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# Comparison of Carbonic Anhydrases for $CO_2$ Sequestration

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## 1. Original SDS-PAGE of CAs in Cleared Cell Lysate and After Purification

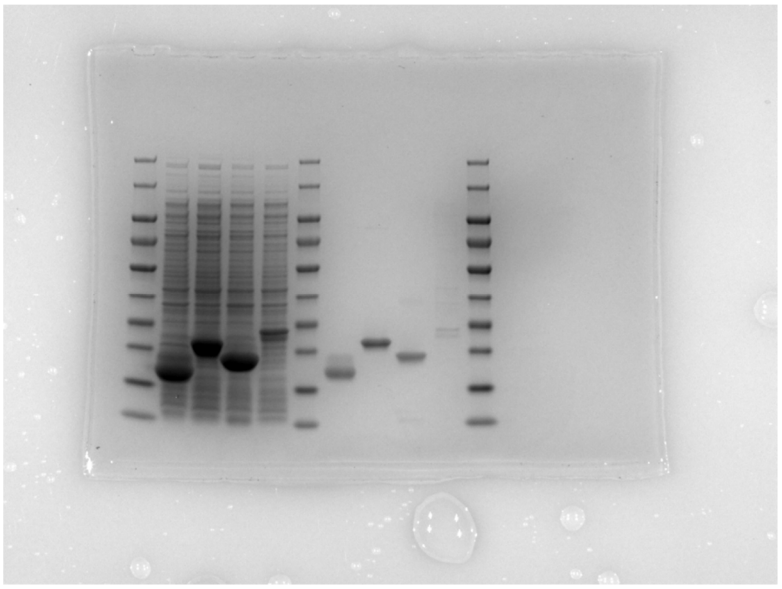

**Figure S1.** original SDS-PAGE of CAs expressed in *E. coli*. Samples of cleared cell lysate and after purification by affinity chromatography.

## 2. SDS-PAGEs of Timepoints During Expression and Purification of CAs

*2.1. AwCA*

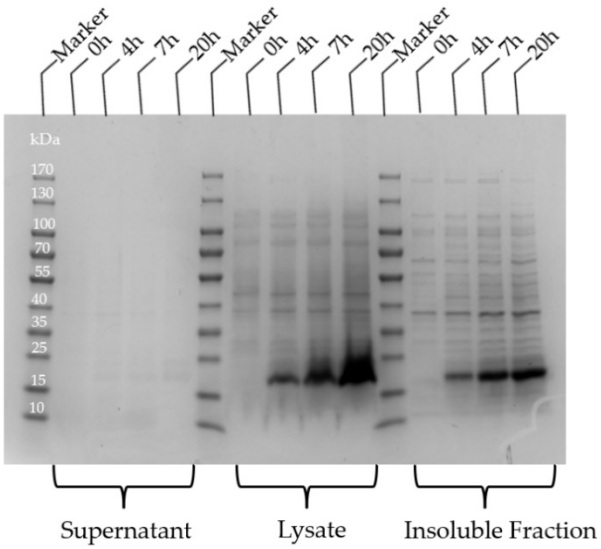


**Figure S2.** SDS-PAGE of supernatant, lysate and insoluble fraction during expression of AwCA in *E. coli*. Expected molecular weight is 22.0 kDa (AwCA).

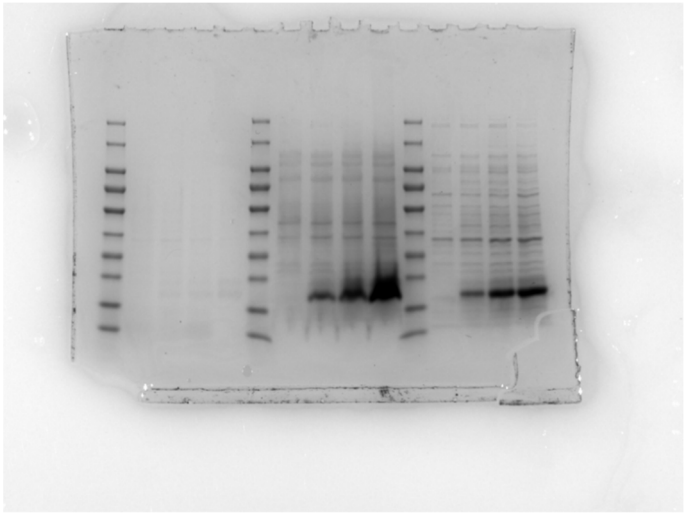

**Figure S3.** original SDS-PAGE of supernatant, lysate and insoluble fraction during expression of AwCA in *E. coli*.

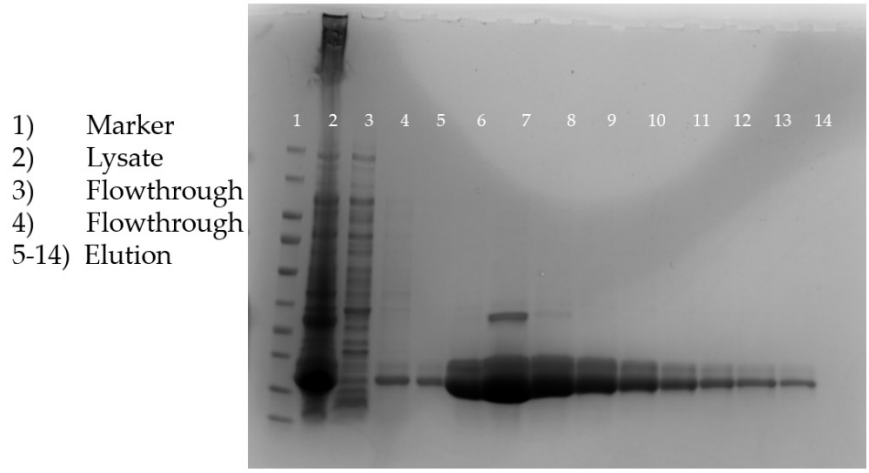


**Figure S4.** SDS-PAGE of lysate (2), flowthrough (3&4) and elution fractions (5-14) during purification of AwCA by affinity chromatography.

*2.2. PmCA*

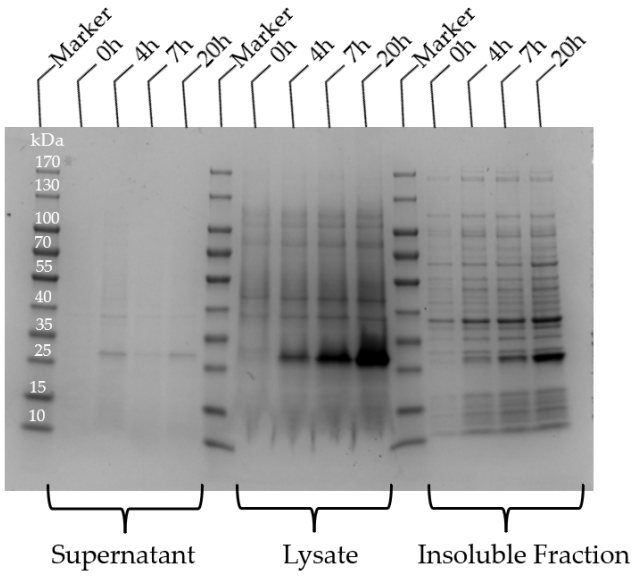


**Figure S5.** SDS-PAGE of supernatant, lysate and insoluble fraction during expression of PmCA in *E. coli*. Expected molecular weight is 26.9 kDa (PmCA).

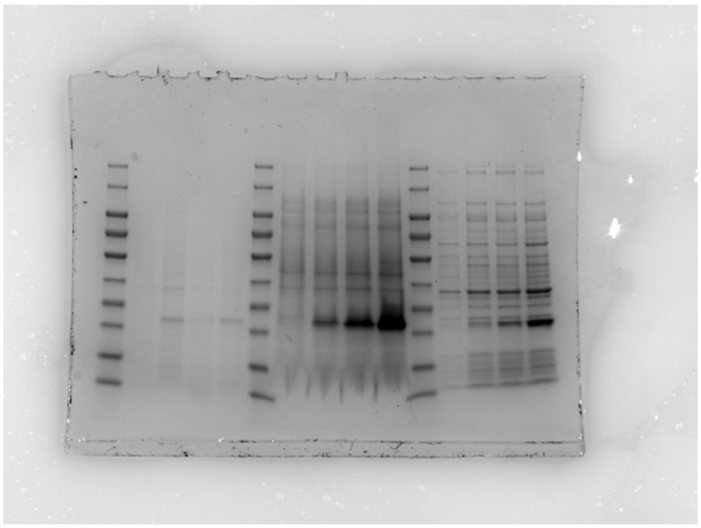

**Figure S6.** original SDS-PAGE of supernatant, lysate and insoluble fraction during expression of PmCA in *E. coli*.

1) Marker
2) Lysate
3) Flowthrough
4-13) Elution

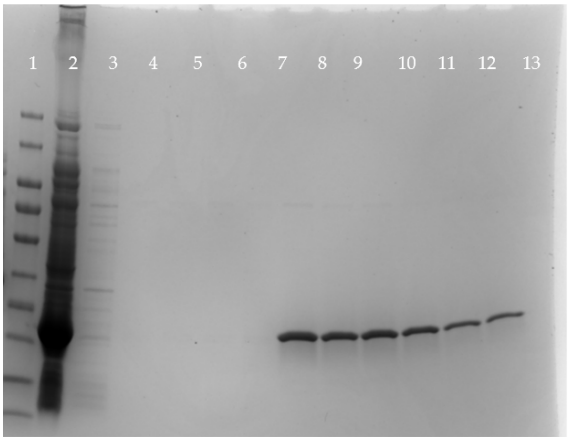

**Figure S7.** SDS-PAGE of lysate (2), flow through (3) and elution fractions (4-13) during purification of PmCA by affinity chromatography.

*2.3. MtaCA*

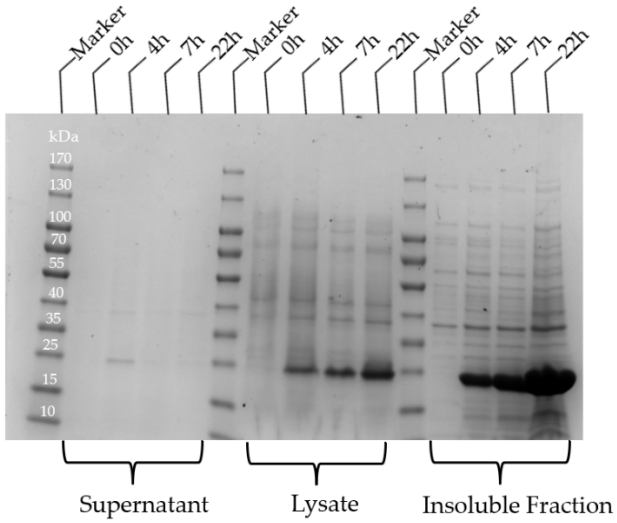


**Figure S8.** SDS-PAGE of supernatant, lysate and insoluble fraction during expression of MtaCA in *E. coli*. Expected molecular weight is 19.9 kDa (MtCA).

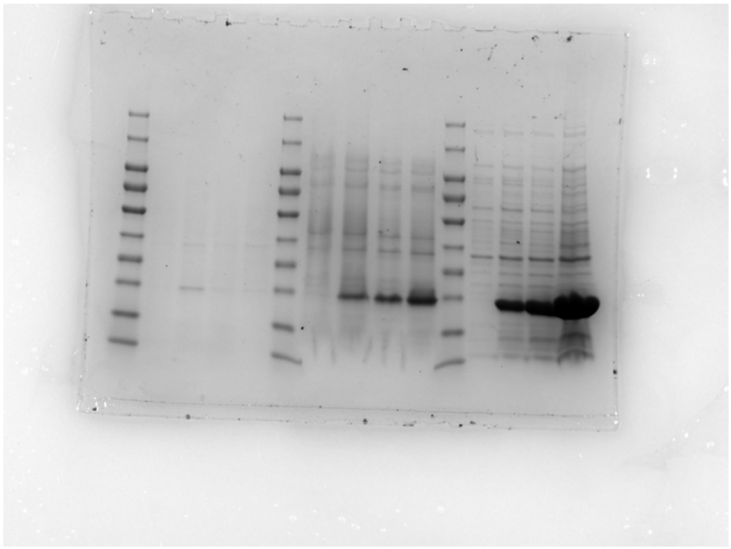

**Figure S9.** original SDS-PAGE of supernatant, lysate and insoluble fraction during expression of MtaCA in *E. coli*.

1) Marker
2) Lysate
3) Flowthrough
4-13) Elution

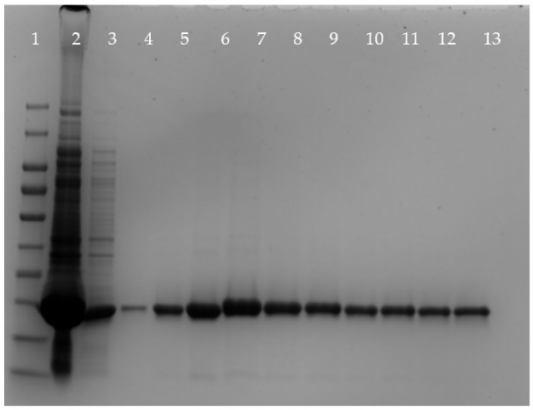

**Figure S10.** SDS-PAGE of lysate (2), flow through (3) and elution fractions (4-13) during purification of MtaCA by affinity chromatography.

*2.4. SspCA*

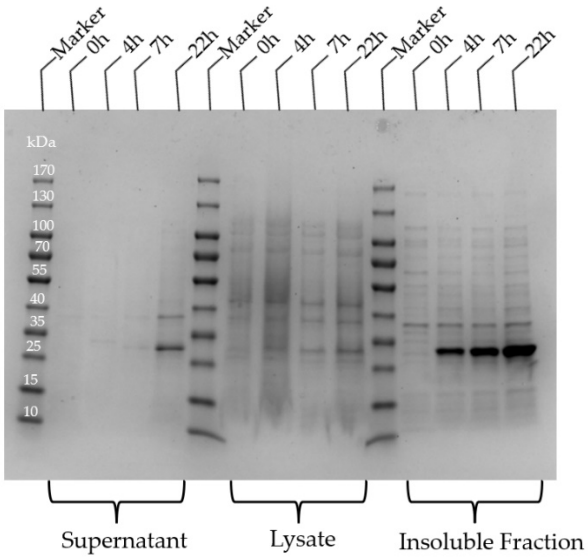


**Figure S11.** SDS-PAGE of supernatant, lysate and insoluble fraction during expression of SspCA in *E. coli*. Expected molecular weight is 27.5 kDa (SspCA).

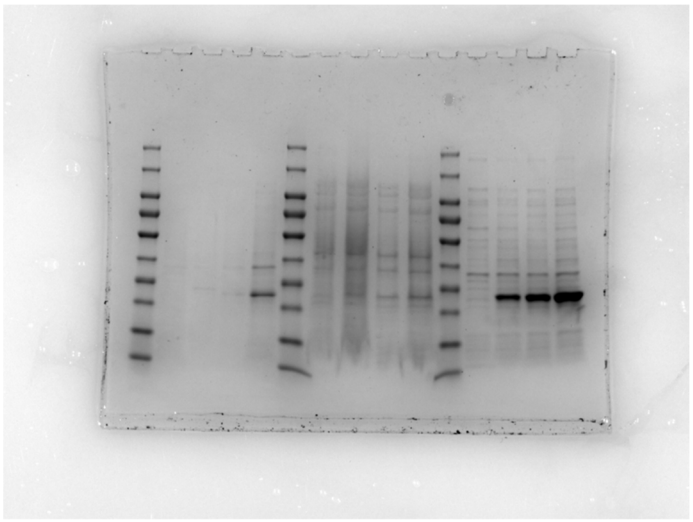

**Figure S12.** original SDS-PAGE of supernatant, lysate and insoluble fraction during expression of SspCA in *E. coli*.

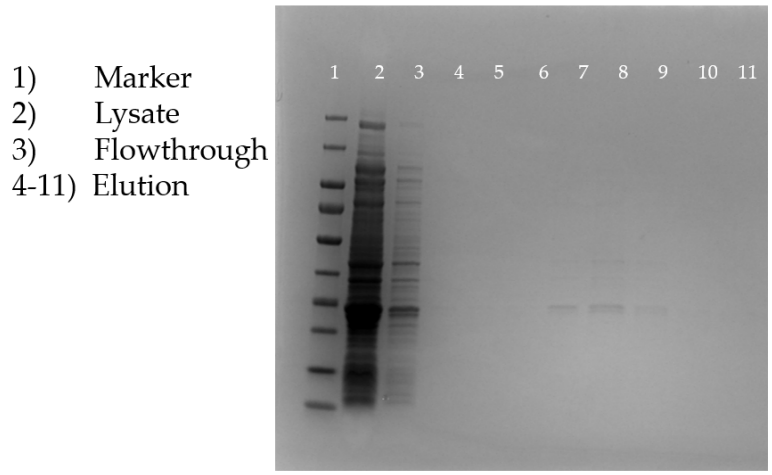


**Figure S13.** SDS-PAGE of lysate (2), flow through (3) and elution fractions (4-11) during purification of SspCA by affinity chromatography.

### 3. Protein Sequence Alignment of CAs as Expressed in this study

```
                 ....|....| ....|....| ....|....| ....|....| ....|....|
                         10         20         30         40         50
PmCA             --------MG GGWSYHGEHG PEHWGDLKDE YIMCKIGKNQ SPVDINR--I
SspCA            MWSHPQFEKE HEWSYEGEKG PEHWAQLKPE FFWCKL-KNQ SPINIDKKYK
Consensus                   WSY GE G PEHW  LK E    CK  KNQ SP  I

                 ....|....| ....|....| ....|....| ....|....| ....|....|
                         60         70         80         90        100
PmCA             VDAKLKPIKI EYRAG-ATKV LNNGHTIKVS YEPGSYIVVD GIKFELKQFH
SspCA            VKANLPKLNL YYKTAKESEV VNNGHTIQIN IKEDNTLNYL GEKYQLKQFH
Consensus        V A L       Y       V  NNGHTI              G K  LKQFH

                 ....|....| ....|....| ....|....| ....|....| ....|....|
                        110        120        130        140        150
PmCA             FHAPSEHKLK GQHYPFEAHF VHADKHGNLA VIGVFFKEGR ENPILEKIWK
SspCA            FHTPSEHTIE KKSYPLEIHF VHKTEDGKIL VVGVMAKLGK TNKELDKILN
Consensus        FH PSEH       YP E HF VH    G    V GV  K G   N  L KI

                 ....|....| ....|....| ....|....| ....|....| ....|....|
                        160        170        180        190        200
PmCA             VMPENAGEEV KLAHKINAED LLPKDRDYYR YSGSLTTPPC SEGVRWIVME
SspCA            VAPAEEGEKI -LDKNLNLNN LIPKDKRYMT YSGSLTTPPC TEGVRWIVLK
Consensus        V P   GE    L    N    L PKD  Y   YSGSLTTPPC  EGVRWIV

                 ....|....| ....|....| ....|....| ....|....| ....
                        210        220        230        240
PmCA             EEMEMSKEQI EKFRKIMGGD TNRPVQPLNA RMIMEKWSHP QFEK
SspCA            KPISISKQQL EKLKSVMVNP NNRPVQEINS RWIIEGF--- ----
Consensus             SK Q  EK    M     NRPVQ  N  R I E
```

**Figure S14:** ClustalW Multiple Alignment (BioEdit Sequence Aligment Editor v7.0.5) of α-CAs from *Persephonella marina* (PmCA) and *Sulphurihydrogenibium yellowstonense* (SspCA) as expressed in this study. Orange: StrepTag. Grey background highlights the zinc-coordinating histidines, red background indicates the proton shuttle residues and blue background shows the „gate-keeper" residues of α-CAs.

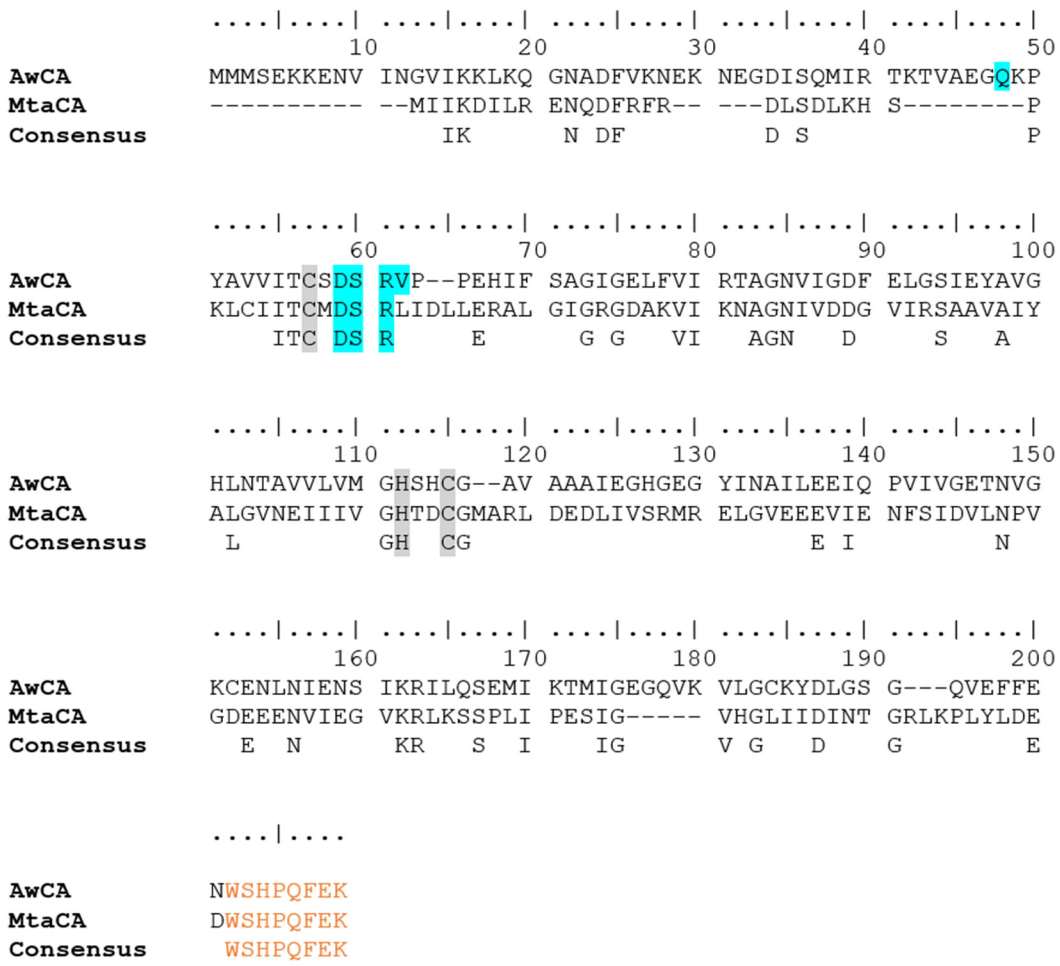


**Figure S15:** ClustalW Multiple Alignment (BioEdit Sequence Aligment Editor v7.0.5) of β-CAs from *Methanobacterium thermoautotrophicum* (MtaCA) and *Acetobacterium woodii* (AwCA). Orange: StrepTag. Grey background highlights the zinc-coordinating histidines and blue background indicates amino acids which are involved in the catalytic reaction.

## 4. Codon Optimized CA Gene Sequences

### AwCA-StrepTag

3`-CATATG

ATGATGAGCGAGAAGAAGGAGAACGTGATCAACGGTGTGATTAAGAAGCTGAAGCAAGGCAACGCGGACTTTGTG

AAGAACGAGAAAAACGAGGGCGACATCAGCCAGATGATTCGTACCAAGACCGTGGCGGAAGGTCAAAAACCGTAC

GCGGTGGTTATCACCTGCAGCGATAGCCGTGTTCCGCCGGAGCACATCTTCAGCGCGGGTATTGGCGAACTGTTT

GTGATCCGTACCGCGGGCAACGTTATTGGTGACTTCGAGCTGGGCAGCATCGAATACGCGGTGGGTCACCTGAAC

ACCGCGGTGGTTCTGGTGATGGGTCACAGCCATTGCGGTGCGGTTGCGGCGGCGATTGAGGGTCATGGTGAAGGT

TATATCAACGCGATTCTGGAGGAAATCCAGCCGGTGATTGTTGGCGAAACCAACGTTGGCAAGTGCGAGAACCTG

AACATCGAAAACAGCATCAAGCGTATTCTGCAGAGCGAGATGATCAAAACCATGATTGGCGAAGGTCAAGTGAAG

GTTCTGGGCTGCAAATATGATCTGGGTAGCGGTCAGGTTGAGTTCTTTGAAAACTGGAGCCATCCGCAATTTGAA

AAATAA

AAGCTT-5`

**PmCA-StrepTag**

3`-CATATG

GGCGGCGGTTGGAGCTACCACGGCGAGCATGGCCCGGAGCATTGGGGTGACCTGAAGGACGAGTACATTATGTGC

AAGATCGGCAAGAACCAGAGCCCGGTGGACATCAACCGTATTGTTGATGCGAAGCTGAAACCGATCAAGATTGAG

TACCGTGCGGGCGCGACCAAAGTGCTGAACAACGGTCACACCATCAAGGTTAGCTACGAACCGGGCAGCTATATC

GTGGTTGACGGTATTAAGTTCGAACTGAAACAGTTCCACTTTCACGCGCCGAGCGAGCACAAGCTGAAAGGCCAA

CACTATCCGTTCGAAGCGCACTTTGTGCACGCGGATAAACACGGTAACCTGGCGGTGATTGGCGTTTTCTTTAAG

GAAGGTCGTGAGAACCCGATCCTGGAAAAGATTTGGAAAGTGATGCCGGAGAACGCGGGCGAGGAAGTTAAGCTG

GCGCACAAAATCAACGCGGAGGACCTGCTGCCGAAAGACCGTGATTACTATCGTTATAGCGGCAGCCTGACCACC

CCGCCGTGCAGCGAGGGTGTGCGTTGGATTGTTATGGAGGAAGAGATGGAAATGAGCAAGGAACAAATCGAGAAG

TTTCGTAAAATTATGGGTGGCGATACCAACCGTCCGGTTCAACCGCTGAATGCGCGTATGATTATGGAGAAATGG

AGCCACCCGCAATTTGAGAAGTAA

AAGCTT-5`

**MtaCA-StrepTag**

3`-CATATG

CATCAAAGACATCCTGCGTGAGAACCAAGATTTCCGTTTCCGTGACCTGAGCGACCTGAAGCATAGCCCGAAGCT

GTGCATCATTACCTGCATGGACAGCCGTCTGATCGATCTGCTGGAACGTGCGCTGGGTATTGGCCGTGGTGACGC

GAAAGTGATTAAAAACGCGGGCAACATCGTGGACGATGGTGTTATTCGTAGCGCGGCGGTTGCGATCTATGCGCT

GGGCGTGAACGAGATCATTATCGTTGGCCACACCGATTGCGGTATGGCGCGTCTGGACGAGGATCTGATCGTGAG

CCGTATGCGTGAACTGGGTGTGGAGGAAGAGGTTATTGAGAACTTCAGCATCGACGTGCTGAACCCGGTTGGCGA

TGAAGAGGAAAACGTGATCGAAGGTGTTAAGCGTCTGAAAAGCAGCCCGCTGATTCCGGAGAGCATCGGCGTTCA

CGGTCTGATTATCGACATTAACACCGGTCGCCTGAAACCGCTGTATCTGGACGAAGACTGGAGCCATCCGCAATT

TGAGAAGTAA

AAGCTT-5`

**StrepTag-SspCA**

3`-CATATG

TGGAGCCACCCGCAATTTGAAAAGGAGCATGAGTGGAGCTACGAAGGCGAGAAAGGCCCGGAGCATTGGGCGCAA

CTGAAGCCGGAGTTTTTCTGGTGCAAACTGAAGAACCAGAGCCCGATCAACATTGACAAGAAATACAAGGTGAAA

GCGAACCTGCCGAAACTGAACCTGTACTATAAGACCGCGAAAGAGAGCGAAGTGGTTAACAACGGCCACACCATC

CAAATTAACATCAAAGAGGACAACACCCTGAACTACCTGGGTGAAAAATATCAGCTGAAGCAATTCCACTTTCAC

ACCCCGAGCGAGCACACCATTGAAAAGAAAAGCTATCCGCTGGAGATCCACTTCGTGCACAAAACCGAAGACGGC

AAGATTCTGGTGGTTGGCGTTATGGCGAAGCTGGGTAAAACCAACAAGGAGCTGGATAAAATTCTGAACGTGGCG

CCGGCGGAGGAAGGCGAAAAAATTCTGGACAAGAACCTGAACCTGAACAACCTGATCCCGAAGGATAAACGTTAC

ATGACCTATAGCGGCAGCCTGACCACCCCGCCGTGCACCGAGGGTGTGCGTTGGATTGTTCTGAAGAAACCGATT

AGCATCAGCAAGCAGCAACTGGAAAAGCTGAAAAGCGTGATGGTTAACCCGAACAACCGTCCGGTTCAGGAGATT

AATAGCCGTTGGATCATCGAAGGTTTCTAA

AAGCTT-5`

## 5. Calculated Hydratase Activities for Thermostability Experiments

**Table S1.** Calculated hydratase activities at 25 °C for AwCA and PmCA after preincubation for thermostability determination in turnover rate per mg enzyme and WAU per mg enzyme.

| Tempe-rature | Incubation Time | AwCA | | | PmCA | | |
|---|---|---|---|---|---|---|---|
| | | Total turnover rate μmol $s^{-1}$ $L^{-1}$ | Turnover rate per mg enzyme μmol $s^{-1}$ $mg^{-1}$ | WAU per mg enzyme | Total turno-ver rate μmol $s^{-1}$ $L^{-1}$ | Turnover rate per mg enzyme μmol $s^{-1}$ $mg^{-1}$ | WAU per mg enzyme |
| 30 °C | 96 h | 1417 ± 37 | 2580 ± 75 | 1400 ± 165 | 1552 ± 59 | 3123 ± 120 | 2079 ± 327 |
| | 120 h | 1408 ± 63 | 2349 ± 137 | 1368 ± 279 | 1530 ± 36 | 2837 ± 90 | 1956 ± 190 |
| | 144 h | 1414 ± 40 | 2520 ± 99 | 1388 ± 185 | 1548 ± 32 | 3057 ± 86 | 2052 ± 175 |
| 40 °C | 96 h | 1289 ± 42 | 2068 ± 86 | 873 ± 160 | 1574 ± 11 | 3211 ± 26 | 2193 ± 58 |
| | 120 h | 1222 ± 35 | 1606 ± 89 | 640 ± 118 | 1495 ± 41 | 2695 ± 99 | 1774 ± 208 |
| | 144 h | 1199 ± 44 | 1662 ± 105 | 567 ± 136 | 1540 ± 8 | 3024 ± 60 | 2005 ± 44 |
| 50 °C | 24 h | 1262 ± 31 | 1944 ± 66 | 775 ± 111 | 1485 ± 25 | 2833 ± 55 | 1721 ± 124 |
| | 48 h | 1090 ± 22 | 1115 ± 54 | 258 ± 53 | 1501 ± 31 | 2757 ± 64 | 1804 ± 156 |
| | 72 h | 888 ± 28 | 489 ± 64 | −105 ± 34 | 1480 ± 12 | 2859 ± 40 | 1700 ± 60 |
| | 96 h | 868 ± 3 | 387 ± 17 | −128 ± 3 | 1507 ± 8 | 2942 ± 23 | 1833 ± 42 |
| | 120 h | 833 ± 26 | 47 ± 75 | −159 ± 20 | 1474 ± 28 | 2610 ± 78 | 1667 ± 138 |
| | 144 h | 813 ± 10 | 116 ± 61 | −175 ± 6 | 1502 ± 9 | 2874 ± 60 | 1810 ± 45 |
| 60 °C | 24 h | 1116 ± 55 | 1204 ± 110 | 328 ± 138 | 1532 ± 28 | 2868 ± 59 | 1964 ± 149 |
| | 48 h | 790 ± 24 | −202 ± 64 | −188 ± 12 | 1490 ± 12 | 2598 ± 49 | 1746 ± 62 |
| | 72 h | n. a. | n. a. | n. a. | 1436 ± 9 | 2757 ± 20 | 1487 ± 41 |
| | 96 h | n. a. | n. a. | n. a. | 1396 ± 2 | 2477 ± 35 | 1307 ± 8 |
| | 120 h | n. a. | n. a. | n. a. | 1524 ± 14 | 2858 ± 37 | 1921 ± 71 |
| | 144 h | n. a. | n. a. | n. a. | 1445 ± 49 | 2090 ± 105 | 1532 ± 231 |
| 70 °C | 24 h | n. a. | n. a. | n. a. | 1560 ± 33 | 3153 ± 68 | 2114 ± 182 |
| | 48 h | n. a. | n. a. | n. a. | 1577 ± 17 | 3030 ± 38 | 2205 ± 96 |
| | 72 h | n. a. | n. a. | n. a. | 1469 ± 10 | 2629 ± 24 | 1644 ± 51 |
| | 96 h | n. a. | n. a. | n. a. | 1477 ± 36 | 2798 ± 79 | 1684 ± 176 |
| | 120 h | n. a. | n. a. | n. a. | 1436 ± 10 | 2461 ± 57 | 1488 ± 45 |
| | 144 h | n. a. | n. a. | n. a. | 1418 ± 26 | 2535 ± 77 | 1403 ± 117 |
| 80 °C | 24 h | n. a. | n. a. | n. a. | 1400 ± 27 | 2486 ± 61 | 1326 ± 119 |
| | 48 h | n. a. | n. a. | n. a. | 1377 ± 33 | 2195 ± 69 | 1225 ± 140 |
| | 96 h | n. a. | n. a. | n. a. | 1413 ± 29 | 2512 ± 59 | 1385 ± 130 |
| | 120 h | n. a. | n. a. | n. a. | 1066 ± 18 | 979 ± 65 | 200 ± 41 |
| | 144 h | n. a. | n. a. | n. a. | 1148 ± 37 | 1451 ± 79 | 412 ± 104 |