Physical stimuli-responsive cell-free protein synthesis

Junzhu Yang, Yuan Lu [*]

Key Laboratory of Industrial Biocatalysis, Ministry of Education, Department of Chemical Engineering, Tsinghua University, Beijing, China

ARTICLE INFO

ABSTRACT

Cell-free protein synthesis has been developed as a critical platform in synthetic biology. Unlike the cell-based synthesis system, cell-free system activates transcriptional and translational mechanisms *in vitro*, and can control protein synthesis by artificially adding components or chemicals. However, the control method puts forward higher requirements in terms of accurate and non-toxic control, which cannot be achieved by chemical substances. For cell-free system, physical signal is a kind of ideal spatiotemporal control approach to replace chemical substances, realizing high accuracy with little side effect. Here we review the methods of using physical signals to control gene expression in cell-free systems, including studies based on light, temperature, electric field, and magnetic force. The transfer of these switches into cell-free system further expands the flexibility and controllability of the system, thus further expanding the application capability of cell-free systems. Finally, existing problems such as signal source and signal transmission are discussed, and future applications in pharmaceutical production, delivery and industrial production are further looked into.

1. Introduction

Cell-free protein synthesis (CFPS) has developed rapidly in recent years [1]. CFPS activates biological machinery without the use of living cells, allowing direct control of transcription, translation and metabolism in an open environment [2]. It eliminates membrane closure [3, 4], and is not restricted by most intracellular metabolic pathways, providing simpler and faster engineering solutions to design with extreme freedom than cell system. In CFPS, by adding specific substances, the biosynthetic reaction is initiated and completes within a few hours [5], offering rapid iterative "design-build-test" cycles [6]. Given the above superiorities, CFPS has been widely used in protein synthesis, including biodrug [7], membrane protein [8], unnatural protein [9,10], biological diagnosis [11–13], genetic circuit design [14,15], and high-throughput analysis [16]. However, different applications have specific requirements in time and space, and the transcription and translation process of the system needs precise initiation and control to better realize these applications.

Therefore, one of the major objectives and challenges for cell-free systems is appropriate spatial and temporal control of the transcription or translation. Openness is a unique characteristic of cell-free

systems. The addition of inorganic ions and organics at a specific time can flexibly control the transcriptional and translation process in a cell-free system. In recent years, to address these challenges, researchers have proposed a spatiotemporal control method based on physical elements as stimulus signals, including light [4,5,17], temperature [18], electric field [19] and magnetic force [20] in CFPS. Physical signals with low toxicity and side effects are better than chemical substances at switching genes on and off [21]. More importantly, the physical signal can be flexibly adjusted in CFPS, so as to achieve immediate and local signal transmission and activation, and the rate will not be limited by other metabolic paths *in vitro*. The introduction of the physical signal switch into CFPS increases the potential of the system in health care, industrial production, synthetic biology education, and eventually achieving creating intelligent synthetic life.

Here, we mainly review the progress on the control of gene transcription and translation in CFPS by using physical signals with two types of classification, including direct response and indirect response (Fig. 1). Direct response involves directly changing the structure of the regulatory elements, while indirect response involves altering the position of the elements. In addition to summarizing the control principles (Table 1) and technical characteristics (Table 2) of each control method,

the application fields of the system also prospect, the possible defects are discussed, and the future research direction is further proposed.

2. Direct response - control of elements

Direct method is to change the state of the corresponding regulatory element by physical stimuli, which may be allosteric, phosphorylated, or specifically altered in the structure of the DNA. Specific elements that respond to physical stimuli can bind to gene promoters to determine whether genes are expressed or not. At present, two physical stimuli, light and temperature, have been well developed to respond directly to genetic elements, on the basis of which the applications of these systems in cell-free systems have been achieved (Table 1).

2.1. Control by light

Light possesses many advantages as an ideal genetic control switch, including fast input speed, good spatiotemporal conversion, low toxicity, and low cost of light source device. Light-controlled gene synthesis belongs to the field of optogenetics technology, which uses light to remotely control modified genes. At present, light control systems are mainly based on two-component systems (TCSs) [22], one-component systems [23] and photoreceptors [24]. Their introduction into CFPS could enrich the optogenetics research means, provide better guidance for the design of light control elements, and provide guidance for studying the interaction between proteins.

The two-component system (TCSs) depends on the phosphorylation between bicomponent proteins under different light conditions, ranging from ultraviolet light to near-infrared light, and light signal controls gene expression under the matched promoter [25]. Zhang et al. constructed two plasmids named pDark and pLight using TCSs YF1/FixJ

that responds to blue light, to realize cell-free light-controlling gene expression with *Escherichia coli* extract under darkness and blue light in the batch, respectively [5]. This work successfully transplanted the TCSs into CFPS and achieved light control, further demonstrating its potential in education, healthcare and artificial cell construction. However, the control signal studied so far is limited to blue light, whose phototoxicity might destroy the expressed proteins, and the subsequent *in vivo* application of artificial cells constructed by CFPS is limited. To address these challenges, an alternative solution is to use TCSs with longer wavelengths, such as CcaS/CcaR responding to green light [26], Cph8/OmpR responding to red light [27], and BphP1/PpsR2 responding to near-infrared light [28]. It also could achieve flexible cooperative regulation of CFPS using different wavelengths of light, to enrich the applications of cell-free light-controlled system. However, the TCSs are intrinsically complex, relatively slow in signal relay and reversal, and less portable. All of these are the directions for further efforts and attempts to construct cell-free optogenetic systems in the future.

To avoid the possible drawbacks brought by TCSs, the system that only requires a single element to achieve control was developed, the so-called one-component system. Jayaraman et al. used EL222 protein-based blue light-inducible promoter to construct the light-controlled system in CFPS with *E. coli* extract [17]. EL222 protein forms homologous dimers when irradiated by blue light, and the dimers would bind to the PBind promoter, recruiting relevant RNA polymerase to the promoter region to initiate transcription [29]. This work proved the possibility of dynamic control of target gene expression, but this light control system is not currently being developed for engineering applications. However, in the one-component system, the components that can be selected to respond to long wavelengths are limited compared with TCSs, which may limit its development for CFPS.

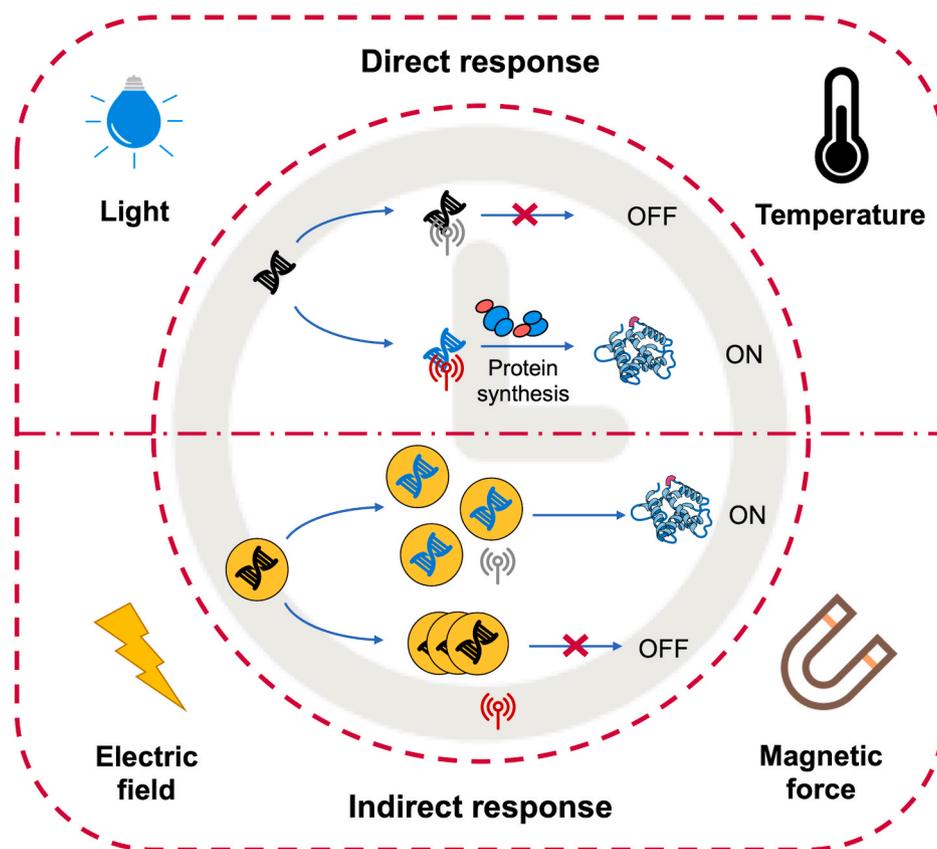In addition to the above light-controlled methods by directly

Fig. 1. Schematic illustration of the gene expression control systems in CFPS. Strategies shown in the top half part are based on the principle of direct response regulated by light and temperature. Strategies shown in the bottom half part are based on the principle of indirect response regulated by electric field and magnetic force.

interacting macromolecular photosensitive proteins with promoters, organic small molecular structures that respond to specific wavelengths of light are also used to control the cell-free expression. Kamiya et al. tethered 2,6-dimethyl-4-(methylthio) azobenzene-4′-carobxylic acid (S-DM-Azo) units to the T7 promoter region and achieved photoswitching of gene expression using visible light in CFPS with PURE system [30]. In this system, azobenzene can induce reversible formation and dissociation of the DNA duplex upon light irradiation [31]. The system avoids complex protein-promoter interactions, but the sample preparation procedure was laborious, and heating to above the Tm of the template was required to induce photoisomerization, limiting its practical applications. In addition to azobenzene, the photoswitches commonly used also include stilbenes [32], hemithioindigos [33], spiropyrans [34], diarylethenes [35], and fulgides [36]. By modularizing these conversion units into DNA strands, it is also possible to reversibly control cell-free gene transcription [37].

At present, cell-free optogenetic system is the main means of direct response, but there are still some challenges to be solved for its development. The establishment of the system requires that the optically responsive elements originally existing *in vivo* should be transplanted into the cell-free system solution. To overcome the differences between *in vivo* and *in vitro* and improve the efficiency, the prototype design should be carried out, and the key influencing factors of the system should be explored. One important problem of cell-free optogenetic system is that light can be refracted in the solution. The effective transmission of light in solution is also the direction of future research.
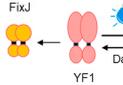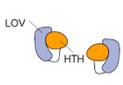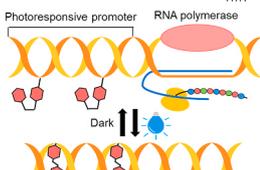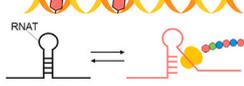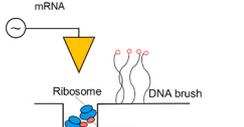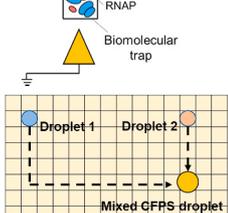
2.2. Control by temperature

Temperature is another physical signal with high flexibility and stability. Compared with the light control, there are more methods to control and change the temperature of the system, including external triggers such as focused ultrasound, infrared light, and magnetic particle hyperthermia [38]. In general, there are heat-shock protein promoters [39], thermo-sensitive repressor [40], and RNA thermometers (RNATs) that could induce the transcription process [41]. Changes in temperature alter the structure of these elements, thereby altering the process of gene transcription. It is of great significance for the intelligent creation of artificial life systems to give cell-free systems with the ability to respond to temperature.

Though there have been various ways to achieve temperature control in cells, only RNATs have been transplanted into cell-free systems in previous studies. RNAT loop unfolds when the temperature exceeds a defined threshold, to release the RBS for subsequent gene expression. Jia et al. designed three different threshold temperature RNAT switches that can be activated at 35 °C, 37 °C, or 40 °C in cell-free PURE system [18]. This study further used this system to create a temperature-sensitive protocell model for potential drug delivery applications. However, the expression of a high threshold switch under thermal activation is relatively low, which may be related to the stability of the secondary structure in the system.

From a technical point of view, heat-shock promoters and heat repressor proteins are also feasible means of abundant cell-free temperature control in the future. Compared with RNA thermometers, the interactions between promoters and related proteins are more complex

Table 1
Summary of the gene expression control systems in CFPS.

Sorting	Physical signal	Key elements	Mechanism	Induction Multiple	Ref.
Direct response	Light	Two-component system: YF1/FixJ		6	[5]
		One-component system: EL222		>10	[17]
		Azobenzene-tethered photoresponsive T7 promoter		7.1	[30]
	Temperature	RNA thermometer		11	[18]
Indirect response	Electric field	DNA compartment on a chip		–	[19]
		DMF board		–	[42]
	Magnetic force	Magnetic beads coated with DNA		–	[20]
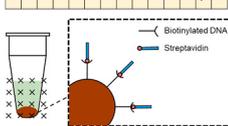
and may be associated with metabolic pathways in the body, making transplantation more difficult. Another idea is about miniaturization, which has a great prospect in the application fields such as therapy and medicine. Currently, magnetic nanoparticles are used to be coated with microcapsules to generate heat under the action of alternating magnetic field, so as to achieve the purpose of heat induction.

3. Indirect response - control of contact

Indirect methods mainly use external physical signals to control the location of elements involved in transcription and translation, thus controlling the process of gene expression. This control mode does not change the activity and state of biological components, but is more like a button that controls the contact between elements and DNA. The indirect methods are mainly achieved via electric field and magnetic force (Table 1), while it is difficult for the two signals to directly control gene expression mainly due to the insufficient research on the related elements.

3.1. Control by electric field

Compared with the precise and complex control of genetic elements by light and temperature, electric field control is simpler and more flexible. The biomolecules under a nonuniform E-field could be trapped and polarized using dielectrophoresis (DEP). The other way is to turn on gene expression by manipulating the droplet's motion directly with the electric field. It is also worth noting that, for the cell-free system, the manipulation using electric field can be carried out without considering the survival and growth of cells.

One way that the electric field controls cell-free gene expression is to use DEP technology. Efrat et al. used DEP to demonstrate the electric field on/off switch response of gene expression in the PURE CFPS system on a chip [19]. Various forms of DEP can be applied to trap, manipulate and separate biomolecules to controlling the contact between the DNA brush attached to the surface in the trap and the elements, such as ribosomes, RNA polymerases, newborn RNAs and proteins, so that controlling protein synthesis. Based on the electronic control system, the biochip was constructed and demonstrated protein synthesis oscillations successfully, which further improved the potential of electric control. However, the electrothermal effect may occur when the electric field interacts with the solution system, which affects the performance of the components in the system. In the future, by combining the design principles of integrated circuits with the powerful information processing capability of biological systems, the capability of biochips to process biological information can be expanded. At the same time,

further development of genetic elements that can accurately respond to electric fields would enrich the means of electric field control.

An alternative approach to controlling gene expression using electric fields takes a more macroscopic approach. There are also studies achieved controlling gene expression into proteins using digital microfluidic (DMF), which can directly manipulate single droplets. Liu et al. used real-time remote-controlled DMF technology to start cell-free synthetic reaction successfully [42]. By controlling the movement and mixing of the drops containing different components through real-time DMF, cell-free protein expression reaction started on the board. This work enables the automation of the preparation of cell-free system solutions, which can save time and manpower costs. However, limited by the number of plate electrode arrays, only the motion of small droplets can be controlled. Moreover, this control plate can only initiate the gene expression by electric fields, while it cannot be stopped without the addition of an exogenous transcriptional inhibitor, because the components in the droplet are sufficiently mixed.

Electric-field regulation is usually used to regulate complex neural signaling pathways *in vivo*, mainly to control the expression of some factors and enzymes [43], which is difficult to be transplanted to *in vitro* system. At present, the method of electric-field control in CFPS is relatively simple. In addition to controlling the location of key components, the combination of electric field and materials science improved the accuracy of spatiotemporal control, such as hydrogels that release substances in response to electric field stimuli [44].

3.2. Control by magnetic force

Magnetic force is a noteworthy control signal. Magnetic force has a stronger ability to penetrate tissues than light signals, and it does not cause global effects or local thermal damage like temperature signals, nor does it need to consider the potential harm of electric current to life activities. Although the regulation of magnetic field on life activities has been studied, the regulatory elements for gene expression have not been developed, so magnetic field control is still indirect.

The magnetic force controlling cell-free gene expression mainly relies on nanomagnetic beads. Finkler et al. developed a cell-free magnetic gene expression control system based on *E. coli extract* using the magnetic beads connected to target DNA [20]. Specifically, the DNA templates bound to magnetic beads can accumulate under magnetic control, thus stopping elements in the solution system from participating in DNA transcription and translation. This method can precisely regulate gene expression only through the spatiotemporal separation of nucleic acids. The magnetic control system is simple in principle and can be applied to microfluidics or non-regular amino acid embedded proteins. However,

Table 2

Summary of technical details in different CFPS system. Citations indicate where more information about the systems in the context of cell-free can be found.

Physical signal	Key elements	Cell-free system	Reaction format	Volume	Pros	Cons	Ref.
Light	YF1/FixJ	*E. coli* extract	Batch; One compartment	20 μL	Abundant components responding to different wavelengths	Slow signal relay and reversal rates; Poor portability	[5]
	EL222	*E. coli* extract	Batch; One compartment	25 μL	Good portability; Fast response	Limited response wavelength	[17]
	Azobenzene-tethered promoter	PURE	Batch; One compartment	–	Avoiding complex protein interactions	Tedious preparation process	[30]
Temperature	RNAT	PURE	Batch; One compartment	–	Flexible and varied means of temperature control	Low expression	[18]
Electric field	DNA brush on a chip	PURE	Batch; Fixed in compartment on a chip	Compartment: R = 35 μm; H = 3 μm	Novel manipulation of cell-free expression	Reduced component performance by electrothermal effect	[19]
	DMF board	*E. coli* extract	Batch; Multiple droplet compartments	20 μL	Remoted control of cell-free expression	Limited control scale	[42]
Magnetic force	Magnetic beads coated with DNAs	*E. coli* extract	Batch; One compartment	24 μL	Simple control method and spatiotemporal principle	The attachment effectiveness of DNA on magnetic beads	[20]

this control method requires attaching the target DNA to the nano-particles, which cannot guarantee that each particle will adhere effectively.

4. Conclusion and perspective

At present, the research on cell-free gene expression by the direct response realized in CFPS is mainly based on the light and temperature signals as switches. The signal response circuit originally existed in the cell is transplanted to the cell-free system, and the protein expression was successfully induced and controlled *in vitro* by using two kinds of physical signals, light and temperature. The direct response method has been preliminarily applied to simple dynamic demonstration, control of pharmaceutical protein synthesis and artificial cells, showing certain application potential. The indirect response is a simple control method that can bypass the complex biological metabolic interaction network to turn on and off the biological response of gene expression. The ability of electric and magnetic forces to control the elements allows them to respond indirectly, and the researchers have built systems for controlling gene expression that is sensitive and fast. Such systems have shown potential for mass control of synthesis and non-natural amino acids embedded in proteins, but have not been further explored for application.

The physical signal-controlling system has been developed with certain control ability, but it still needs to be further improved. For a system that responds directly, overcoming differences between *in vivo* and *in vitro* can improve the control effect of components, so it is necessary to screen out key influencing factors to reduce such differences. And rapid prototyping and high-throughput screening are also advantaging cell-free systems. Besides, direct methods require several hours to respond, and the development of systems with shorter response time will greatly improve its application ability. For systems that respond indirectly, the flexibility of the control system can be broadened by combining the control system with new materials. For example, the electro-responsive hydrogels are currently being carried out in drug delivery, and the combination of material and elements is worth considering.

In addition, this system has great application potential in both engineering manufacturing and medical health. CFPS controlled by physical signals has not been put into engineering manufacturing practice. Engineering proteins with complex structures put forward higher requirements on the synthesis ability, so it is necessary to further improve the ability to modify the high-level structure of proteins in cell-free systems. In recent years, the construction of artificial cells with cell-free system has increased. Studies have used light and temperature signals to control protein expression in artificial cells, preliminarily proving that protein synthesis can be controlled at the cell scale. Since artificial cells may be responsible for the production and delivery of drugs *in vivo*, the transmission of physical signals *in vivo* and the activity of protein production require further study.

In general, the development of controlled protein synthesis in cell-free systems will greatly promote the development of targeted drug synthesis and delivery technologies, and the flexible control of protein production *in vitro* will further promote the progress in the field of industrial protein manufacturing.

Ethics approval

This article does not contain any studies with human participants or experimental animals performed by any of the authors.

CRediT authorship contribution statement

Junzhu Yang: Conceptualization, Validation, Writing - original draft. **Yuan Lu:** Writing - review & editing, Supervision, Project administration, Funding acquisition.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

References

[1] Silverman AD, Karim AS, Jewett MC. Cell-free gene expression: an expanded repertoire of applications. Nat Rev Genet 2020;21:151–70. https://doi.org/10.1038/s41576-019-0186-3.

[2] Lu Y. Cell-free synthetic biology: engineering in an open world. Synth Syst Biotechnol 2017;2:23–7. https://doi.org/10.1016/j.synbio.2017.02.003.

[3] Yanagisawa T, Takahashi M, Mukai T, Sato S, Wakamori M, Shirouzu M, et al. Multiple site-specific installations of Nε-monomethyl-L- lysine into histone proteins by cell-based and cell-free protein synthesis. Chembiochem 2014;15:1830–8. https://doi.org/10.1002/cbic.201402291.

[4] Karig DK, Iyer S, Simpson ML, Doktycz MJ. Expression optimization and synthetic gene networks in cell-free systems. Nucleic Acids Res 2012;40:3763–74. https://doi.org/10.1093/nar/gkr1191.

[5] Zhang P, Yang J, Cho E, Lu Y. Bringing light into cell-free expression. ACS Synth Biol 2020;9:2144–53. https://doi.org/10.1021/acssynbio.0c00211.

[6] Moore SJ, MacDonald JT, Freemont PS. Cell-free synthetic biology for in vitro prototype engineering. Biochem Soc Trans 2017;45:785–91. https://doi.org/10.1042/BST20170011.

[7] Pardee K, Slomovic S, Nguyen PQ, Lee JW, Donghia N, Burrill D, et al. Portable, on-demand biomolecular manufacturing. Cell 2016;167:248–59. https://doi.org/10.1016/j.cell.2016.09.013. e12.

[8] Jacobs ML, Boyd MA, Kamat NP. Diblock copolymers enhance folding of a mechanosensitive membrane protein during cell-free expression. Proc Natl Acad Sci U S A 2019;116:4031–6. https://doi.org/10.1073/pnas.1814775116.

[9] Martin RW, Des Soye BJ, Kwon YC, Kay J, Davis RG, Thomas PM, et al. Cell-free protein synthesis from genomically recoded bacteria enables multisite incorporation of noncanonical amino acids. Nat Commun 2018;9:1–9. https://doi.org/10.1038/s41467-018-03469-5.

[10] Gao W, Bu N, Lu Y. Efficient incorporation of unnatural amino acids into proteins with a robust cell-free system. Methods Protoc 2019;2:16. https://doi.org/10.3390/mps2010016.

[11] Wen KY, Cameron L, Chappell J, Jensen K, Bell DJ, Kelwick R, et al. A cell-free biosensor for detecting quorum sensing molecules in P. Aeruginosa-infected respiratory samples. ACS Synth Biol 2017;6:2293–301. https://doi.org/10.1021/acssynbio.7b00219.

[12] Salehi ASM, Shakalli Tang MJ, Smith MT, Hunt JM, Law RA, Wood DW, et al. Cell-free protein synthesis approach to biosensing hTRβ-specific endocrine disruptors. Anal Chem 2017;89:3395–401. https://doi.org/10.1021/acs.analchem.6b04034.

[13] Pardee K, Green AA, Takahashi MK, Braff D, Lambert G, Lee JW, et al. Rapid, low-cost detection of zika virus using programmable biomolecular components. Cell 2016;165:1255–66. https://doi.org/10.1016/j.cell.2016.04.059.

[14] Pardee K, Green AA, Ferrante T, Cameron DE, Daleykeyser A, Yin P, et al. Paper-based synthetic gene networks. Cell 2014;159:940–54. https://doi.org/10.1016/j.cell.2014.10.004.

[15] Takahashi MK, Chappell J, Hayes CA, Sun ZZ, Kim J, Singhal V, et al. Rapidly characterizing the fast dynamics of RNA genetic circuitry with cell-free transcription-translation (TX-TL) systems. ACS Synth Biol 2015;4:503–15. https://doi.org/10.1021/sb400206c.

[16] Saha A, Acharya BN, Priya R, Tripathi NK, Shrivastava A, Rao MK, et al. Development of nsP2 protease based cell free high throughput screening assay for evaluation of inhibitors against emerging Chikungunya virus. Sci Rep 2018;8:1–13. https://doi.org/10.1038/s41598-018-29024-2.

[17] Jayaraman P, Yeoh JW, Jayaraman S, Teh AY, Zhang J, Poh CL. Cell-free optogenetic gene expression system. ACS Synth Biol 2018;7:986–94. https://doi.org/10.1021/acssynbio.7b00422.

[18] Jia H, Heymann M, Härtel T, Kai L, Schwille P. Temperature-sensitive protein expression in protocells. Chem Commun 2019;55:6421–4. https://doi.org/10.1039/c9cc02734c.

[19] Efrat Y, Tayar AM, Daube SS, Levy M, Bar-Ziv RH. Electric-field manipulation of a compartmentalized cell-free gene expression reaction. ACS Synth Biol 2018;7:1829–33. https://doi.org/10.1021/acssynbio.8b00160.

[20] Finkler M, Ott A. Bead-based assay for spatiotemporal gene expression control in cell-free transcription–translation systems. Biotechniques 2019;66:29–33. https://doi.org/10.2144/btn-2018-0097.

[21] Lu Y, Sun W, Gu Z. Stimuli-responsive nanomaterials for therapeutic protein delivery. J Contr Release 2014;194:1–19. https://doi.org/10.1016/j.jconrel.2014.08.015.

[22] West AH, Stock AM. Histidine kinases and response regulator proteins in two-component signaling systems. Trends Biochem Sci 2001;26:369–76. https://doi.org/10.1016/S0968-0004(01)01852-7.

[23] Liu Z, Zhang J, Jin J, Geng Z, Qi Q, Liang Q. Programming bacteria with light-sensors and applications in synthetic biology. Front Microbiol 2018;9. https://doi.org/10.3389/fmicb.2018.02692.

[24] Kong SG, Okajima K. Diverse photoreceptors and light responses in plants. J Plant Res 2016;129:111–4. https://doi.org/10.1007/s10265-016-0792-5.

[25] Möglich A, Ayers RA, Moffat K. Design and signaling mechanism of light-regulated histidine kinases. J Mol Biol 2009;385:1433–44. https://doi.org/10.1016/j.jmb.2008.12.017.

[26] Hirose Y, Shimada T, Narikawa R, Katayama M, Ikeuchi M. Cyanobacteriochrome CcaS is the green light receptor that induces the expression of phycobilisome linker protein. Proc Natl Acad Sci U S A 2008;105:9528–33. https://doi.org/10.1073/pnas.0801826105.

[27] Tabor JJ, Levskaya A, Voigt CA. Multichromatic control of gene expression in escherichia coli. J Mol Biol 2011;405:315–24. https://doi.org/10.1016/j.jmb.2010.10.038.

[28] Ong NT, Olson EJ, Tabor JJ. Engineering an E. coli near-infrared light sensor. ACS Synth Biol 2018;7:240–8. https://doi.org/10.1021/acssynbio.7b00289.

[29] Motta-Mena LB, Reade A, Mallory MJ, Glantz S, Weiner OD, Lynch KW, et al. An optogenetic gene expression system with rapid activation and deactivation kinetics. Nat Chem Biol 2014;10:196–202. https://doi.org/10.1038/nchembio.1430.

[30] Kamiya Y, Takagi T, Ooi H, Ito H, Liang X, Asanuma H. Synthetic gene involving azobenzene-tethered t7 promoter for the photocontrol of gene expression by visible light. ACS Synth Biol 2015;4:365–70. https://doi.org/10.1021/sb5001092.

[31] Liang X, Mochizuki T, Asanuma H. A supra-photoswitch involving sandwiched DNA base Pairs and azobenzenes for light-driven nanostructures and nanodevices. Small 2009;5:1761–8. https://doi.org/10.1002/smll.200900223.

[32] Waldeck DH. Photoisomerization dynamics of stilbenes in polar solvents. J Mol Liq 1993;57:127–48. https://doi.org/10.1016/0167-7322(93)80051-V.

[33] Lougheed T, Borisenko V, Hennig T, Rück-Braun K, Woolley GA. Photomodulation of ionic current through hemithioindigo-modified gramicidin channels. Org Biomol Chem 2004;2:2798–801. https://doi.org/10.1039/B408485C.

[34] Minkin VI. Photoswitchable molecular systems based on spiropyrans and spirooxazines. In: Feringa BL, Browne WR, editors. Molecular switches. second ed. Weinheim: Wiley-VCH; 2011. p. 37–80. https://doi.org/10.1002/9783527634408.ch2.

[35] Irie M. Diarylethenes for memories and switches. Chem Rev 2000;100:1685–716. https://doi.org/10.1021/cr980069d.

[36] Yokoyama Y. Fulgides for memories and switches. Chem Rev 2000;100:1717–40. https://doi.org/10.1021/cr980070c.

[37] Szymański W, Beierle JM, Kistemaker HAV, Velema WA, Feringa BL. Reversible photocontrol of biological systems by the incorporation of molecular photoswitches. Chem Rev 2013;113:6114–78. https://doi.org/10.1021/cr300179f.

[38] Piraner DI, Abedi MH, Moser BA, Lee-Gosselin A, Shapiro MG. Tunable thermal bioswitches for in vivo control of microbial therapeutics. Food, Pharm Bioeng Div 2017;2:695–702. https://doi.org/10.1038/nchembio.2233. 2017 Core Program Area 2017 AIChE Annu Meet.

[39] Rome C, Couillaud F, Moonen CTW. Spatial and temporal control of expression of therapeutic genes using heat shock protein promoters. Methods 2005;35:188–98. https://doi.org/10.1016/j.ymeth.2004.08.011.

[40] Valdez-Cruz NA, Caspeta L, Pérez NO, Ramírez OT, Trujillo-Roldán MA. Production of recombinant proteins in E. coli by the heat inducible expression system based on the phage lambda pL and/or pR promoters. Microb Cell Factories 2010;9:1–16. https://doi.org/10.1186/1475-2859-9-18.

[41] Sen S, Apurva D, Satija R, Siegal D, Murray RM. Design of a toolbox of RNA thermometers. ACS Synth Biol 2017;6:1461–70. https://doi.org/10.1021/acssynbio.6b00301.

[42] Liu D, Yang Z, Zhang L, Wei M, Lu Y. Cell-free biology using remote-controlled digital microfluidics for individual droplet control. RSC Adv 2020;10:26972–81. https://doi.org/10.1039/D0RA04588H.

[43] Nguyen EB, Wishner J, Slowinska K. The effect of pulsed electric field on expression of ECM proteins: collagen, elastin, and MMP1 in human dermal fibroblasts. J Electroanal Chem 2018;812:265–72. https://doi.org/10.1016/j.jelechem.2018.01.050.

[44] Tomer R, Dimitrijevic D, Florence AT. Electrically controlled release of macromolecules from cross-linked hyaluronic acid hydrogels. J Contr Release 1995;33:405–13. https://doi.org/10.1016/0168-3659(94)00115-B.