Supporting Information

Programmable Biofilm-Cellulose Hybrid Platform for Specifically Clustering of Microbial Catalysts with Optimized Cellular Synergy

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Experimental Procedures

Induction and cultivation of engineered strains in the liquid media containing cellulose materials

Engineered strains was initially grown in LB liquid medium (+corresponding antibiotics) at 37°C for 12 h as the seed liquid. The seed liquid was then added in the M63 minimal medium (supplemented with cotton or gauze (2.5%, w/v), glucose (0.2%, w/v), MgSO$_4$ (1 mM), and antibiotics) and incubated at 37°C until the value of OD$_{600}$ reached 0.8. Subsequently, 0.3 mM IPTG was added into the medium and incubated at 30°C for 36 h to promote the expression of fusion proteins, followed by cultivating the medium statically for 24 h to promote the assembly of curli nanofibers. After incubation, the cotton or gauze was recovered and washed with PBS (pH 7.4) three times for further experiments.

CBD-mediated the anchoring of BL21:ΔCsgA strains onto cellulose materials

The nucleic acid sequence of the cellulose binding domain (CBD) from *Clostridium thermocellum* DSMZ 2360 was optimized and synthesized, followed by inserting it into the autotransporter unit AIDA-I including the N-terminal cholera toxin B signal peptide (CtxB), resulting the AIDA-CBD fusion protein.[1, 2] The nucleic acid sequence of AIDA-CBD was finally linked to the pACYCDuet-1 plasmid with *Nco*I and *Hin*dIII restriction sites, resulting in the recombinant plasmid pACYCDuet-*AIDA-CBD*. Furthermore, the pACYCDuet-*AIDA-CBD* plasmid was transformed into BL21:ΔCsgA competent cells and screened by agar plate with chloramphenicol (17 μg/mL), resulting the EX02 strain. EX02 strain was cultured in the medium with the addition of gauze or cotton, and EX01 strain without CBD was used as a negative control. After cultivation, field emission scanning electron microscopy (FE-SEM, GeminiSEM 500, Germany) analysis was used to determine their morphological differences. For further locating the cells, the recombinant plasmid pET28a-*mCherry* encoding the red fluorescent protein mCherry was transformed into EX01 and EX02 cells, resulting IN01 and IN02 strains, respectively. mCherry would be expressed under IPTG induction, which could be determined under the confocal laser scanning microscopy (CLSM, Nikon, Japan). By comparing the difference of fluorescence intensity, the function of CBD could be further identified.

Construction of the biofilm-cellulose hybrid materials

The functional peptide EFCA was used to modify CsgA proteins, and the nucleic sequence of CsgA(EFCA) was linked to the pACYCDuet-*AIDA-CBD* plasmid with *Nde*I and *Xho*I restriction sites, resulting the pACYCDuet-*AIDA-CBD-CsgA(EFCA)* plasmid. The pET21a-*CsgB-CsgA(SpyTag)* plasmid was constructed in our previous report.[3] Furthermore, the pACYCDuet-*AIDA-CBD-CsgA(EFCA)* and pET21a-*CsgB-CsgA(SpyTag)* plasmids were co-transformed into the BL21:ΔCsgA cells, and the resulting strain was named EX05. After cultivated into biofilms on cotton or gauze, FE-SEM was used to analyze the topological structure of the prepared hybrid materials.

Function analysis of the prepared biofilm-cellulose hybrid materials

In order to determine the functions of the hybrid material, green fluorescent protein (GFP) and mCherry were used to localize the functional peptides (SpyTag and EFCA) in biofilms. Firstly, we constructed two recombinant plasmids (pET28a-*GFP-*

SpyCatcher and pET28a-*mCherry-InaD*), which were separately transformed into BL21(DE3) for expression. After induced by IPTG for 24 h, the cells (IN04 or IN06) expressing GFP-SpyCatcher or mCherry-InaD were ruptured by sonication. Furthermore, the lysis solution was centrifugated (8000 × g) at 4°C for 15 min, and the supernatant of the cell lysis containing GFP-SpyCatcher and mCherry-InaD was co-incubated with the hybrid materials for 6 h. Sterile water was then used to wash the hybrid materials to remove the unbound fluorescent proteins. Finally, CLSM analysis was carried out to determine the green and red fluorescence. In this section, the supernatant of cell lysis (IN03 and IN05) containing GFP and mCherry was used as a negative control to locate the functional peptides (SpyTag and EFCA) by means of the specific recognition between conjugation pairs (SpyCatcher/SpyTag or InaD/EFCA).

Construction of whole-cell platforms for further immobilization

A membrane-bound protein, truncated ice nucleation protein InaK from *Pseudomonas syringae*, was used to achieve the surface display of SpyCatcher and InaD on the *E. coli* cell surface separately.[4, 5] The full-length genes of InaK-SpyCatcher and InaK-InaD were linked to the pET21a (+) plasmid with *Nde*I and *Xho*I restriction sites, resulting pET21a-*InaK-SpyCatcher* and pET21a-*InaK-InaD* plasmids, respectively.

Verification of the assembly of engineered cells onto hybrid materials

The pET28a-*GFP*/pET21a-*InaK-SpyCatcher* and pET28a-*mCherry*/pET21a-*InaK-InaD* plasmid groups were co-transformed into BL21(DE3) strains, resulting IN08, and IN10 strains, respectively. IN08 and IN10 strains were separately inoculated into LB liquid medium (+ ampicillin + kanamycin) and incubated at 37°C overnight. Then, the seed liquid (1%) was inoculated into 100 mL LB medium and incubated at 37°C until OD_{600} reached 0.3–0.7. IPTG was added into the medium to induce the expression of heterologous proteins with the final concentration of 300 μM. After induced at 25°C for 24 h, cells were recovered and washed with PBS (pH 7.4) three times. The induced IN08 and IN10 cells were re-suspended in PBS and co-incubated with the prepared hybrid materials at 20°C for 6 h. Unbound cells were removed by washing with PBS, and CLSM was finally used to determine the green and red fluorescence from the hybrid materials. Induced IN07 and IN09 cells were used as negative control, which were lack of surface-displayed SpyCatcher and InaD, respectively.

Construction of recombinant plasmids for synthesis of D-PLA

In this section, based on the co-expression plasmid pACYCDuet-1, two recombinant plasmids encoding *Pa*TA + *Cg*TD and *Gra*FDH2 + D-HicDH were constructed. The pET21a-*InaK-SpyCatcher* and pACYCDuet-*PaTA-CgTD* were transformed into BL21(DE3) to obtain EX06. The pET21a-*InaK-SpyCatcher* and pACYCDuet-*CgTD-PaTA* were transformed into BL21(DE3) to obtain EX07. The pET21a-*InaK-InaD* and pACYCDuet-*GraFDH2-D-HicDH* were transformed into BL21(DE3) to obtain EX08. The pET21a-*InaK-InaD* and pACYCDuet-*D-HicDH-GraFDH2* were transformed into BL21(DE3) to obtain EX09. As described above, engineered cells (EX06, EX07, EX08, and EX09) were cultivated and induced into whole-cell biocatalysts for further immobilization onto the prepared hybrid materials.

Immobilization of whole-cell biocatalysts

For synthesis of D-PLA, whole-cell biocatalysts of EX06 and EX08 were first immobilized onto the hybrid materials. The initial immobilization system was as follows: 350 mg hybrid materials, 30 mg EX06 cells, 30 mg EX08 cells, 1 mL PBS (pH 7.4). Then, the above system was incubated at 20°C for 12 h to promote the immobilization of whole-cell biocatalysts. The absorbance of cell solutions under 600 nm at 0 h and 12 h was recorded, which were defined as A0 and A1, respectively. The amount of the whole-cell biocatalysts bound to the hybrid materials was calculated through the subtraction of A1 from A0. The resulting hybrid materials containing two whole-cell biocatalysts were washed with PBS thoroughly to remove unbound cells, which were used for further cascade transformations.

Synthesis of D-PLA

The assembled whole-cell biocatalysts were put into the reaction media for D-PLA synthesis, and the initial reaction system was as follows: 350 mg immobilized whole-cell catalysts, 100 mM glycine, 10 mM benzaldehyde, 10 mM ammonium formate, 100 μM PLP, 1 mM NAD^+, 0.1% Tween-80, 1 mL potassium phosphate buffer (pH 8.0, 100 mM). The reaction mixture was incubated at 25°C with shaking for 36 h, and high-performance liquid chromatograph (HPLC) was further used for the determination of D-PLA.

Optimization of the immobilization conditions

Under the initial immobilization conditions described above, the optimal group of whole-cell biocatalysts, the optimal ratio of two whole-cell biocatalysts, and the optimal concentration of whole-cell biocatalysts were examined. The effects of the reaction parameters were specifically studied as follows: For the optimal group of two whole-cell biocatalysts, four groups of EX06 + EX08, EX06 + EX09, EX07 + EX08, and EX07 + EX09 were studied. The ratio of EX07 to EX09 was varied by fixing the total amount of EX07 and EX09 at 60 mg/mL. The ratio of EX07 to EX09 was set to 3:1, 2:1, 1:1, 1:2, and 1:3. Furthermore, the total amount of EX07 and EX09 was set as 10, 20, 40, 60, 80, 100, and 120 mg/mL. The immobilization amount of whole-cell biocatalysts was determined as described above. The immobilized whole-cell biocatalysts were then used to do the transformation reaction, and the production of D-PLA was used as an evaluation index to get the optimal immobilization conditions.

Optimization of the reaction conditions

Under the optimal immobilization conditions (350 mg immobilized catalysts, 30 mg EX07, 30 mg EX09, 1 mL PBS (pH 7.4)), the optimal addition of ammonium formate, NAD^+, and PLP were examined as well as the optimal ratio of glycine to benzaldehyde. For optimizing the addition of ammonium formate, the concentration of ammonium formate was set to 5, 7.5, 10, 12.5, 15, 17.5, 20, 30 and 40 mM. To evaluate the effect of NAD^+, the addition amount of NAD^+ was set to 5, 7.5, 10, 12.5, 15, 17.5, 20, 30, and 40 mM. For the effect of PLP addition, the amount of PLP was set as 20, 40, 60, 80, 100, 120, 140, 160, 180, and 200 μM. Furthermore, the optimal ratio of glycine to benzaldehyde was set to 16:1, 14:1, 12:1, 10:1, 8:1, 6:1, 4:1, 2:1, and 1:1 to evaluate its effect on the production of D-PLA. After reaction, the yield of D-PLA was determined as described above, and the highest yield was defined as 100%.

Synthesis of D-PLA under optimal conditions

Under optimal immobilization (EX07 + EX09 as the optimal pair at a ratio of 1:1, with the cell density maintained at 60 mg/mL) and reaction (17.5 mM ammonium formate, 1.0 mM NAD^+, 10:1 ratio of glycine to benzaldehyde, and 40 μM PLP) conditions, immobilized EX07 and EX09 whole-cell catalysts were used to synthesize D-PLA. Furthermore, free EX07 (4.2 mg/mL) and EX09 (4.2 mg/mL) whole-cell catalysts were as control to compare the transformation efficiency and the final yield of D-PLA.

Measurements and characterization

FE-SEM: The samples were directly taken from induced M63 media and washed with sterile H_2O several times to remove unbound cells. For FE-SEM analysis, the samples were fixed overnight with 2% glutaraldehyde and 2% paraformaldehyde at 4°C, followed by washing several times with sterile H_2O. After dehydrated with an increasing ethanol step gradient, GeminiSEM 500 (Germany) was used to analyze the morphological structures of the samples sputtered with gold.

CLSM: After immobilized with fluorescent proteins or cells, the samples were washed with sterile water thoroughly to remove unbound fluorescent proteins and cells. Then, the samples were placed between two cover slides and further analyzed with CLSM. The green and red fluorescence was determined at 488 nm and 561 nm, respectively.

HPLC analysis: After reaction, the reaction liquid was obtained and centrifugated at 10,000 × g to remove insoluble fractions. Then, the supernatant was mixed with the pretreatment liquid (ultra-pure water : acetonitrile = 1:1). The mixture was further centrifugated at 10,000 × g for 15 min to remove insoluble fractions. Finally, the supernatant of the mixture was analyzed using a HPLC system (Agilent) equipped with a VWD detector. The chromatographic column was TSKgel ODS-100 V 5 μm (4.6 mm × 15 cm, TOSOH). The detector wavelength was set to 254 nm and the temperature was kept at 30°C. The mobile phase was acetonitrile and ultra-pure water (+0.1% TFA). Detailed gradient elution conditions were shown in Table S3.
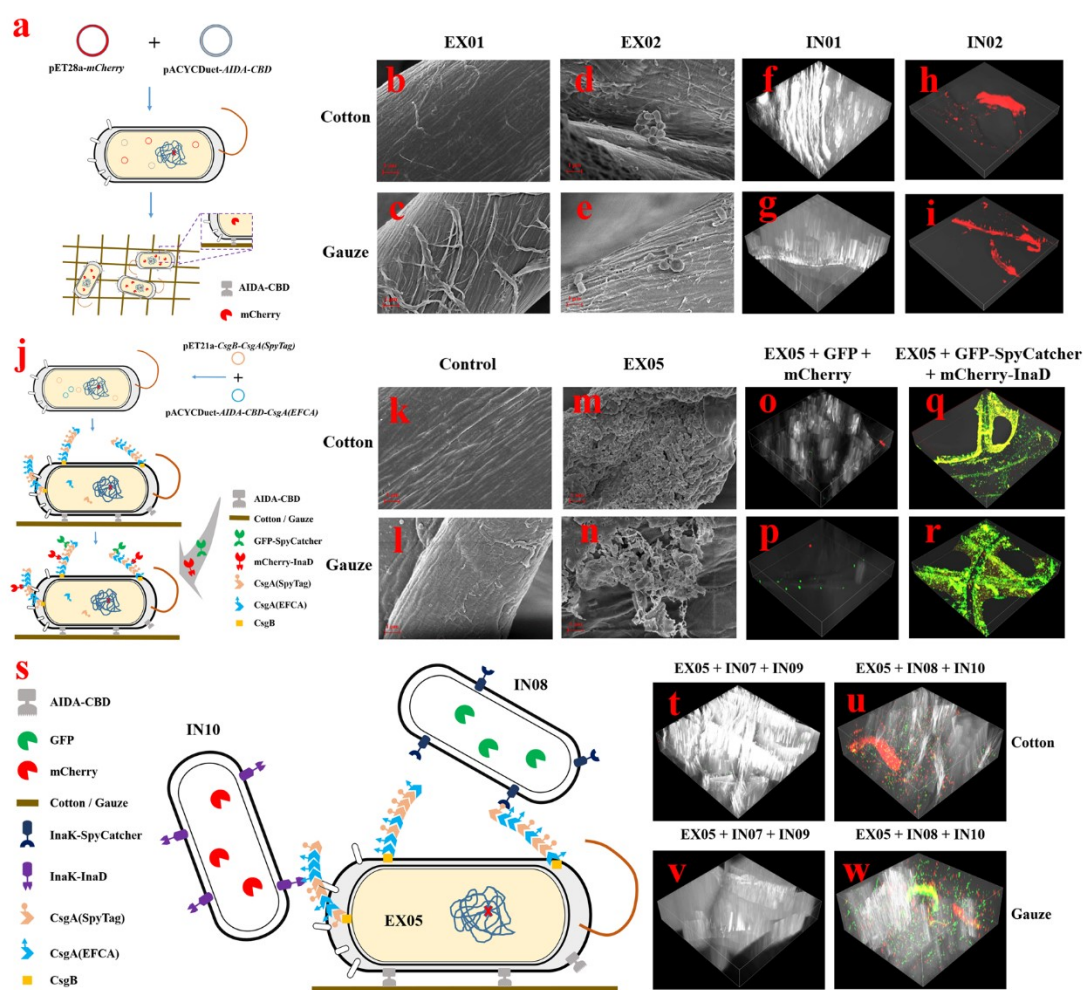
Supplementary Figures and Tables

Fig. S1: Construction and determination of the bifunctional biofilm-cellulose hybrid platform and whole-cell surface display systems. a) Illustration of the strategy for improving the anchoring of BL21:ΔCsgA onto cellulose materials, where mCherry was used for locating cells under CLSM. b-e) FE-SEM results after EX01 or EX02 was co-incubated with cellulose materials. f-i) CLSM results after IN01 or IN02 was co-incubated with cellulose materials. j) Illustration of the construction, formation and function analysis of *E. coli* biofilms on cellulose materials. k-l) FE-SEM results of cellulose materials. m-n) FE-SEM results after EX05 was co-incubated with cellulose materials. o-p) CLSM results after EX05 biofilm-based hybrid materials were co-incubated with GFP and mCherry. q-r) CLSM results after EX05 biofilm-based hybrid materials were co-incubated with GFP-SpyCatcher and mCherry-InaD. s) Illustration of the specific immobilization of two cells on the prepared biofilm-cellulose hybrid platform, where SpyCatcher and InaD were separately surface-displayed via InaK. GFP and mCherry were expressed in IN08 and IN10 cells, respectively, for location via CLSM. t-w) CLSM results after the two cells were co-incubated with EX05 biofilm-cellulose hybrid platform.
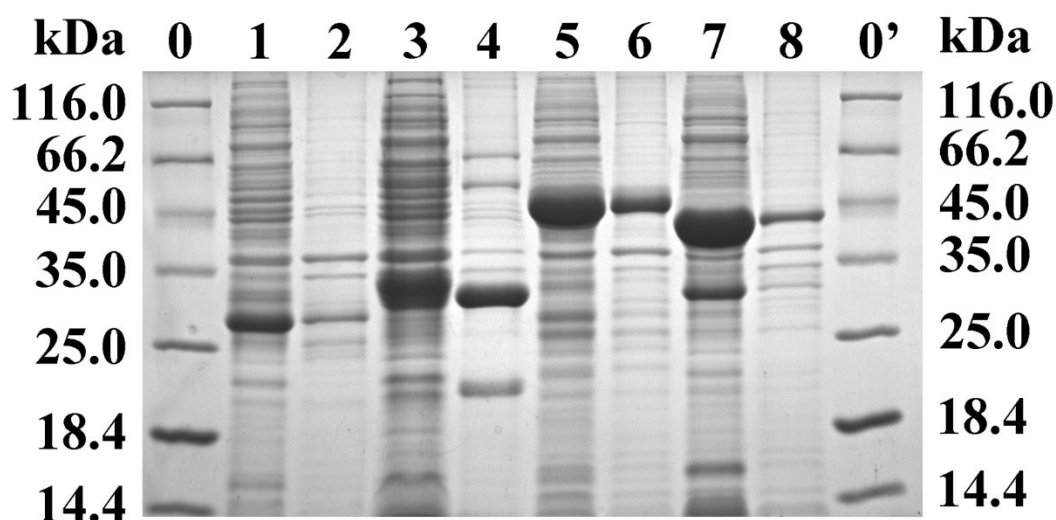
Fig. S2 SDS-PAGE analysis of the expression of fluorescent proteins. lane 0, protein marker; lane 1, the supernatant of the cell lysis of IN03; lane 2, the precipitant of the cell lysis of IN03; lane 3, the supernatant of the cell lysis of IN04; lane 4, the precipitant of the cell lysis of IN04; lane 5, the supernatant of the cell lysis of IN05; lane 6, the precipitant of the cell lysis of IN05; lane 7, the supernatant of the cell lysis of IN06; lane 8, the precipitant of the cell lysis of IN06; lane 0', protein marker.
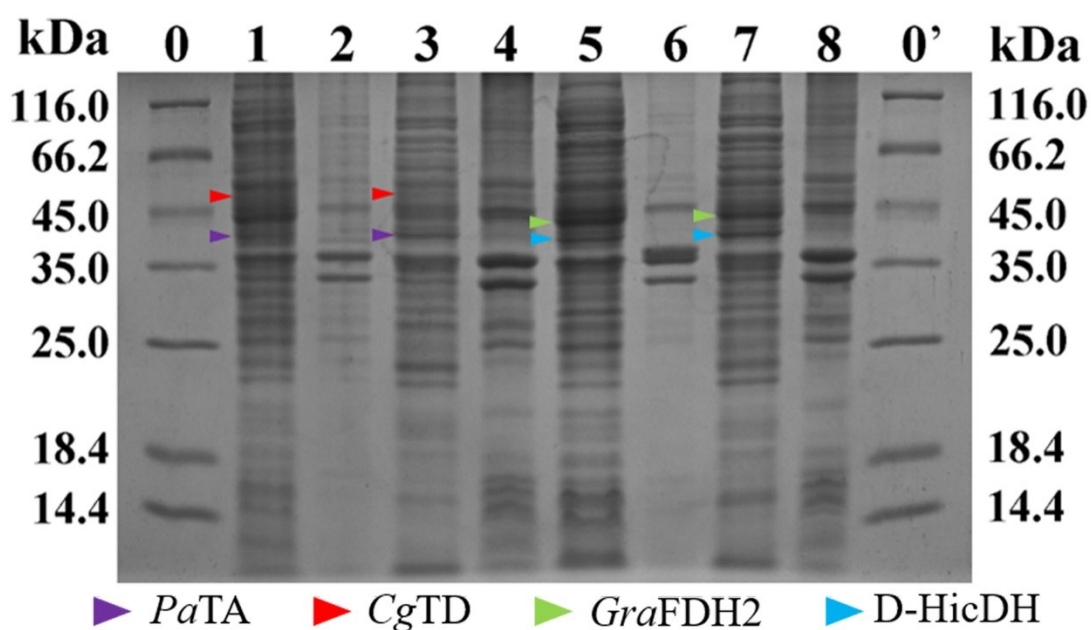

Fig. S3 SDS-PAGE analysis of the expression of enzymes. lane 0, protein marker; lane 1, the supernatant of the cell lysis of EX06; lane 2, the precipitant of the cell lysis of EX06; lane 3, the supernatant of the cell lysis of EX07; lane 4, the precipitant of the cell lysis of EX07; lane 5, the supernatant of the cell lysis of EX08; lane 6, the precipitant of the cell lysis of EX08; lane 7, the supernatant of the cell lysis of EX09; lane 8, the precipitant of the cell lysis of EX09; lane 0', protein marker.
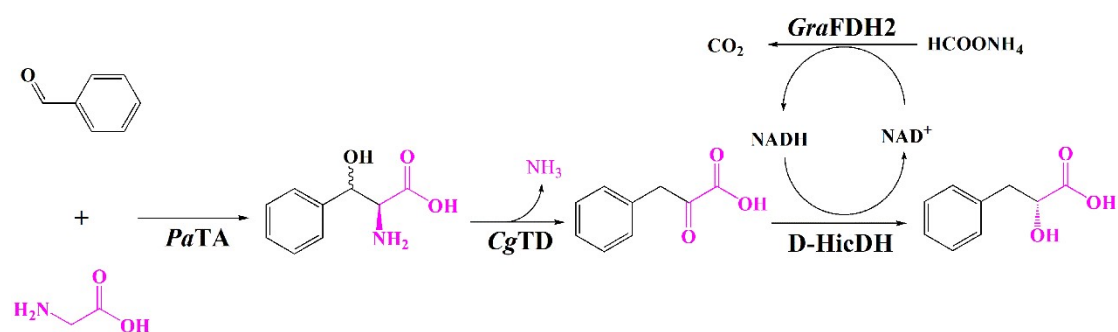
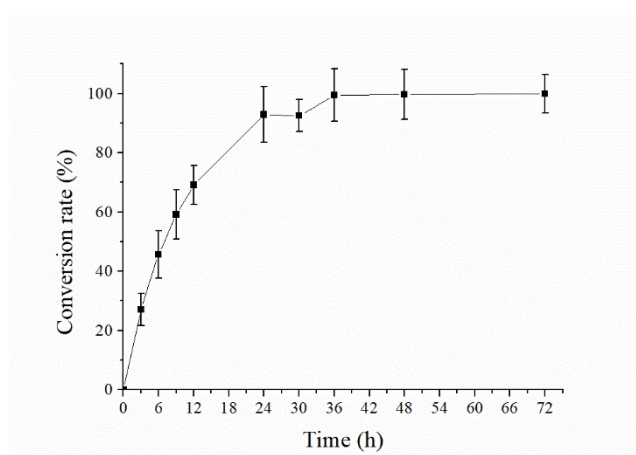**Fig. S4** Synthesis of D-PLA through cascade reactions using benzaldehyde and glycine as substrates.

Fig. S5 Synthesis curve of D-PLA production catalyzed by free EX07 +EX09 catalysts.
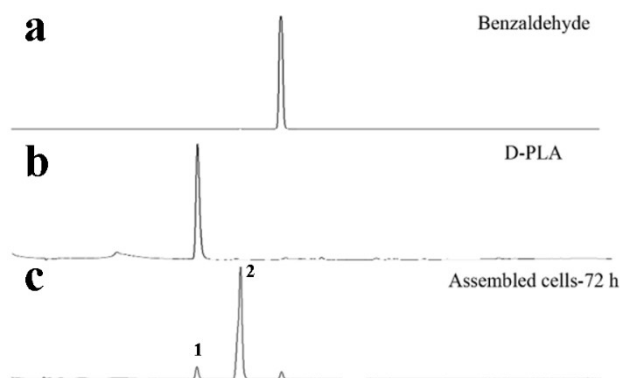
Fig. S6 HPLC analysis of the production of D-PLA. a) The standard of benzaldehyde. b) The standard of D-PLA. c) The HPLC result after reaction for 72 h. Peak 1, D-PLA; peak 2, by-products (identified as benzoic acid).

Table S1 Amino acid sequences of functional proteins

Name	Amino acids sequence	Length (aa)
CsgA[6]	MKLLKVAAIAAIVFSGSALAGVVPQYGGGGNHGGGGNNSGPN SELNIYQYGGGNSALALQTDARNSDLTITQHGGGGNGADVGQGS	151

	DDSSIDLTQRGFGNSATLDQWNGKNSEMTVKQFGGGNGAAVD QTASNSSVNVTQVGFGNNATAHQY	
CsgB[7]	MKNKLLFMMLTILGAPGIAAAAGYDLANSEYNFAVNELSKSSF NQAAIIGQAGTNNSAQLRQGGSKLLAVVAQEGSSNRAKIDQTG DYNLAYIDQAGSANDASISQGAYGNTAMIIQKGSGNKANITQY GTQKTAIVVQRQSQMAIRVTQR	151
mCherry[8]	MAIIKEFMRFKVHMEGSVNGHEFEIEGEGEGRPYEGTQTAKLK VTKGGPLPFAWDILSPQFMYGSKAYVKHPADIPDYLKLSFPEGF KWERVMNFEDGGVVTVTQDSSLQDGEFIYKVKLRGTNFPSDG PVMQKKTMGWEASSERMYPEDGALKGEIKQRLKLKDGGHYD AEVKTTYKAKKPVQLPGAYNVNIKLDITSHNEDYTIVEQYERA EGRHSTGGMDELYK	227
GFP[9]	MGHHHHHHMVSKGEEDNMASLPATHELHIFGSINGVDFDMVG QGTGNPNDGYEELNLKSTKGDLQFSPWILVPHIGYGFHQYLPY PDGMSPFQAAMVDGSGYQVHRTMQFEDGASLTVNYRYTYEG SHIKGEAQVKGTGFPADGPVMTNSLTAADWCRSKKTYPNDKTI ISTFKWSYTTGNGKRYRSTARTTYTFAKPMAANYLKNQPMYV FRKTELKHSKTELNFKEWQKAFTDVMGMDELYKLAAALEEEE EAYGWMDF	261
Linker	GGGGSGGGGS	10
SpyTag[10, 11]	AHIVMVDAYKPTK	13
SpyCatcher[10, 11]	MSYYHHHHHHDYDIPTTENLYFQGAMVDTLSGLSSEQGQSGD MTIEEDSATHIKFSKRDEDGKELAGATMELRDSSGKTISTWISD GQVKDFYLPGKYTFVETAAPDGYEVATAITFTVNEQGQVTV NGKATKGDAHI	139
EFCA[11]	EFCA	4
InaD[11]	GELIHMVTLDKTGKKSFGICIVRGEVKDSPNTKTTGIFIKGIVPD SPAHLCGRLKVGDRILSLNGKDVRNSTEQAVIDLIKEADFKIELE IQTFDHHHHHH	101
CBD[1, 12]	PVSGNLKVEFYNSNPSDTTNSINPQFKVTNTGSSAIDLSKLTLRY YYTVDGQKDQTFWCDHAAIIGSNGSYNGITSNVKGTFVKMSSS TNNADTYLEISFTGGTLEPGAHVQIQGRFAKNDWSNYTQSNDY SFKSASQFVEWDQVTAYLNGVLVWGKEP	159
InaK[4, 5]	MGTLDKALVLRTCANNMADHCGLIWPASGTVESRYWQSTRR HENGLVGLLWGAGTSAFLSVHADARWIVCEVAVADIISLEEPG MVKFPRAEVVHVGDRISASHFISARQADPASTSTSTSTLTPM PTAIPTPMPAVASVTLPVAEQARHEVFDVASVSAAAAPVNTLP VTTPQNLQT	180
AIDA-I[2]	MGIKLKFGVFFTVLLSSAYAHGTPQNITDPWL<u>N</u>P<u>TKES</u>A<u>GNTLT</u> <u>VSNYTGTPGSVISLGGVLEGDNSLTDRLVVKGNTSGQSDIVYV</u> <u>NEGGSGGQTREGINIISVEGNSDAEFSLKNRVVAGAYDYTLQK</u> <u>GNVSGTDNKGWYLTSHLPTSDTRQYRPENGSYATNMALANSL</u> FLMDLNERKQFSAVNDSTQPESASVWMKITGGRTSGKLSDGQ NKTITNQFINQLGGDIYKYHAEKLGDFTLGIMGGYANAKGKTI	478

	NHTSKKGARNTLDGYSAGLYGTWYQNGANATGLFAETWMQY NWFNASVKGDGLEEEKYNLNGLTASVGGGYNLNVHTWTSPE GIKGEFWLQPHLQAVWMGVTPDTHQEVNGTVVQGTGKNNLQ TKAGIRASWNVKSTLDKDTGREFRPYIEANWIHNTHEFGVKMS GDSQLLSGSRNQGEIKTGIEGVITQNLSVNGGVAYQAGGHGSN AISGALGIKYSF	
*Pa*TA[13]	MGTDHTQQFASDNYSGICPEAWAAMAEANRGHERAYGDDQW TARASDYFRQLFETDCEVFFAFNGTAANSLALAALCQSYHSVIC SETAHVETDECGAPEFFSNGSKLLLAQTEVGKLTPASIRDIALKR QDIHYPKPRVVTLTQATEVGTVYRPDELKAISATCKELGLHLH MDGARFSNACAFLGCSPAELSWKAGVDVLCFGGTKNGMAVG EAILFFNRDLAEDFDYRCKQAGQLASKMRFLAAPWVGVLQDD AWLRYADHANRCARLLAELVADVPGVSLMFPVEANGVFLQLS EPAIEALRARGWRFYTFIGEGGARFMCSWDTDIERVRELARDIR LVMGAHHHHHH	353
*Cg*TD[13, 14]	MSETYVSEKSPGVMASGAELIRAADIQTAQARISSVIAPTPLQY CPRLSEETGAEIYLKREDLQDVRSYKIRGALNSGAQLTQEQRDA GIVAASAGNHAQGVAYVCKSLGAQGRIYVPNQTPSQKRDRIM VHGGEFISLVVTGNNFDEASAAAHEDAERTGATLIEPFDARNTV IGQGTVAAEILSQLTSMGKSADHVMPVGGGGLLAGVVSYMA DMAPRTAIVGIEPAGAASMQAALHNGGPITLETVDPFVDGAAV STVGDLNYTIVEKNQGRVHMMSATEGAVCTEMLDLYQNEGII AEPAGALSIAGLKEMSFAPGSVVVCIISGGNNDVLRYAEIAERS LVHRGLKHYFLVNFPQKPGQLRHFLEDILGPDDDITLFEYLKRN NRETGTALVGIHLSEASGLDSLLERMEESAIDSRRLEPGTPEYEY LTHHHHHH	442
*Gra*FDH2[15]	MAKILCVLYDDPITGYPKSYARADVPKIDHYPGGQTAPTPKQID FTPGELLGSVSGELGLRKYLEGLGHTLVVTSDKEGEDSVFEREL PDAEIVISQPFWPAYLTPERIAKAKKLKLAVTAGIGSDHVDLEA AIKNGITVAEVTYSNSISVSEHVVMMILSLVRNYIPSYQWVIKG GWNIADCVERSYDLEAMHVGTVAAGRIGLAVLKRLKPFDVKL HYFDQHRLPESVENELGLTYHPSVEDMVKVCDVVTINAPLHPG TLDLFNDELISKMKRGAYLVNTARGKICNRDAVVRALESGQLA GYAGDVWFPQPAPKDHPWRTMPHHGMTPHISGTSLSAQARYA AGTREILECWFEERPIREEYLIVDGGKLAGTGAHSYTVSKHHHH HH	392
D-HicDH[13]	MKIIAYGARVDEIQYFKQWAKDTGNTLEYHTEFLDENTVEWA KGFDGINSLQTTPYAAGVFEKMHAYGIKFLTIRNVGTDNIDMT AMKQYGIRLSNVPAYSPAAIAEFALTDTLYLLRNMGKVQAQLQ AGDYEKAGTFIGKELGQQTVGVMGTGHIGQVAIKLFKGFGAK VIAYDPYPMKGDHPDFDYVSLEDLFKQSDVIDLHVPGIEQNTHI INEAAFNLMKPGAIVINTARPNLIDTQAMLSNLKSGKLAGVGID TYEYETEDLLNLAKHGSFKDPLWDELLGMPNVVLSPHIAYYTE TAVHNMVYFSLQHLVDFLTKFKPARKLLVQQVVNHHHHHH	341

Note: Underlined sequence is the linker.

Table S2 Strains and plasmids used in this study

Strains	Description	Source
DH5α	Cloning	Stored in our laboratory
BL21(DE3)	Protein expression	Stored in our laboratory
BL21::ΔCsgA	Protein expression; no resistance gene toward antibiotic	Constructed in our previous work[3]
Plasmids	**Description**	**Source**
pET21a (+)	Ampicillin; pBR322 origin	Stored in our laboratory
pET28a (+)	Kanamycin; pBR322 origin	Stored in our laboratory
pACYCDuet-1	Chloramphenicol; p15A origin	Stored in our laboratory
Recombinant strains (Abbreviations)	**Description**	**Source**
EX01 (Chl)	BL21:ΔCsgA pACYCDuet-*AIDA*	This work
EX02 (Chl)	BL21:ΔCsgA pACYCDuet-*AIDA-CBD*	This work
EX03 (Chl)	BL21:ΔCsgA pACYCDuet-*AIDA-CBD-CsgA(EFCA)*	This work
EX04 (Amp)	BL21:ΔCsgA pET21a-*CsgB-CsgA(SpyTag)*	This work
EX05 (Amp + Chl)	BL21:ΔCsgA pET21a-*CsgB-CsgA(SpyTag)* pACYCDuet-*AIDA-CBD-CsgA(EFCA)*	This work
EX06 (Amp + Chl)	BL21(DE3) pET21a-*InaK-SpyCatcher* pACYCDuet-*PaTA-CgTD*	This work
EX07 (Amp + Chl)	BL21(DE3) pET21a-*InaK-SpyCatcher* pACYCDuet-*CgTD-PaTA*	This work
EX08 (Amp + Chl)	BL21(DE3) pET21a-*InaK-InaD* pACYCDuet-*GraFDH2-D-HicDH*	This work
EX09	BL21(DE3)	This work

(Amp + Chl)	pET21a-*InaK-InaD* pACYCDuet-*D-HicDH-GraFDH2*	
IN01 (Kan + Chl)	BL21:ΔCsgA pET28a-mCherry pACYCDuet-*AIDA*	This work
IN02 (Kan + Chl)	BL21:ΔCsgA pET28a-mCherry pACYCDuet-*AIDA-CBD*	This work
IN03 (Kan)	BL21(DE3) pET28a-*GFP*	Constructed in our previous work
IN04 (Kan)	BL21(DE3) pET28a-*GFP-SpyCatcher*	Constructed in our previous work
IN05 (Kan)	BL21(DE3) pET28a-*mCherry*	Constructed in our previous work
IN06 (Kan)	BL21(DE3) pET28a-*mCherry-InaD*	Constructed in our previous work
IN07 (Amp + Kan)	BL21(DE3) pET21a-*InaK* pET28a-*GFP*	This work
IN08 (Amp + Kan)	BL21(DE3) pET21a-*InaK-SpyCatcher* pET28a-*GFP*	This work
IN09 (Amp + Kan)	BL21(DE3) pET21a-*InaK* pET28a-*mCherry*	This work
IN10 (Amp + Kan)	BL21(DE3) pET21a-*InaK-InaD* pET28a-*mCherry*	This work

Table S3 Gradient elution system

Time/min	Acetonitrile	Water (+0.1% TFA)
0	0	100
22	100	0
24	0	100

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