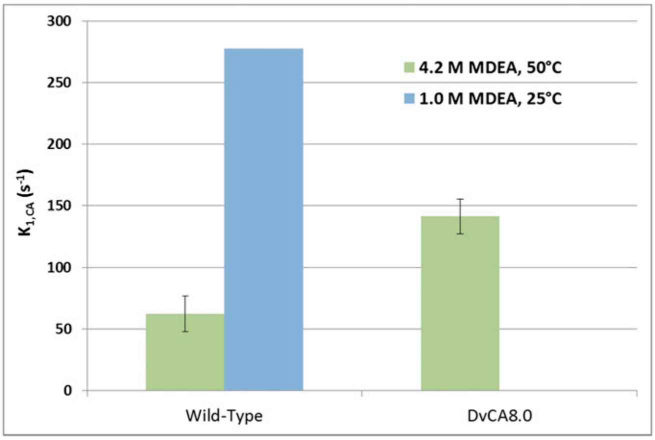

Fig. S4. Initial stirred cell reactor rate data for the wild-type enzyme and the DvCA8.0 evolved variant. At 4.2 M MDEA and 50 °C, the relatively higher activity of DvCA8.0 relative to the wild-type enzyme is attributed to the wild type's instability at higher MDEA concentration and temperature. However, comparison of the wild-type's activity in 1.0 M MDEA and 25 °C shows that it is more active than the evolved DvCA8.0 variant.

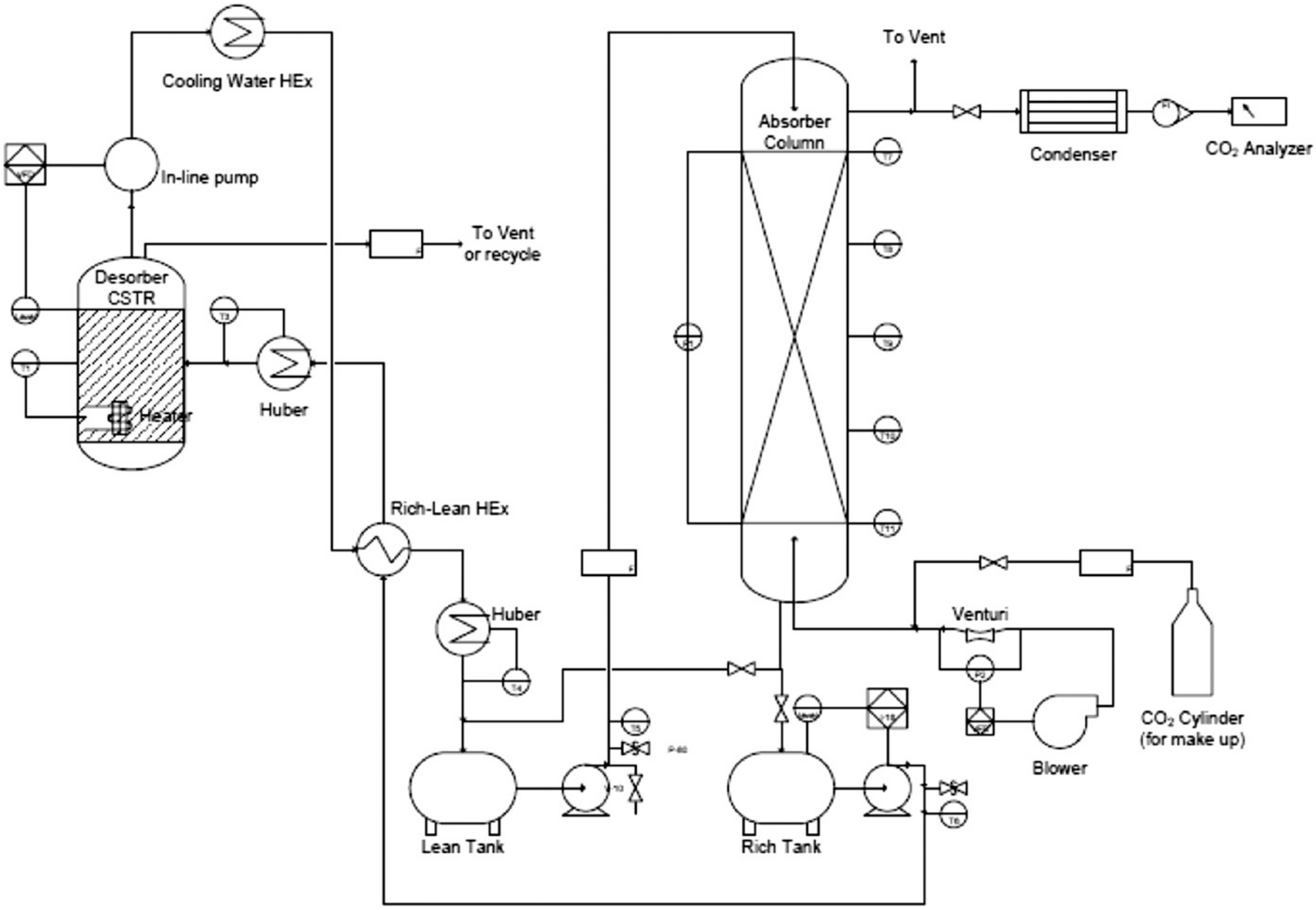

Fig. S5. Pilot test unit was custom-built for in-house testing and easy transport and installation at the National Carbon Capture Center. It was designed to be a small-scale amine scrubber–stripper system operating over a flexible range of process conditions. Solvent flow rates were variable from 1 to 5 LPM and flue-gas flow rates from 30 to 500 SLPM. Absorber temperature was controlled via the lean feed temperature, from 20 to 40 °C. The desorber temperature could be controlled up to 120 °C. The system was typically operated to capture ~150 lb CO_2/d at steady-state conditions. The absorber column was 150 mm in diameter and had 6.3 m of total packed height using 16-mm pall rings.

Table S1. Round 1 beneficial sequence diversity

WT AA	Position	Mutation	Δ Charge	FIOPC*	SD
G	2	R	1	1.42	0.13
		T		1.72	0.03
T	4	F		2.16	0.03
R	16	S	−1	1.78	0.04
T	30	L		1.38	0.09
		A		1.44	0.04
		Q		2.23	0.17
T	30	R	1	3.04	0.35
R	31	P	−1	1.7	0.08
Q	32	R	1	1.66	0.08
		K	1	1.89	0.07
L	34	H		1.98	0.28
S	35	R	1	1.3	0.07
		A		1.39	0.02
K	37	R		1.58	0.03
A	40	L		1.36	0.09
		W		1.8	0.09
Q	43	V		1.19	0.01
		M		1.45	0.03
T	47	R	1	1.29	0.04
A	56	S		1.94	0.04
A	60	C		1.43	0.01
E	68	G	1	1.16	0.04
		A	1	1.38	0.07
V	70	I		1.36	0.09
A	84	R	1	1.23	0.13
		S		1.52	0
		N		1.67	0.01
		Q		2.32	0.08
Y	93	W		1.1	0.01
A	95	V		1.54	0.09
D	96	K	2	1.2	0.1
		A	1	1.29	0.06
		E		1.38	0.03
		C	1	1.49	0.04
H	97	F		1.4	0.09
Q	119	T		1.33	0.03
		M		2.05	0.01
		K	1	1.28	0.02
G	120	R	1	2.31	0.06
A	121	W		1.56	0.05
		V		1.48	0.05
		T		1.3	0.04
		Q		1.49	0.1
		L		1.3	0.06
		K	1	1.65	0.05
		H		1.42	0.07
H	124	R	1	1.24	0.05
		G		1.3	0.07
V	131	L		1.96	0.11
		F		1.15	0.04
V	138	L		1.29	0.06
T	139	Q		1.65	0.01
		M		2.44	0.11
		K	1	1.64	0.04
		H		1.31	0.05
E	142	L	1	1.72	0.05
S	144	L		1.68	0.01
		A		1.3	0.05
N	145	W		1.78	0.03
		F		1.62	0.08
		C		1.29	0.08
K	147	T	−1	1.9	0.07

Table S1. Cont.

WT AA	Position	Mutation	Δ Charge	FIOPC*	SD
		G	−1	1.47	0.01
		F	−1	1.21	0.11
		E	−2	3.31	0.07
H	148	T		1.63	0.05
		A		1.25	0.08
V	157	A		1.15	0.07
E	159	V	1	1.27	0.06
		R	2	1.21	0.05
		H	1	1.67	0.05
D	168	E		1.25	0.06
M	170	F		1.83	0.05
A	178	G		1.22	0.02
E	200	R	2	1.49	0.05
M	207	H		1.13	0.07
		E	−1	1.11	0.02
N	213	Q		1.29	0.13
		E	−1	1.74	0.02
A	221	C		1.61	0.12
H	222	C		1.94	0.15
R	223	Q	−1	1.11	0.05
		C	−1	1.49	0.02

*Fold improvement over the positive control (wild-type enzyme).

Table S2. High-throughput screening conditions

		Challenge				Assay				
Evolution round	Parent	MDEA, M	Temperature, °C	pH	Duration of challenge, h	MDEA, mM	pH	Temperature, °C	$KHCO_3$, mM	Phenolphthalein, μM
1	DvCA	3.0	42.0	10.0	24	300	8.0	25	200	400
2	DvCA 2.0	4.0	50.0	10.0	24	300	8.0	45	200	400
3	DvCA 3.0	4.2	65.0	10.5	24	1,000	8.0	45	300	400
4	DvCA 4.0	4.2	70.0	10.5	24	500	8.0	45	400	400
5	DvCA 5.0	4.2	82.5	10.5	24	750	8.0	45	300	1,100
6	DvCA 6.0	4.2	87.0	10.5	24	500	8.8	25	300	500
7	DvCA 7.0	4.2	101.0	10.5	1	500	8.8	25	300	500
8	DvCA 8.0	4.2	103.0	10.5	1	500	8.8	25	300	500
9	DvCA 9.0	4.2	107.0	10.5	1	500	8.8	25	300	500