Intrinsic Proton Transfer Activation of L-DOPA Encoded Carbonic Anhydrase for Efficient CO_2 Sequestration.

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Supporting Information

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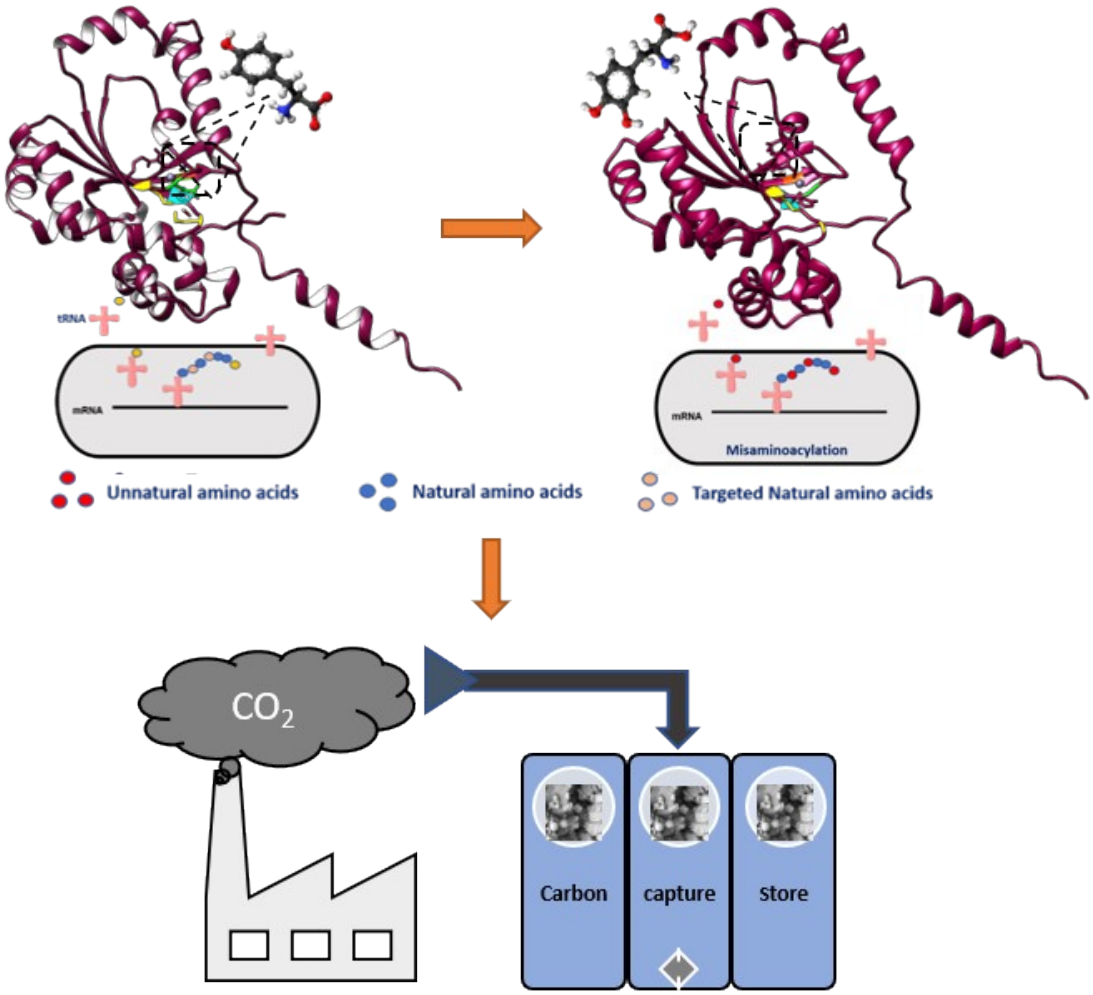
1. Graphical Abstract



Scheme 1. Graphical abstract of L-DOPA encoded carbonic anhydrase through genetic code expansion method for CO_2 sequestration.

2. Results and Discussion

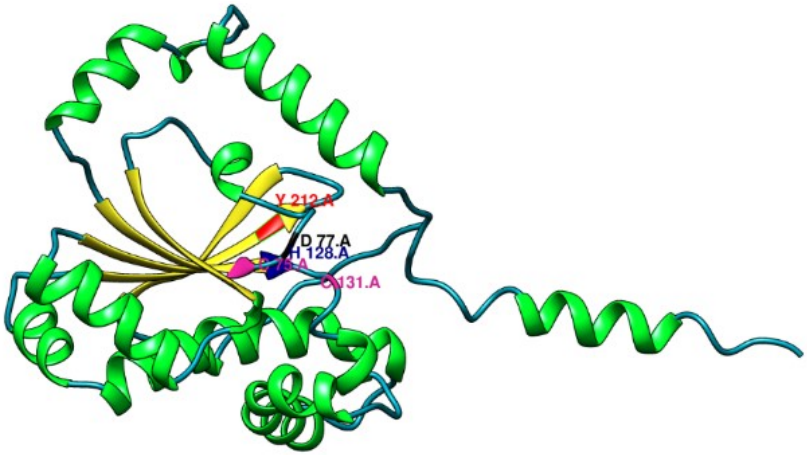


Fig. S1 Alphafold2 Predicted structure of CA visualized in UCSF Chimera v 1.18

The presence of DOPA (DAH) at 212 position (instead of tyrosine) is hypothesized to enhance enzymatic activity by improving proton transfer efficiency and stabilizing interactions within the active site cleft, particularly through its ability to facilitate hydrogen bonding and hydrophilic interactions.

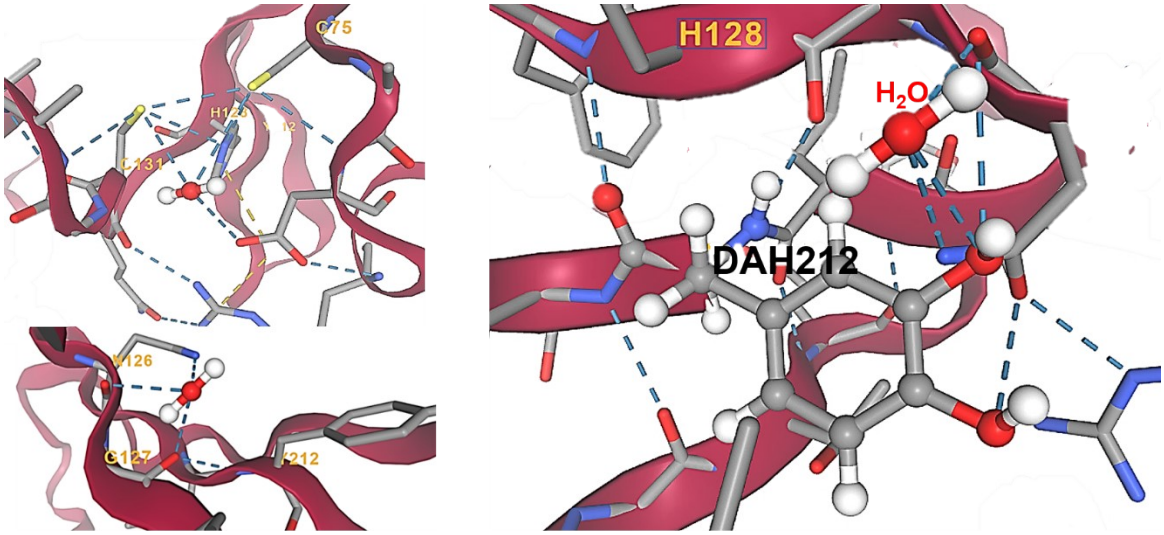


Fig. S2 Molecular interaction prediction between active site and cleft residues with water molecule through SwissDock – Autodock vina. Calculated affinity of water molecule with rCA and rDCA is -1.01 kcal/mol and -1.35 kcal/mol respectively.

The congener DOPA encoded CA enzymes (rDCA) were purified using Ni-NTA affinity column and desalted using G25 Sephadex column chromatography in AKTA FPLC system. The desalted enzymes were concentrated and observed at ~28 kDa on SDS-PAGE. In NBT, the positive control L-DOPA (lane 1) and activator-incorporated CA (rDCA) (lane 2) showed a purple spot during NBT staining (Fig. S3), indicating the presence of quinone substances that catalyze redox cycling at alkaline pH 10 with glycine in NBT as a reductant.

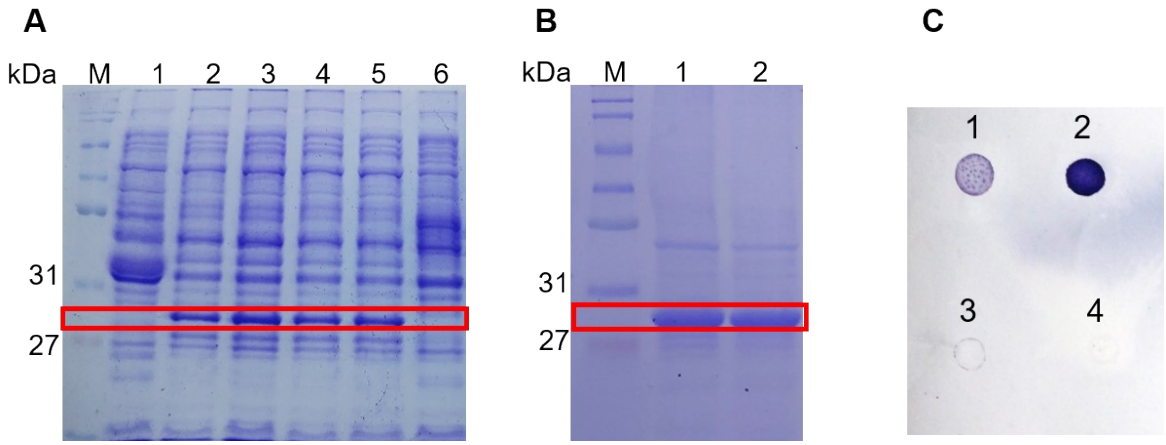


Fig. S3 (A) Expression of rCA and rDCA. (B) Purified rCA and rDCA. (C) Confirmation of DOPA by NBT staining.

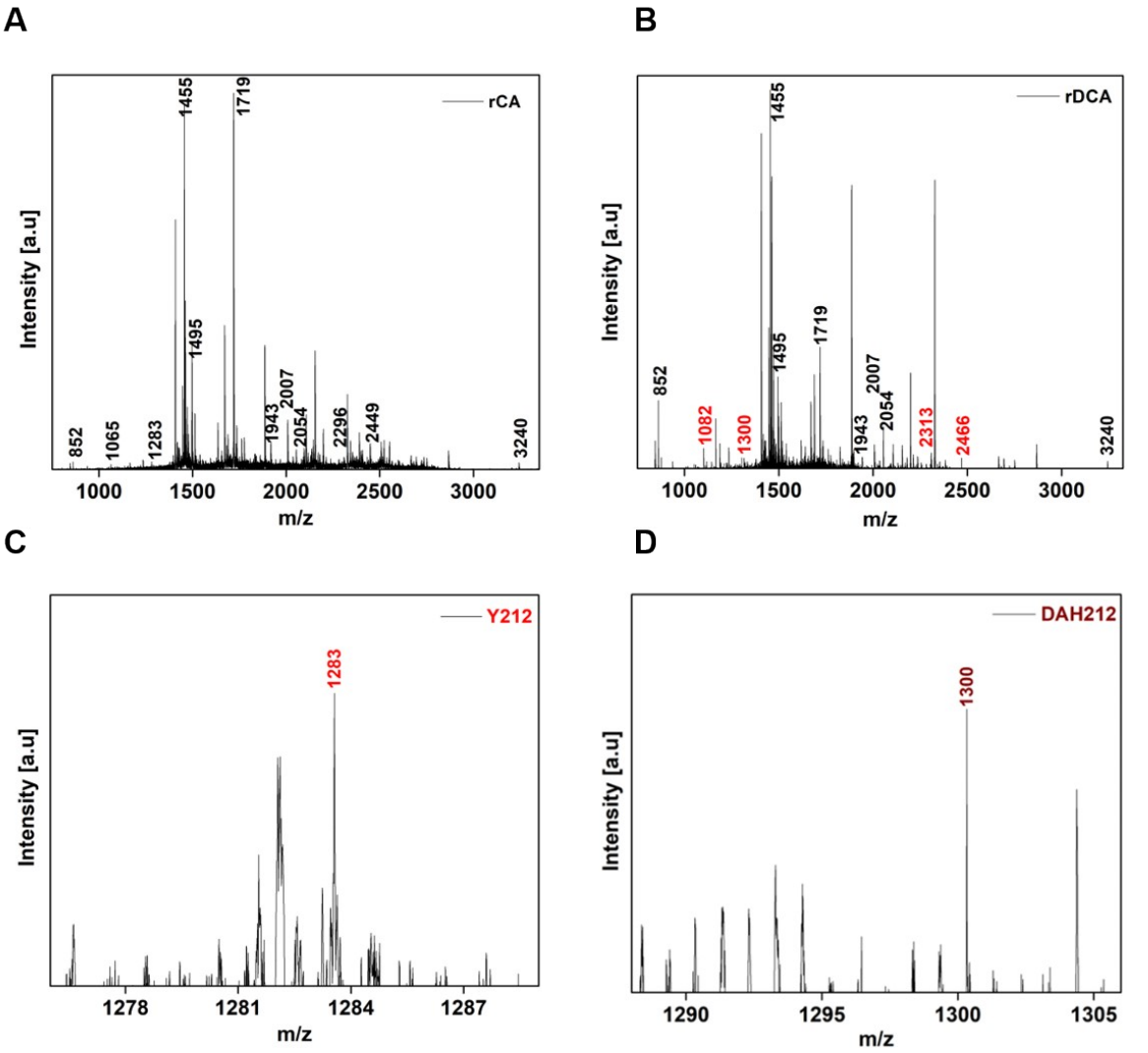


Fig. S4 Confirmation of DOPA incorporation in CA from tryptic digested rCA and rDCA peptides through MALDI-ToF-ToF. (A) Mass of ingel digested rCA peptides. (B) Mass of ingel digested rDCA peptides. (C) Peptide mass containing tyrosine at 212th position in rCA. (D) Peptide mass containing DOPA replace tyrosine at 212th position in rDCA.

Enzyme activity assay

$$CA\ Activity\ (W-A\ Units) = \frac{T_b - T_s}{T_s} * d.f\ \frac{U}{mg} \quad (1)$$

Where T_b and T_s refer to the time taken for the blank and sample to reach stationary phase at OD_{430} respectively, d.f represents the dilution factor used in the assay reaction. This activity was estimated for all the samples including rCA, rDCA, and both with an external activator L-DOPA.

$$Turnover\ number\ (K_{cat}) = \frac{V_{max}}{[E]_o} \quad (2)$$

$$Catalytic\ efficiency = \frac{K_{cat}}{K_m} \quad (3)$$

Where, $[E]_o$ is the total enzyme concentration in the reaction mixture; K_{cat} is the turnover number of the enzyme and K_m is the michaelis constant (Enzyme – Substrate binding affinity).

Table S1. Modified activity assay of rCA and rDCA using time course measurement in UV spectroscopy.

Samples	Average Time (seconds)	Activity (U/mg)
Blank	64	-
rCA	25	78
rDCA	12	217

Enzyme kinetics

The observed tripling of enzymatic activity can be attributed to alterations in the microenvironment within the enzyme's active site cleft. The results suggest that the additional hydroxyl group introduced into the active site interacts with water molecules, thereby enhancing the rate of proton transfer. To further validate this enhancement, L-DOPA was externally added as an activator to the unmodified enzyme, and its kinetics were compared with the genetically encoded L-DOPA variant of recombinant carbonic anhydrase (rDCA). The rDCA variant exhibited superior enzymatic activity compared to the unmodified CA enzyme, both in the presence and absence of external L-DOPA activation.The comparative kinetics were carried out using rCA, rDCA, and rCA with an external activator (rCA + DOPA) to determine the efficiency of the enzyme-bound activator as well as the external activator.

Table S2. Kinetic parameters for DOPA incorporated CA, without incorporated CA, and with external activators respectively. Enzyme concentration used in the reaction for all the samples was 3.8×10^{-4} mM, DOPA concentration was 20μM used.

Samples	**V_{max} (W-A Units)**	**K_m (mM)**	**K_{cat} (S^{-1})**	**K_{cat}/K_m (mM^{-1} S^{-1})**
rDCA	370	20	9.6×10^5	4.8×10^4
rDCA + DOPA	166	33	2.1×10^5	0.65×10^4
rCA	181	40	1.84×10^5	0.69×10^4
rCA + DOPA	108	40	4.7×10^5	1.1×10^4

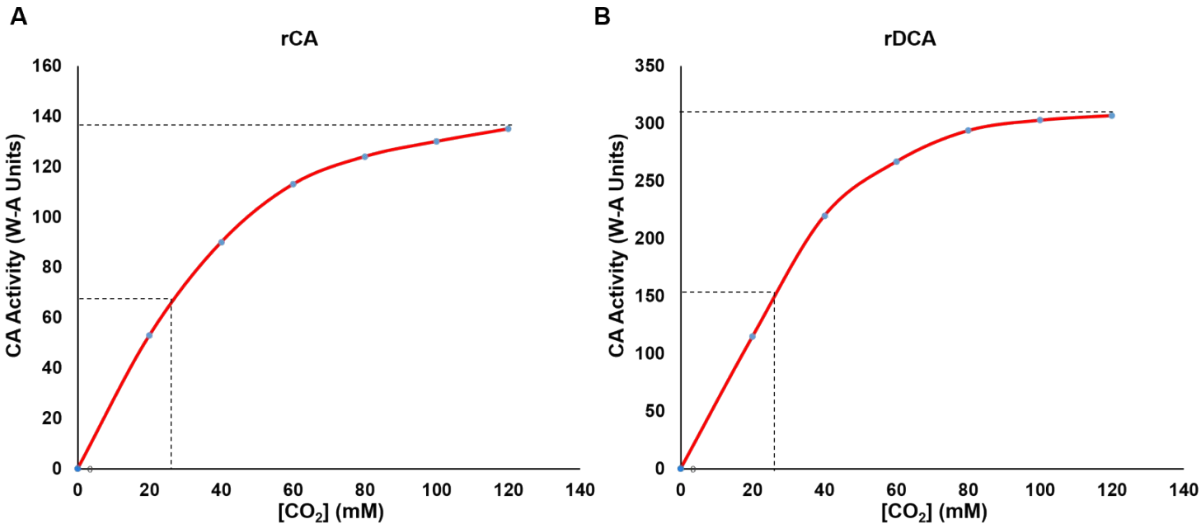


Fig. S5 Michaelis – Menten kinetics for enzyme (A) rCA and (B) rDCA.

Table S3. Statistic summary of Lineweaver-Burk Plot kinetics plotted using both origin and graphpad prism software.

Statistics Summary	rCA	rDCA
Reduced Chi-Sqr	2.48979E-10	1.94934E-9
Adj. R-Square	0.99409	0.9858
Max. no of Y replicates	3	3
P Value	<0.0001	<0.0001
Fit Status	Succeeded	Succeeded

Thermostability assay

Residual activity (%) was calculated to check the temperature stability of the enzyme by the ratio of CA activity of heated samples (A_h) to the activity of unheated samples (A_{uh}) and converted into percentage. Furthermore, residual activity analysis revealed that rDCA maintained 100% of its activity in the temperature range of 95-110°C, whereas rCA exhibited a substantial reduction in residual activity under the same conditions

$$Residual\ activity\ \% = \frac{A_h}{A_{uh}} * 100 \quad (4)$$

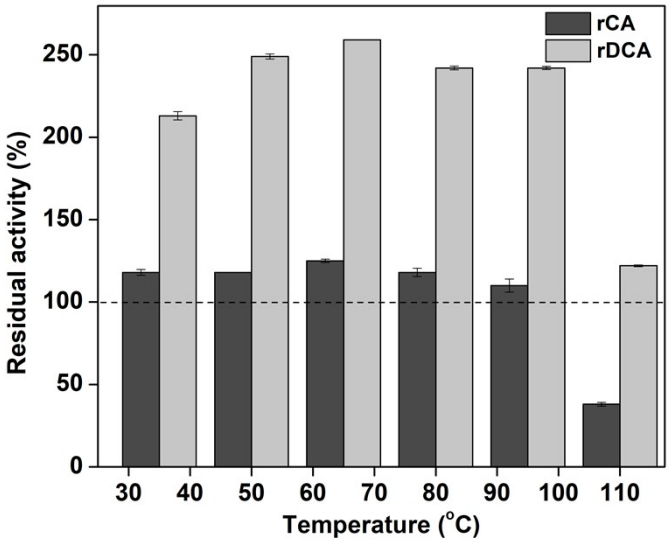


Fig. S6 Short-term thermal stability through residual activity assay for rCA and rDCA

Table S4. Summary of mean relative activity and 95% confidence intervals for both rCA and rDCA enzymes

Temperature(°C)	rCA Mean	rCA CI (95%)	rDCA Mean	rDCA CI (95%)
35	94.67	93.23 – 96.1	90.00	87.52 – 92.48
50	94.67	93.23 – 96.1	95.67	94.23 – 97.1
65	99.67	98.23–101.1	99.67	98.23 – 101.1
80	94.33	92.90 – 95.77	94.67	93.23 – 96.1
95	87.67	78.94 – 96.39	94.33	90.54 – 98.13
110	29.33	26.46 – 32.2	47.67	43.87 – 51.46

CO_2 Sequestration and its confirmation

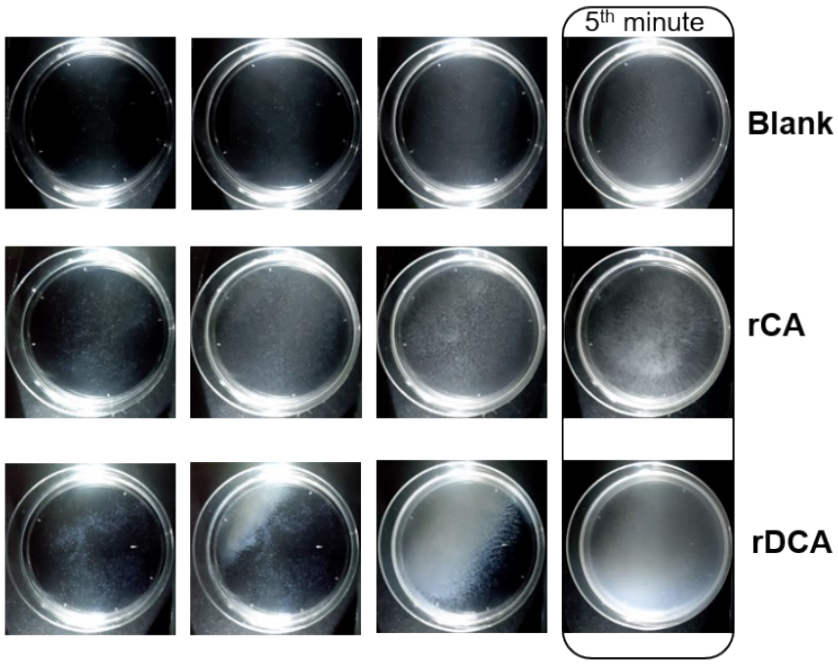


Fig. S7 Video snap of $CaCO_3$ formation inside CO_2 incubator for 5 minutes.

CO_2 sequestration ability of the congener enzyme (rDCA) was examined by detecting the formation of $CaCO_3$ in the reaction mixture containing $CaCl_2$. $CaCO_3$ also visualized in Energy Dispersive X-ray Analysis (EDX) shown in figure S7.

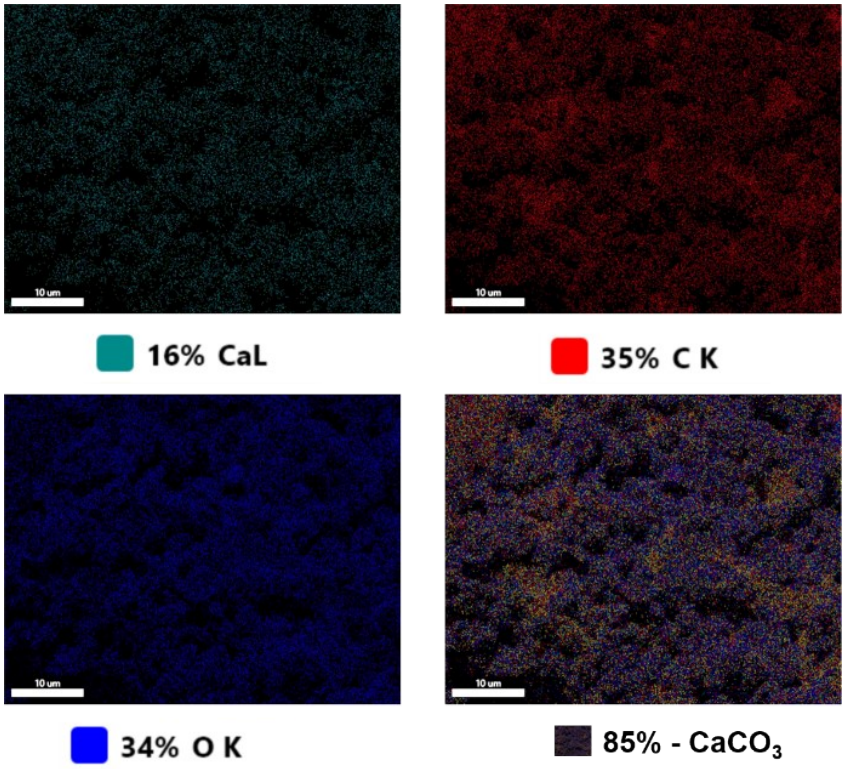


Fig. S8 Energy dispersive X-ray analysis (EDX) result of calcium carbonate formation through carbon capture using rDCA enzyme.