Supplementary Information

Stepwise optimization of genetic RuBisCO-equipped Escherichia coli for low carbon-footprint protein and chemical production

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Supplementary Note 1

For calculation of CO_2 assimilation, we build up the mass balance method as following:

First, we defined the input and output carbon listed below based on the Figure S1

$Input\ carbons$: $0.27 \times [CO_2]_{input} + 0.4 \times [Xylose]$

$Output\ carbons$: $0.27 \times [CO_2]_{output} + C_{biomass} \times [Biomass] + \sum [C_{n,\ metabolites}]$

For a steady state assumption, the input was equal to output as following in which the net carbon was determined as the input carbon subtracted by the output carbon:

$$[CO_2]_{net} = [CO_2]_{input} - [CO_2]_{output} = \frac{C_{biomass} \times [Biomass]}{0.27} + \sum \frac{[C_{n,\ metabolites}]}{0.27} - 1.48 \times [Xylose]$$

Next, the metabolites were neglected since the there was no significant metabolites, thus the calculation could be simplified as following:

$$[CO_2]_{net} = \frac{C_{biomass} \times [Biomass]}{0.27} - 1.48 \times [Xylose]$$

To compare each system, the net carbon was normalized by the biomass as the CO_2 assimilation capability calculated as following:

$$CO_2\ assimilation\ capability = [CO_2]^*_{net} = \frac{C_{Biomass} \times [Biomass] - 0.4 \times [Xylose]}{[Biomass\}} \times \frac{1}{0.27}$$

Following two tables showed the examples to calculate the CO_2 assimilation capability. The first table was the raw data containing the final OD of the culture and that from the analysis of the moisture dryer and the HPLC. The second table illustrated our detailed calculation of CO_2 assimilation capability.

Sample	Final OD (OD_f)	Empty plate (g) (W_0)	After drying (g) (W_d)	OD used for drying (OD_d)	Cell volume (mL) (V_d)	Xylose (g/L)
BD-hCAII	1.038	0.1985	0.2224	0	3	0
I2/SSCI-CA	1.275	0.2058	0.2425	0	3	0

Formula	$\frac{(Wd - W0) \times 1000}{ODd \times Vd}$	$\frac{Biomass}{OD} \times ODf$	$\frac{C_{Biomass} \times [Biomass] - 0.4 \times [Xylos}{[Biomass\}}$
Sample	Biomass/OD (g/L/OD)	Final Biomass (g/L)	CO_2 assimilation capability (g-CO_2/g-biomass)
BD-hCAII	0.7967	0.827	**-7.27**
I2/SSCI-CA	1.2233	1.560	**-3.06**

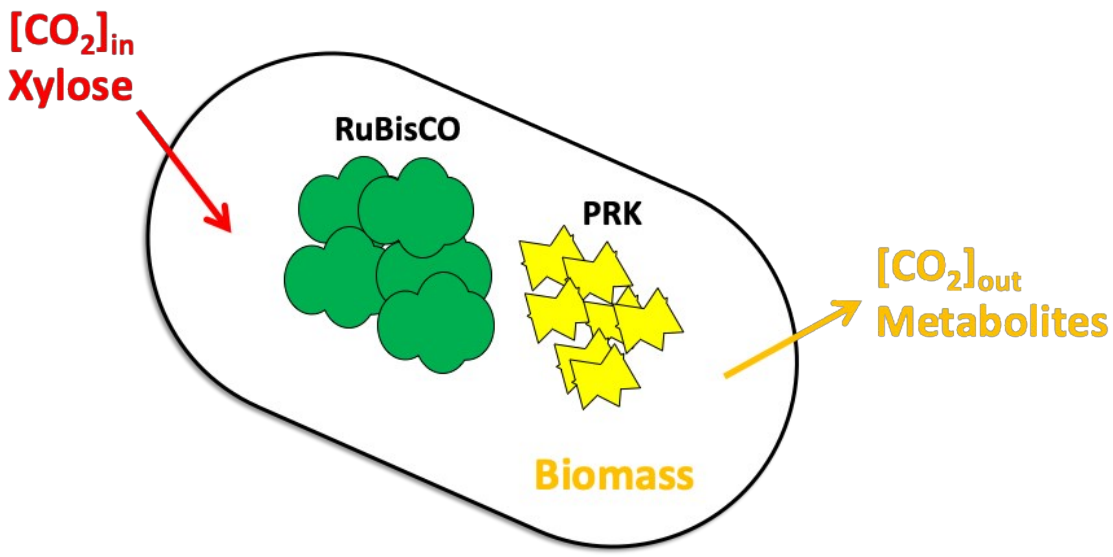
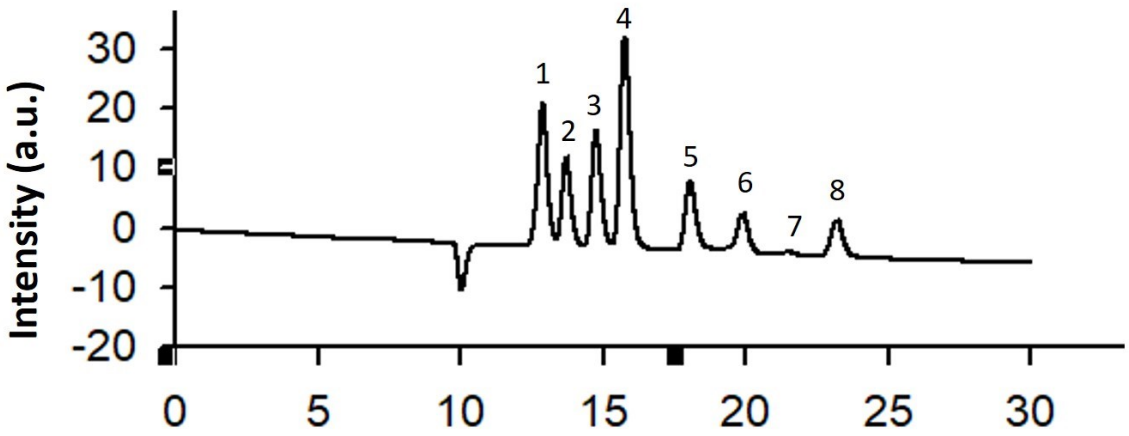


Fig. S1 mass balance for calculation of CO_2 assimilation capability



No.	Chemical	RT (min)	No.	Chemical	RT (min)
1	Citrate	12.89	5	Succinate	18.1
2	Malate	13.78	6	Lactate	19.12
3	Glucose	14.78	7	Formate	21.51
4	**Xylose**	**15.74**	8	Acetate	23.22

Fig. S2 HPLC spectrum of 8 chemicals separated by our HPLC condition and detected by RI detector.

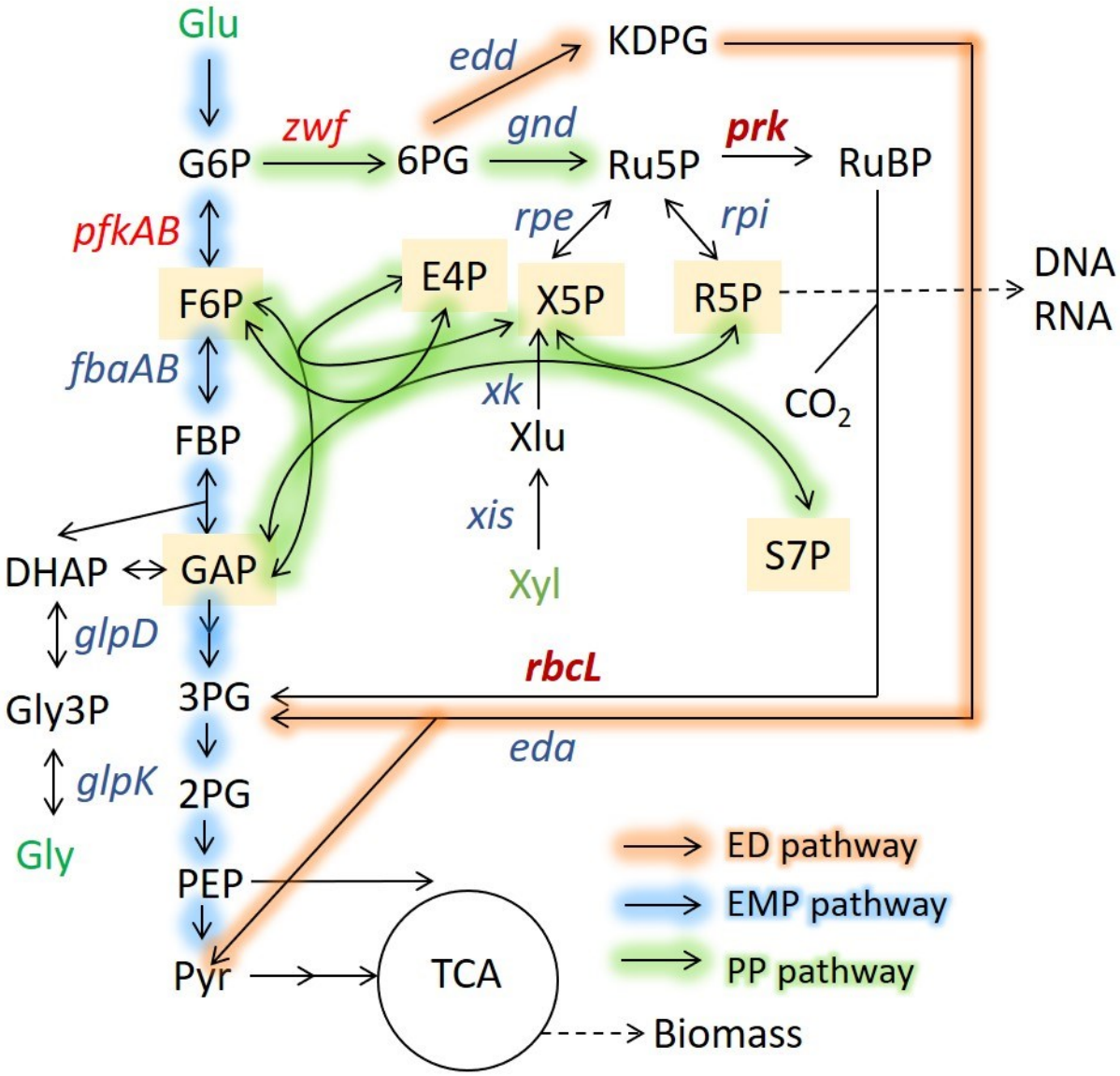


Fig. S3 The PP pathway is used for enhancing the carbon flux to the RuBisCO-equipped *E. coli.*

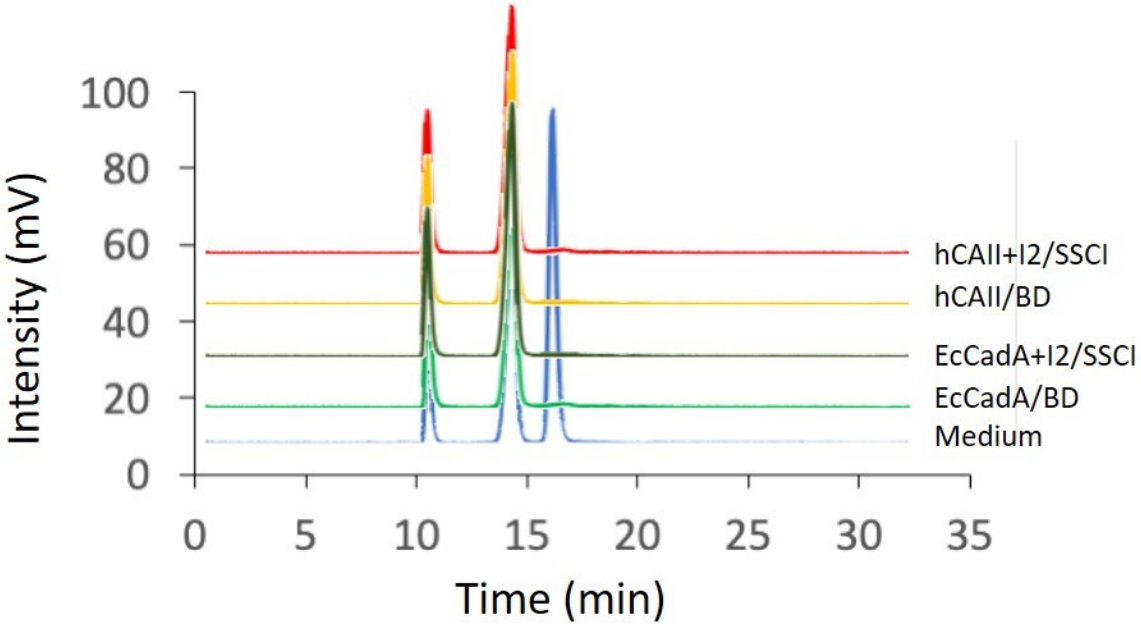


Fig. S4 HPLC analysis of the cell expressing the EcCadA or hCAII in I2/SSCI and BD, respectively.

Table S1 Primers used in this study.

Primers	Sequence (5'-3')
HindIII-SePRK(7942)-F	GCAAGCTTATGAGTAAACCAGACCGCGTAGTGCTG
PstI-SpeI-SePRK(7942)-R	CACTGCAGCGGCCGCTACTAGTTCAAACGGAGGCGGCAACTGGAGCA
HindIII-CaPRK-F	GCAAGCTTATGACTCAAGATAGAGTAGTTATTATCGG
PstI-CaPRK-R	GACTGCAGTTATACTTGAGCAACTTCTTCTTCTGTG
SePRK(7002)-VF	GAAAGGGGCGATCGCCGCAAGCGTATAGCTGCAGGAGTCACTAAGG GTTAG
SePRK(7002)-VR	CTACACGGTCTGTCTGACTGGTCATAAGCTTTTTCTCCTCTTTGGATCC TC
SePRK(7002)-IF	GAGGATCCAAAGAGGAGAAAAAGCTTATGACCAGTCAGACAGACCG TGTAG
SePRK(7002)-IR	CTAACCCTTAGTGACTCCTGCAGCTATACGCTTGCGGCGATCGCCCCT TTC
RcPRK-VF	GATCGAGCGTCTGGTGCGCGAAGGCCGTTGACTGCAGGAGTCACTAA GGGTTAG
RcPRK-VR	CGGAAGATCTGGTCGAAGGTCATAAGCTTTTTCTCCTCTTTGGATCCT C
RcPRK-IF	GAGGATCCAAAGAGGAGAAAAAGCTTATGACCTTCGACCAGATCTTC CG
RcPRK-IR	CTAACCCTTAGTGACTCCTGCAGTCAACGGCCTTCGCGCACCAGACGC TCGATC
28a-VF	GTTGCTCTGGGCGTGAACTGAAAGCTTTGAGATCCGGCTGCTAACAA AGCCC
28a-VR	GCGTAACGGTTAGACTGATCCATCATATGGCTGCCGCGCGGCACCAG
RcCbbM-IF	CTGGTGCCGCGCGGCAGCCATATGATGGATCAGTCTAACCGTTACGC
RcCbbM-IR	GGGCTTTGTTAGCAGCCGGATCTCAAAGCTTTCAGTTCACGCCCAGAG CAAC
SacI-pSB4A3-BioB-F	CTGAGCTCGATTACTTCGCGTTTGCCACCTG
SalI-pSB4A3-BioB-R	CTGTCGACCGGAGGCTTTTGACTTTCTGCTAATC
Cm-short-F	ATGATGAACCTGAATCGCCAGC
Cm-short-R	CAAGATGTGGCGTGTTACGGTG
Km-short-F	GTAATGGCTGGCCTGTTGAACAAG
Km-short-R	GTTCCATAGGATGGCAAGATC
Ap-short-F	CAGAATGACTTGGTTGAGTACTC
Ap-short-R	CAACGATCAAGGCGAGTTACATG
GroELS-short-F	GCATGATCGCGTGATCGTCAAGC
GroELS-short-R	CACACCGTAGCCATCGTTGAAAA

Table S2 Elemental analysis of BD and SSA strains harboring pSIT-RcHemA for ALA production.

Elements	Element percentage (%)					
	BD			SSA		
	Test I	Test II	Average	Test I	Test II	Average
Nitrogen (N)	13.40	13.16	12.39	13.79	13.84	11.82
Carbon (C)	43.73	43.52	43.63	45.66	45.81	45.73
Hydrogen (H)	7.28	7.45	7.35	7.53	7.39	7.46

Table S3 The calculation of accumulated carbon and CO_2 assimilation capability.

Strain	Biomass (g/L)	ALA (g/L)	Carbon content in biomass (%)*	C^{13}/C^{12} ratio#	C^{12} (g/L)	Carbon in biomass (g/L)	Carbon in ALA (g/L)**	Accumulated carbon (g/L)	CO_2 assimilation capability (g-CO_2/g-DCW)
BD	2.8	5.0	43.626	99.71	0.012	1.222	2.290	3.51	-9.10
SSA	5.5	3.8	45.732	72.21	0.034	2.515	1.740	4.25	-4.13

*Carbon content is the data from elemental analysis.

C^{13}/C^{12} ratio is provided from Elemental Analyzer coupled with isotope ratio mass spectrometer (EA–IRMS).

** Carbon in ALA is calculated by ALA amount with the ratio of 60/131 (i.e., the ratio of molecular weight for C5 and ALA).

Table S4 Elemental analysis of cell expressing the EcCadA and hCAII.

Elements	Element percentage (%)					
	EcCadA			hCAII		
	Test I	Test II	Average	Test I	Test II	Average
Nitrogen (N)	12.50	12.27	12.39	11.96	11.98	11.97
Carbon (C)	46.22	46.12	46.17	45.47	45.49	45.48
Hydrogen (H)	7.14	7.02	7.08	6.92	6.941	6.93