Rapid regulations of metabolic reactions in Escherichia coli via light-responsive enzyme redistribution

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Abstract

Protein-based condensates have been proposed to accelerate biochemical reactions by enriching reactants and enzymes simultaneously. Here, we engineered those condensates into a Photo-Activated Switch in E. coli (PhASE) to regulate enzymatic reactions via tuning the spatial correlation of enzymes and substrates. In this system, scaffold proteins undergo liquid-liquid phase separation (LLPS) to form light-responsive compartments. Tethered with a light-responsive protein, enzymes of interest (EOIs) can be recruited by those compartments from cytosol within only a few seconds after a pulse of light induction and fully released in 15 minutes. Furthermore, we managed to enrich small molecular substrates simultaneously with enzymes in the compartments and achieved the acceleration of luciferin and catechol oxidation by 2.3 folds and 1.6 folds, respectively. We also developed a quantitative model to guide the further optimization of this de-mixed regulatory system. Our tool can thus be used to study the rapid redistribution of proteins, and reversibly regulate enzymatic reactions in E. coli.

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Protein-based condensates have been proposed to accelerate biochemical reactions by enriching reactants and enzymes simultaneously. Here, we engineered those condensates into a Photo-Activated Switch in *E. coli* (PhASE) to regulate enzymatic reactions via tuning the spatial correlation of enzymes and substrates. In this system, scaffold proteins undergo liquid-liquid phase separation (LLPS) to form light-responsive compartments. Tethered with a light-responsive protein, enzymes of interest (EOIs) can be recruited by those compartments from cytosol within only a few seconds after a pulse of light induction and fully released in 15 minutes. Furthermore, we managed to enrich small molecular substrates simultaneously with enzymes in the compartments and achieved the acceleration of luciferin and catechol oxidation by 2.3 folds and 1.6 folds, respectively. We also developed a quantitative model to guide the further optimization of this de-mixed regulatory system. Our tool can thus be used to study the rapid redistribution of proteins, and reversibly regulate enzymatic reactions in *E. coli* .

Introduction

Up to now, there have been many endeavors contributed to expanding the toolbox of enzymatic reaction regulation in bacterial chassis like*E. coli* [1]. Tuning the expression level of enzymes is straightforward but has many limitations including more metabolic burden of protein synthesis[2]. Recently, regulations on the spatial arrangement of enzymes have been proposed as a solution for adjusting reaction rate at a fixed enzyme concentration[3-5]. With the enzymes in the same pathway assembled on a DNA- or protein-based scaffold, the reaction productivity can be enhanced via the adjustment of spatial correlation of enzymes in the same

pathway, as the result of minimizing the diffusion loss of intermediates[3, 6, 7]. In addition to those *de novo* designed proximity-based strategies, naturally existed bioreactors, known as bacterial microcompartments (BMCs), were hijacked to pack target enzymes of the same pathway together in a protein shell to mitigate intermediate losses[8]. However, the complexed properties of BMCs, made it challenging to be reused [9-11]. Furthermore, for all those strategies mentioned, it is hard to switch among different regulatory states in case it is needed to balance different metabolic pathways regularly[12].

Recently, LLPS-based technologies have been utilized as a convenient method to regulate the spatial distribution of target proteins, since a single protein segment can readily form compartments in cells[13], [14, 15]. There has been an integration of LLPS and proximity-based strategy to regulate pathway production[16, 17]. *In vitro*condensates were also constructed to regulate enzymatic reactions via protein redistribution, and some of them proved it possible to regulate reactions via tuning the spatial correlation between enzymes and substates [15, 18, 19]. However, the attempt of regulating *in vivo* reactions based on the same strategy, especially those catalyzing the conversion of small organic molecules, was still at its infancy, [18, 20, 21], though there are over 30 important metabolic pathways involving small molecular reactions found to be regulated by condensates, including acetyl-CoA carboxylation and glutamine synthesis[22-24].

The main strategy of enriching molecules into protein-based condensates was specific molecular recognition. For example, substrate RNAs could be directly enriched by interior RNA binding proteins, while SUMOylation enzymes could be recruited to synthetic condensates via protein-protein recognitions[18, 25, 26]. Beyond macro-biomolecules, recent studies also observed the direct incorporation of organic dyes and other small molecules like ATP and GTP into the condensates formed *in vitro* , providing the potential of enriching substrates in enzymatic reactions via non-specific protein-substrate interactions (Figure 1A)[19, 27]. With the enrichment of substrate inside the condensates, the overall productivity of an enzymatic reaction can be regulated by switching on the redistribution of enzymes (Figure 1B).

Here, we developed a photo-activated switch in *E. coli* (PhASE) to dynamically regulate enzymatic reactions by tuning the spatial correlation of enzymes and substrates (Figures 1C and 1D). To start with, a scaffold protein was harnessed to form artificial compartments based on LLPS in *E. coli* , followed by fusing it and protein of interest (POI) with either member of an optogenetic protein pair, termed phase module and light-responsive module (Figures 1C). POI was confirmed to be reversibly recruited into LLPS-based compartments within seconds via light-activated interaction between two modules (Figure 1C). Taking pi-pi interaction as the scaffold-client interaction, we tested the enrichment of small molecules and found that their reaction rates could be changed by about 2 folds (Figure 1D)[28]. The PhASE strategy can thus be used for sensitively and reversibly regulating wide range of enzymatic reactions in *E. coli* via light-induced protein rearrangement.

Results

2.1 Introduction of LLPS-based compartments into E. coli

Since the establishment of LLPS-based compartment in prokaryotes has not been recorded in detail in previous studies[4], we introduced several phase modules and recorded condensate nucleation, growth, and maintenance in *E. coli* . The major phase module we tested included FUSLCD (fused in sarcoma low complexity domain) fused with truncated GCN4. FUSLCD was known for its ability to phase separate via pi-pi interaction[28, 29], while GCN4 could further facilitate phase separation via oligomerization[30] (Figures 2A and 2B). This fusion protein formed membraneless compartments in the following experiments. During the dynamic formation process, some condensates were observed to nucleate around cell poles and move around them (Figure 2C, Video S1), while others emerged elsewhere in the cytosol, then moved around along the cellular membrane, and fused with each other, especially with the ones near cell poles (Figure 2D, Video S2). These phenomena are much like the behavior of liquid droplets[31]. Interestingly, nearly all condensates finally took up the intracellular space in a regular pattern, much like polar bodies[32], and the pattern was homogeneous across different cells and different expression levels of this phase module (Figure 2E, Supplementary Figure 1). Other phase separating proteins, such as cryptochrome 2 (CRY2), known for

its homo-oligomerization after a period of blue light induction, formed compartments at only one pole of the cell (Supplementary Figure 2)[33].

To further investigate the mechanism underlying the pole-localized pattern of LLPS-based compartment, it was attempted to dissect genome exclusion and pole attraction of protein aggregates by expressing phase module in elongated *E. coli* (Δ*ftsZ*), which had duplicated genomes but no septum between them. It turned out that the condensates formed regularly among genomes rather than only targeting the poles (Supplementary Figure 3). This phenomenon was similar to the results of Winkler *et. al* , indicating that the curvature of a cell wall did not play a role in the pole-localization[34, 35]. Additionally, it was found that the compartments formed only along one side of the cellular major axis, opposite to the side nucleoids occupied (Supplementary Figure 3). Combining those results together, it was reasoned that the unfavorable contact between nucleoid and phase module and the stochastic movements of the droplet-like condensates could determine its polar localization.

Then, the fluidity of the compartment formed by the phase module was investigated, which showed similar recovering capability as that in eukaryotes (Figure 2F)[36]. Remarkably, the compartments formed in our experiments were smaller compared to those formed inside eukaryotic cells or *in vitro* due to the limited space inside a bacterial cell[19, 21, 37], which could be the reason why they showed whole condensate bleaching. The recovery of fluorescence could be the result of protein exchange from the other compartments in the cells mediated by diffusing scaffold proteins in cytosol, instead of the periphery of the bleached site (Figure 2F). This property indicated that protein (and other molecules) were allowed to diffuse across the condensate boundary, providing the basis for POI recruitment[19].

2.2 Light-induced recruitment of POI into LLPS-based compartments

To recruit POI into compartments rapidly and dynamically, the light-responsive module was designed to sense the dynamic light signal. Together with the phase module, the system was termed the Photo-Activated Switch in *E. coli* (PhASE) (Figure 3A). The phase module acted as a scaffold to form isolated compartments from the cytosol of *E. coli* , while the light-responsive module remained evenly distributed in the cell until triggered by induction signals. After the light induction, the light-responsive module would be recruited into the compartments formed by the phase module (Figure 1C). Light-responsive protein pair CIB1 and CRY2 were chosen to respond to the blue light signal, between which the binding affinity would increase with the light intensity[38]. CIB1 was included as a part of the phase module, localizing permanently in compartments isolated from the cytosol, while CRY2 was included as a part of the light-responsive module, distributing evenly inside the cell in darkness. POI was represented by mCherry (Figure 3A). During the light induction process, the light-responsive module was captured and fixed when diffused into the LLPS-based compartment. Since protein diffusion is the major time-consuming step in this process, the regulation can be completed in the time scale of seconds theoretically, estimated by the protein diffusion rate in *E. coli* [39, 40]. Indeed, it was found that right after 8 seconds of 488 nm laser intense stimulation, the reorganization of mCherry into the compartments was completed. The concentration of mCherry inside could reach more than 15 folds compared to the cytosol (Figure 3B) and maintained almost unchanged over 30 minutes (data not shown). The LLPS-based compartments labeled with GFP were captured after the stimulation process, showing perfect overlap with mCherry signal (Figure 3B). When stimulated with the laser of lower intensity, the recruited amount of mCherry reduced, with the enrichment reaching only about 2 folds (Figure 3C). Furthermore, this state was highly reversible, with the fastest recovery time less than 10 minutes, and a fully reversed state was attained within less than 15 minutes (Figure 3C, Video S3). We next tried to stimulate the system several times after reversion and found that the induction effect was consistent and robust at least in the first three rounds (Figure 3C, Video S3). The overall reduction of the mCherry signal could be the effect of laser bleaching, yet the fold enrichment of POI in the compartment remained roughly the same under the same intensity of light during different induction rounds. Remarkably, like other condensates formed by proteins[41], their aging and solidification were observed. Their fluidity decreased along time and was almost undetectable after 10-hour IPTG induction at 16°C. However, under such states, POIs could still be recruited in seconds, suggesting a different mechanism for protein entrance other than the dynamic

component exchange between interior and exterior layer (Figure 3, Supplementary Figure 4). In summary, PhASE#1 was verified reorganizing POI within seconds in a reversible manner.

PhASE#2 system was constructed using similar light-responsive components as PhASE#1 but substituting FUSLCD with tandem SIM and SUMO repeats (Figure 3D), known for phase separating via multi-valent interactions[42]. The fold enrichment of POI inside the compartment after light induction could be over 10-folds (Figure 3E). Additionally, the interaction between phase module and light-responsive module in this system can be conveniently redesigned by adding different number (valency) of SIM or SUMO to light-responsive module genetically[42], so that the fold enrichment of POI can still be tuned even light source is fixed.

2.3 Regulation of metabolic reactions via mass action

Besides the enzyme concentration, another critical component influencing a biochemical reaction rate is the substrate concentration. If both enzymes and substrates are enriched in the same compartment, the reactions can be accelerated. If they are separated from each other, the enzymatic reactions can be shut down (Figure 1B). Considering that the main driving force of FUSLCD LLPS is pi-pi interaction, compounds enriched with pi-electrons could be included in the compartment as clients theoretically[13, 28]. To explore the regulatory effect of PhASE#1 on enzymatic reactions, the enzyme of interest (EOI) was tethered to the light-responsive module as the experimental group (EXP), so that it could be recruited to the membraneless compartments according to light-induced CRY2-CIB1 interaction. In the control groups (CTRL), EOIs were fused with mCherry to perform catalysis in a fully dispersed state, excluding the influence of protein fusion on enzymatic activity (Figure 4A). Then, both groups were treated with light and the reaction rate difference between them was derived.

As a proof of concept, the coelenterazine (CLZ) oxidation reaction catalyzed by Renilla luciferase (Rluc) was tested, which was a reporter system frequently harnessed for *in vivo* studies[43] (Figure 4B), and the luminescence generated during the process was used as a direct measurement of reaction rate[44] (Figure 4C). After the light induction, EOIs were successfully recruited into the membraneless compartments (Supplementary Figure 4A). In the first 20 minutes after CLZ addition, the EXP group displayed a significantly higher oxidation rate compared to that of the CTRL group, with 2.6 times of coelenteramide (CLM, the oxidative product of CLZ) accumulation difference. Since the overall substrate and enzyme concentration remained the same between these two groups (Figure 4B), we deduced that CLZ should have been enriched by the compartment from the cytosol, otherwise the limitation of enzyme movement in cells would not benefit the reaction efficiency. Remarkably, the concentration of bacteria did not have a major impact on this reaction at a proper range as long as the overall enzyme concentration remained at the same level (Supplementary Figure 5). With four biological replicates, it was concluded that PhASE#1 could indeed regulate this enzymatic reaction.

Subsequently, more biochemical reactions were conducted for possible practical applications. Catechol 2,3-dioxygenase (XylE) catalyzes the oxidation of catechol, which is a key metabolite in many organic degradation pathways[45, 46] (Figure 4D). Like the design above, a modified light-responsive module tethered with XylE was constructed and was demonstrated to be successfully enriched into the LLPS-based compartments (Supplementary Figure 4B). Both incubated under daylight, the EXP group showed a higher reaction rate compared to that of the CTRL group at the same enzyme and substrate levels, with 1.6 times 2-hydroxymuconate semialdehyde (2-HMS, the oxidative product of catechol) accumulation difference 4.5 minutes after the substrate addition (Figure 4E). From these results, the regulatory ability of PhASE on enzymatic reactions was demonstrated. Additionally, they also provided evidence for the principle of regulating metabolic reactions via changing the distribution correlation of enzymes and substrates.

Furthermore, we have built a quantitative model based on mass action and Michaelis-Menten equation to explore the parameters affecting the regulatory capacity of a de-mixed system on biochemical reactions, in which the acceleration of the reaction via the colocalization of the substrate and enzyme has been verified. Interestingly, we also found that the size of the compartments had significant effect on the regulatory capacity.

Discussion

Tuning metabolic reactions via regulations on the enzyme expression level has been studied comprehensively[1, 47]. Yet in many cases, changing the concentration of protein can cause additional metabolic burden and a waste of time[2, 40]. Here, we engineered protein-based condensates into a reaction regulator named PhASE, changing reaction rate via the mechanism of mass action. Without the need for protein synthesis during regulation, our tool enabled the control of enzymatic reactions at a higher temporal resolution, completing in a few seconds. Also, the reversibility of PhASE allows the dynamic regulation of reaction velocity, while the traditional regulations via chemical inducers and non-inducible scaffolds can hardly be reversed[3, 6]. Additionally, our exploration of chemical interactions between small molecular client and protein scaffold provided the potential of regulating vast metabolic reactions via condensate-based approach[24] (Figures 1D, 4B and 4E).

However, there are still some considerations that should be taken into using PhASE. It can be difficult to find such protein scaffolds for every specific chemical to enrich them at arbitrary folds. Remarkably, too strong interactions between chemical client and protein scaffold may increase reaction efficiency either since the scaffold will compete substrate binding with enzymes. Besides, to design those compartments in specific sizes can be difficult, except for tuning the expression level of scaffold proteins (Figure 2). Inappropriate fractional volume of the condensates can lead to limited regulatory capacity (Figure S7).

Nevertheless, our system has many potential applications. With a very high regulatory temporal resolution and reversibility, the PhASE system allows the switch of enzymatic reactions in a higher frequency, while the pulsatile activation of different pathways has been proved to enhance biosynthetic production[12]. Besides metabolic regulations, PhASE system also has the potential to regulate and study various biological activities. By quickly changing the spatial correlation of substrates and enzymes, it is suitable for studying transient biochemical reactions and the switch of signaling pathways (Figure 2). Some of these processes last only for a few seconds, which can hardly be rebuilt and studied via transcriptional and translational control, which achieves the maximum regulatory effect in minutes or even hours[42, 48]. Additionally, the fold change of reaction rate between two regulatory states may be increased even more if our approach can be integrated with the proximity-based regulation of metabolic pathways[16].

Material and Methods

4.1 DNA manipulation

All constructs were assembled using available restriction enzymes and T4 ligase, or Hi-Fi assembly from New England BioLabs (NEB) and other basic molecular cloning approaches. To construct the light-responsive LLPS system PhASE#1, we acquired gene sequences of FUSLCD (FUS residues 1-212, NP_004951.1), CRY2 (NP_171935.1), CIB1 (NP_195179.2), and GCN4 (residues 2-34, 1W5I_A) from Pilong Li's lab at Tsinghua University. The sequences of the other system, PhASE#2, contained SIM (E3 SUMO-protein ligase PIAS2 isoform X1 residues 505-527, XP_006722634.1) and SUMO (XP_012635485.1), were synthesized by Ruibiotech directly. Since we only used CRY2 as a light-responsive element, we added an MBP tag to avoid its aggregation[49]. Plasmids (Supplementary Plasmid list) contain genes encoding Rluc (Renilla luciferase, AGU01696.1), XylE (catechol 2,3-dioxygenase, WP_011005909.1), fluorescence proteins were acquired from iGEM parts distribution. All sequence ID could be found on NCBI.

4.2 Plasmid transformation

For plasmid amplification, 1 μL plasmid (approximately 100 ng μL^{-1}) was transformed to 50 μL *E. coli* DH5a competent strain (TIANGEN). It was then incubated on ice for 25 minutes followed by 90 seconds heat shock at 42. Subsequently, it was mixed with 450 μL LB medium and incubated for an hour using a rotary shaker (ZQZY-BG, Shanghai Zhichu). For protein expression, *E. coli*BL21(DE3) strain was employed (TIANGEN and TSINGKE). 100 μL Competent cell culture was used for co-transformation of two plasmids, the cultures were added with 900 μL LB medium to recover the cells. For single plasmid transformation, the protocol was the same as for *E. coli* DH5a amplification. For plasmid construction using HiFi assembly (NEB), 5 μL

product were transformed to 100 μL bacterial culture, followed the same protocol as that for amplification.

4.3 Strains, media, and culture conditions

LB medium and plates were used for *E. coli* cultivation. Kanamycin and ampicillin were added at 100 mg L^{-1} when used alone, 50 mg L^{-1} when combined. For plasmid amplification and molecular cloning, we used *E. coli* DH5a, cultivated at 37, 220 r.p.m. For protein expression, *E. coli*BL21(DE3) strains (TIANGEN and TSINGKE) were used with regulations on protein expression by *lacO* in the presence of different concentrations of IPTG, respectively. Seed cultures were grown at 37, 220 r.p.m, and adjusted to OD_{600} of 0.6-1.0 for IPTG induction. Since intrinsically disordered proteins were known to form inclusion bodies at high expression levels and may cause the misfolding of fused proteins, all of them were expressed at 16 and 220 r.p.m[49], while the light responses and enzymatic reactivities were studied at room temperature or appropriate temperatures.

4.4 Confocal microscopy

All images shown in this paper were captured by Nikon A1 LFOVT. To assay compartment formation (Figures 2C and 2D) and light responses (Figures 3B, 3C and 3E) for a long period of time, we used an LB agar pad to fix 1-5 μL overnight *E. coli* under a microscope. The solid pad layer was made from 400 μL 1% LB agarose, on a glass coverslip-bottomed (20 mm in diameter) 35 mm Petri dish (D35-20-1-N). To observe bacterial morphology and compartment localization pattern (Supplementary Figure 2-4), recruitment of enzymes into compartments (not shown), and other short period observation, we used glass slides to fix them. All images captured were under a 100X oil immersed lens. 488 nm laser was used both for the observation of phase module distribution and the inducing light signal to trigger POI recruitment. 561 nm laser was used for the observation of POI recruitment. ProLong Live Antifade Reagent (P36975, Invitrogen) was used to avoid photo-bleaching of mCherry (1:50 dilution). Intense light induction was performed using region of interest (ROI) to guide 488 nm lasers, 3% lasted for 8 seconds (Figures 3B and 3E), while weak light induction was conducted under 2% laser intensity for 6 seconds. FRAP assays were carried out using slightly higher laser power with ROI as a single pixel.

4.5 Image analysis

All confocal data analyses were performed on NIS Element Analysis. All parameters in the same time series were maintained the same. All quantified fluorescence intensity was calculated before images were smoothed for display usage. The contrast of images was enhanced by adjusting LUT.

4.6 Live staining of bacterial DNA

NucBlue Live Cell Stain ReadyProbes reagent (R37605, Invitrogen) was used to label *E. coli* nucleoid. 2 drops of the solution were added to 1 mL bacterial culture and incubated in darkness for 15 minutes. The observation should be completed in 45 minutes.

4.7 Coelenterazine oxidation assay

CTRL and EXP bacteria were collected by centrifugation of 5,000-8,000 r.p.m for 2 minutes and washed twice using PBS. Then, 100 μL PBS culture was added to each well of the 96-well plate, and the mCherry fluorescence level of EXP groups and CTRL groups were adjusted to match each other. Prior to each assay, 10 mM CLZ ethanol solution was diluted in PBS to a final concentration of 250 μM and incubated for 45 minutes to an hour. During the study, 3 μL CLZ diluted solution was added to each well[43]. The luminescence of 490 nm was measured at 21-27. CLZ was purchased from Invitrogen (C2944). Thermo Scientific Varioskan LUX was used for quantitating reactions. SkanIt software was used for data management.

4.8 Catechol oxidation assay

CTRL and EXP group bacteria were collected by centrifugation of 5,000-8,000 r.p.m for 2 minutes and washed twice using PBS. Then, 100 μL PBS culture were added to each well of 96-well plate, and the mCherry fluorescence level of EXP groups and CTRL groups were matched to each other. Prior to each

assay, 100 mM catechol water solution was diluted to 10 mM working solution, 2.5 μL of which was then added to each well. The absorbance of oxidation product 2-HMS at 377 nm can be measured at 19-22. Catechol was purchased from Damas-beta (120-80-9). Thermo Scientific Varioskan LUX was used for quantitating reactions. SkanIt software was used for data management.

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Figure legend

Figure 1. Photo-activated switch for controlling enzymatic reactions.

A. Illustration of a model enzymatic reaction. Substrates (S) can be converted to product (P) by enzymes (E), during which light emission (yellow) by non-stable intermediates directly reflects reaction rate. **B. Enzymatic reaction organized by subcellular compartments.** Under the same overall concentration of enzymes and substrates, a reaction will be accelerated if enzymes and substrates are co-localized in a limited space, resulting in increasing local substrates concentration, thus increasing effective collision between substrates and enzymes (right). On the other hand, if enzymes are isolated from substrates, the reaction rate will decrease (left).**C. General design of rearranging enzymes distribution by light.** Each member in a light-responsive protein pair is fused with either phase separating protein or an enzyme of interest (EOI) to form phase module or light-responsive module. The phase module can phase separate into a membraneless compartment. Upon light induction, these two modules will bind to each other, and thus

EOI can entry the compartment. **D. General principle of concentrating chemicals into the compartment.** Substrates will be enriched in the compartment if the substrate-protein interactions are similar to protein-protein interactions promoting phase separation (e.g., pi-pi interaction).

Figure 2. Construction of LLPS-based compartment in *E. coli*.

A. Illustration of PhASE#1 phase module design.FUSLCD can phase separate *in vivo* , while GCN4 can further facilitate it. CIB1 is one of the two members in the light-responsive protein pair. See **Figure 3A** for further explanation.**B. Illustration of the mechanism behind compartment construction.** As its concentration rises beyond a threshold, the phase module will self-aggregate into membraneless compartments with a unique chemical environment. **C, D. The process of compartment formation in *E. coli*.** Most compartments nucleated around cell poles and could move around (C). Some aggregates emerged elsewhere, moved towards an end of the bacteria cell, and finally fused with other condensates (D). Scale bar, 1 μm. **E. Homogeneous compartment formation in *E. coli*.** Almost every bacterium contains compartments localized at their poles, when expressing PhASE#1 phase module. Phase module was under T7 promoter controlled by *lacO* . Either 0.25 mM or 1 mM IPTG was added to induce its expression. For more IPTG concentration gradients, please refer to Supplementary Figure 1. Scale bar, 2.5 μm. **F. Fluidity of PhASE#1 phase module*in vivo*.** Fluorescence Recovery After Photo-bleaching (FRAP) was used to characterize the fluidity of the fusion protein. The recovery of fluorescence could be due to rapid protein exchange with the surrounding cytoplasm. The fluorescence signal of the highlighted condensate was normalized to that on the other side of the bacterium. Scale bar, 1 μm.

Figure 3. The recruitment of POI into protein compartment.

A. Illustration of PhASE#1 system design. CIB1 and CRY2 could bind to each other under 488 nm light. **B, C. Induction of PhASE#1 system by light of different intensity.** With intense 488 nm laser irradiation for 8 seconds, the protein compartments could enrich POI up to 15-fold compared to cytosol concentration. GFP signal was not shown since its capture also need 488 nm laser but note that the compartment formed by phase module had formed before the induction process. The merged image was captured after the end of stimulation process. More confirmations of the co-localization of two modules after light were shown in Supplementary Figure 4 (B). With laser irradiation of lower intensity for 3 seconds, the protein compartments enriched POI at about 2-fold. After 10 to 15 minutes, the system fully reversed to previous state and was ready for the second round of induction (data not shown) and the third round of induction (1:08:19). With the same set of parameters, this system could be induced to almost the same enrichment fold as the first round. Since the bacteria were actively dividing, the intensity profile of the last time point was not precisely overlapped with that of former time points (C). **D. Illustration of PhASE#2 system design.** SIM and SUMO are utilized for compartment formation. **E. Induction of PhASE#2 system by light.** With intense laser irradiation for 8 seconds, the protein compartments could enrich POI for more than 10-fold. Scale bar, 1 μm.

Fig 4. Regulation of single-step enzymatic reactions by PhASE#1 system.

A. Module design of PhASE#1 system. In the experimental strain, both phase module and light-responsive module tethered with enzymes of interest (EOI) were expressed, resulting in cells containing a LLPS-based compartment that could enrich both enzymes and substrates. In the control (CTRL) strain, only a mCherry fused EOI was transformed, mimicking traditional manufacturing process, with enzymes and substrates dispersed in cells**. B, C. Coelenterazine (CLZ) oxidation kinetics.** CLZ can be oxidized to CLM by Renilla Luciferase (Rluc). During the reaction test, the overall concentration of Rluc in both groups has no significant difference (B). Reaction rate from 4 to 20 time points from both groups, reflected by luminescence at 490 nm, were compared to each other using paired t-test (1-3 time points were before the addition of substrates). The experimental group has a significantly higher reaction efficiency (C). This experiment has four biological replicates. **D, E. Catechol oxidation kinetics.** 1, 2-Dihydroxybenzene (Catechol) can be oxidized to 2-hydroxymuconate semialdehyde (2-HMS) by catechol 2,3-dioxygenase (XylE)[31]. During the reaction test, the overall concentration of XylE in both groups has no significant difference (D). Reaction

rates from 4 to 20 time points were calculated in both groups from the light absorbance of 2-HMS and compared using paired t-test (1-3 time points were before the addition of substrates). The experimental group has a significantly higher reaction rate compared to control group. (E). This experiment has four biological replicates.

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Author contributions

Z.H, G.L, H.L, G.Q.C contributed to the overall design of the PhASE system. Z.H, L.S, G.L, Z.Z, S.F, J.G, C.C, C.Q contributed to molecular cloning; Z.H, L.S, G.L, H.L collected and analyzed imaging data together; Z.H, L.S, J.G and X.W tested the application of PhASE system on metabolic reactions and analyzed the data. Z.H drafted the article and figures, which were revised by X.W and G.Q.C. Under the approval of X.W and G.Q.C, the article was submitted to scientific journals.

Data availability

All original data presented in this paper can be requested from the corresponding authors.

Competing interests

The authors declare no competing interests.

Disclosure

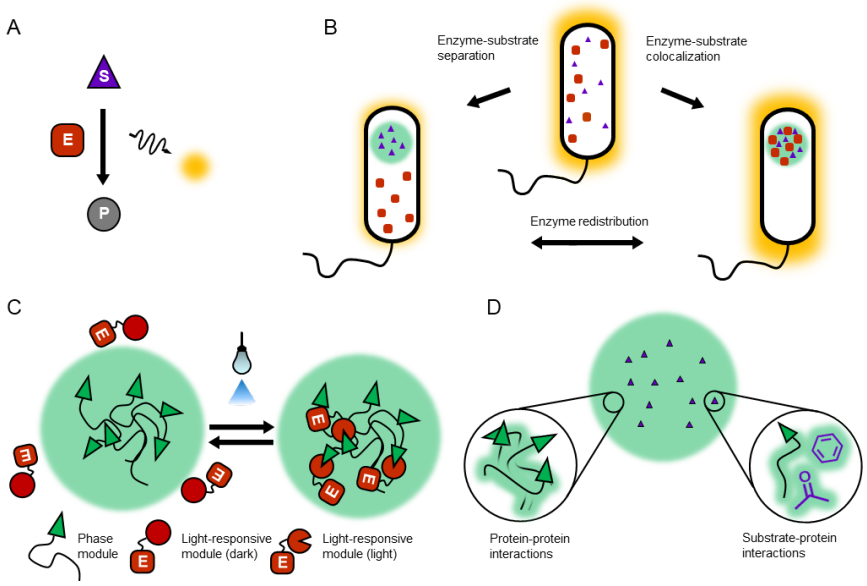
The manuscript does not contain experiments using animals.

The manuscript does not contain human studies.

Additional information

Supplemental material is available.

Figure



Figure

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Figure 2

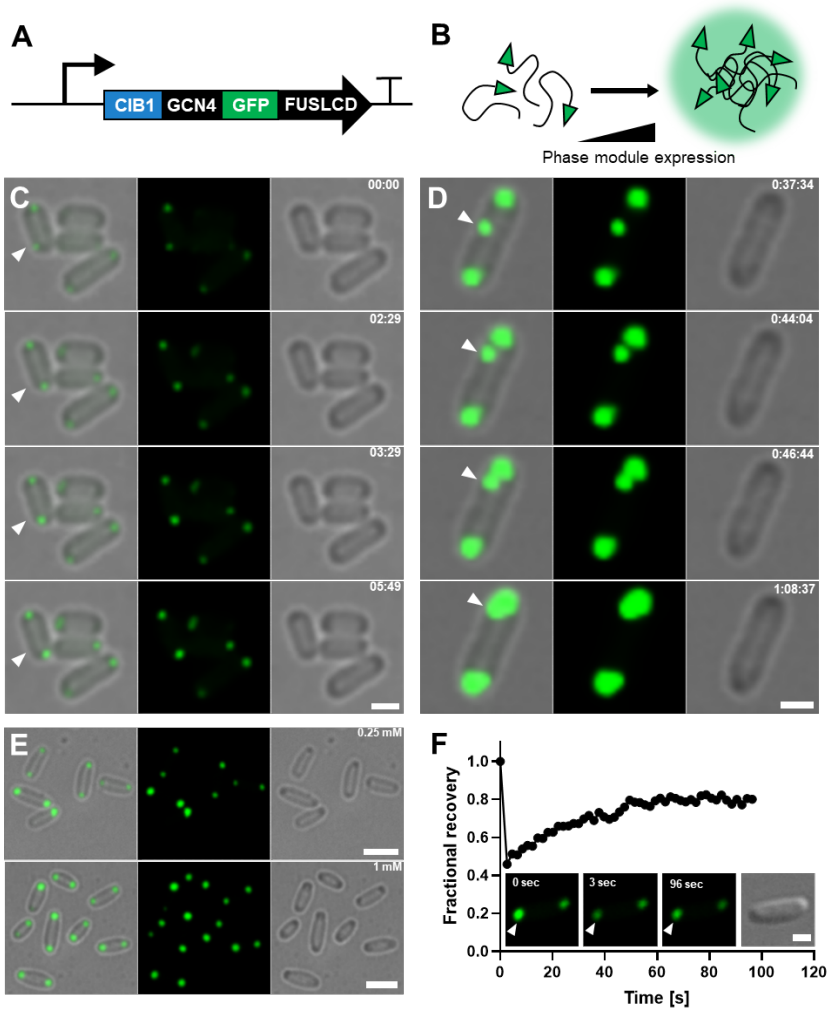

Figure 3

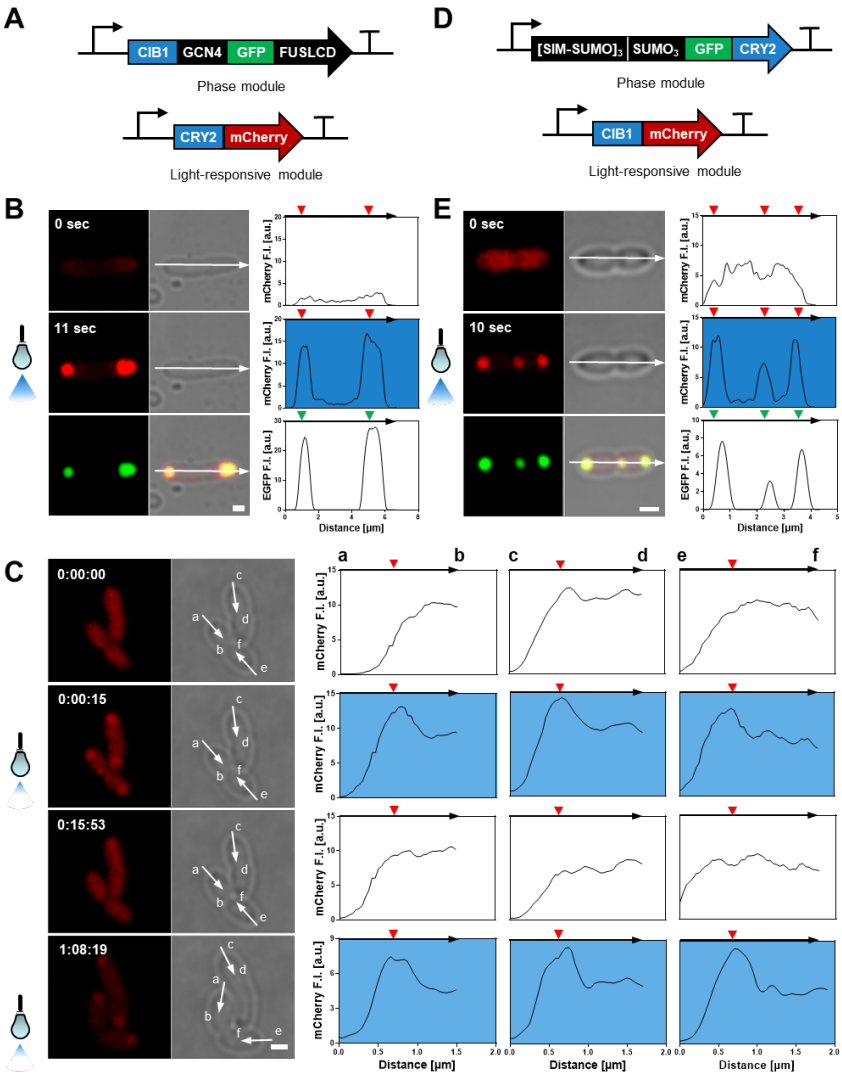

Figure 4