Letter

Dual-signal microbial biosensor for the detection of dopamine without inference from other catecholamine neurotransmitters

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Dual-signal microbial biosensor for the detection of dopamine without inference from other catecholamine neurotransmitters

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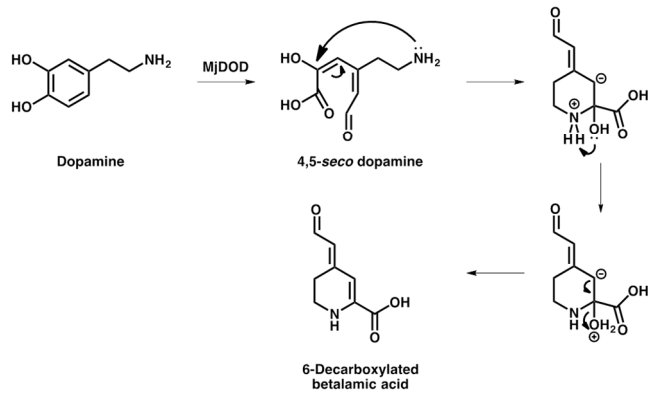
ABSTRACT

Dopamine, one of catecholamine neurotransmitters, plays an important role in many brain functions and behavioral responses. In this study, we developed a novel dual-signal whole-cell biosensor for the detection of dopamine through the generation of red fluorescent proteins and 6-decarboxylated betaxanthin pigments. The proposed system responses specifically to dopamine with a detection limit of 1.43 μM. Furthermore, a combination of dual output signals makes it possible to reduce the interference from other catecholamine neurotransmitters, including L-DOPA, epinephrine, and norepinephrine.

KEYWORDS. L-DOPA, dopamine, *E. coli*, DOPA-dioxygenase, catecholamine, betaxanthin

The central catecholamine neurotransmitter, dopamine (DA), has been shown to play an important role in multiple cognitive functions and researchers have previously investigated how variations in DA neurotransmission correlate with neurodegenerative diseases such as Parkinson's and Huntington's diseases.[1-4] The formation of dopamine is first by hydroxylalation of l-tyrosine and subsequently by the decarboxylation of L-DOPA. Numerous quantitative methods have been developed to estimate DA level accurately in the diagnosis and continuous monitoring of neurological disorders including microdialysis, electrochemistry, and fluorescence approaches.[5-15] Unfortunately, these efforts have often been confounded by compounds such as ascorbic acid, uric acid, epinephrine (EPI), and norepinephrine (NE) that are oxidized at similar potentials or present a similar structure. For this reason, it is important to develop a biosensor that could give rapid responses without interference from other catecholamine neurotransmitters.

In plant, L-DOPA is a substrate in the biosynthesis of betalamic acid, a precursor of a yellow betaxanthin pigment, through the catalysis of DOPA 4,5-dioxygenase.[16,17] Specifically, the DOPA 4,5-dioxygenase catalyzes the extradiol cleavage of L-DOPA to form the intermediate 4,5-*seco*-DOPA. Betalamic acid then conjugates with an amino acid or amine to form yellow betaxanthin through a spontaneous non-enzymatic reaction.[18] The bright yellow pigments have been used to serve as colorimetric signals in biosensors.[19,20] Therefore, we proposed to use DA instead of L-DOPA as substrates in the production of 6-decarboxylated betalamic acid (scheme 1).



Scheme 1 6-decarboxylated betalamic acid produced from dopamine through the catalysis of DOPA 4,5-dioxygenase. MjDOD: a DOPA 4,5-dioxygenase from *Mirabilis jalapa*.

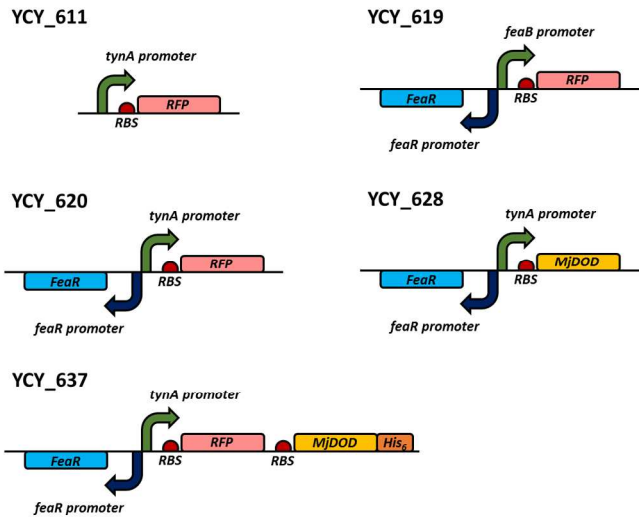


Figure 1 FeaR-based biosensor platform. Arrows indicate the direction of transcription and putative position of promoters. The genetic organization of the feaR -induced RFP/Mjdod expression in YCY_611, 619, 620, and 628 plasmids. RBS indicates ribosomal binding site.

In *E. coli*, the oxidation of aromatic amines, including dopamine, phenylethylamine (PEA), and tyramine to the corresponding aldehydes is catalyzed by the periplasmic amine oxidase (TynA). The aldehydes are further oxidized to the corresponding carboxylic acids by FeaB (PAL dehydrogenase).[21] Zeng et al. reported that *tynA* and *feaB* promoters were coordinately regulated by the FeaR. FeaR is a transcriptional regulator in *E. coli* that belongs to the AraC family and is upregulated by carbon or nitrogen limitation for the catabolism of aromatic amines.[22,23] In the presence of FeaR coactivators, C-terminal DNA binding domain of FeaR can bind to DNA and can activate the FeaR-regulating promoters. We designed our biosensor by using DA to activate FeaR-regulating promoters to induce the expression of red fluorescent protein (RFP) (Figure 1). FeaR consensus binding sites TGNCA-N8-AAA were found in the tynA and feaB promoter regions.[24] We therefore constructed strains YCY_619 and 620 in order to examine the FeaB/TynA driven expression of RFP in the presence of DA. Full DNA sequence between FeaR and RFP of YCY_620 is shown in Scheme S1. Conversely, strain YCY_611 was constructed to examine the induction of P_{TynA} under the endogenous expression of FeaR. Details of the plasmid constructions and primers are provided as supporting information (Table S1-3). During the exponential-phase, cells were grown in M9 minimal medium supplemented with 2% glycerol with or without DA at 500 μM for 5 h. The end-point fluorescence intensity values of RFP of three strains were recorded (Figure S1). In the presence of DA, YCY_619 produced a 6.2-fold increase in intensity values, whereas YCY_620 produced a 10.8-fold enhancement of induction. Plasmids without FeaR did not show any induction of fluorescence intensity, which suggests that additional copies of transcriptional regulators are necessary to produce an increase in gene transcription. All three strains presented relatively low background induction in the absence of DA

To evaluate the specificity of the system, we examined the fluorescence induction in this biosensor for interfering compounds (500 μM) including ascorbic acid and (i) aromatic amino acids (tryptophan, tyrosine, phenylalanine), (ii) aromatic derivatives (benzoic acid, PAL, benzaldehyde), (iii) aromatic monoamines (tryptamine, 3,4-dimethoxyphenethylamine, 4-methoxyphenethylamine, benzylamine, aniline, PEA), and (iv) structural analogues of DA (NE, EPI, L-DOPA, and DA). We found that only the native substrates of FeaB/TynA (including PAL, PEA, and DA) induced significant red fluorescence (Figure 2A). The fluorescence intensity of PEA was the strongest followed by DA.

We subsequently sought to determine whether a dose-response relationship existed between the concentration of DA and the red fluorescence intensity/OD600 ratio. As shown in Figure 2B, fluorescence increased as the concentration of DA was raised from 1 to 200 μM, and no further change in fluorescence was observed when the concentrations of DA increase. As shown in the photograph, RFP can be observed by the naked eye in the presence of DA (Figure 2C). The fluorescence response presented a linear relationship with the correlation coefficient (R^2) 0.972 with the concentration of DA between 0-10 μM (Figure 2B, inset). The limit of detection (LOD) calculated to be 1.43 μM.

Next, we proposed a scheme in which DA was used as a substrate for DOPA 4,5-dioxygenase in the production of 6-decarboxylated betaxanthin pigments. The goal was to reduce interference from PEA and PAL. To test how effectively DA can be used in pigment biosynthesis, we examined dioxygenase activity in the presence of various substrates. For this, cell lysates of BL21 carrying Mjdod-his were mixed with 1 mM of PEA, DA, L-DOPA, and EPI. The mixtures were incubated at 37°C for 30 min and examined. As shown in Figure S2, the UV–Vis absorption spectra in the range 300–700 nm were recorded. We observed an increase in

absorbance at 432 nm (A_{432}) in the presence of L-DOPA and DA, which is indicative of the betaxanthin pigment production. Notably, the A_{432} of the betaxanthin pigments which was generated form L-DOPA was about 10-fold stronger than that of DA.

Thus, we replaced *rfp* with *Mjdod* to construct strain YCY_628 (Figure 1). We again examined the increase of A_{432} after cells had been incubated in the presence of interference compounds at concentration of 500 μM for 5 h. We did not observe any increase of A_{432} when PEA or PAL was added to the media (Figure S3). Among samples, only DA and L-DOPA were detected by the enhancement of A_{432}; however, the induction of DA is relatively low. Thus, we also investigated how higher DA concentrations affected A_{432}. As the concentration of DA ranged of 200-5000 μM, an increase of the A_{432} was observed (Figure 3A). Furthermore, the absorbance values were linearly correlated with the concentration of DA (R^2=0.995) at doses between 0-2000 μM and the LOD was 11.1 μM (Figure 3B). As shown in the inset of Figure 3B, pigment production was visible to naked eye. We validated the production of 6-decarboxylated betaxanthin pigment from DA, by subjecting the cell culture to centrifuge, collecting the bacterial supernatant and analyzing the supernatant through high resolution electrospray mass spectrometry (Figure S4).

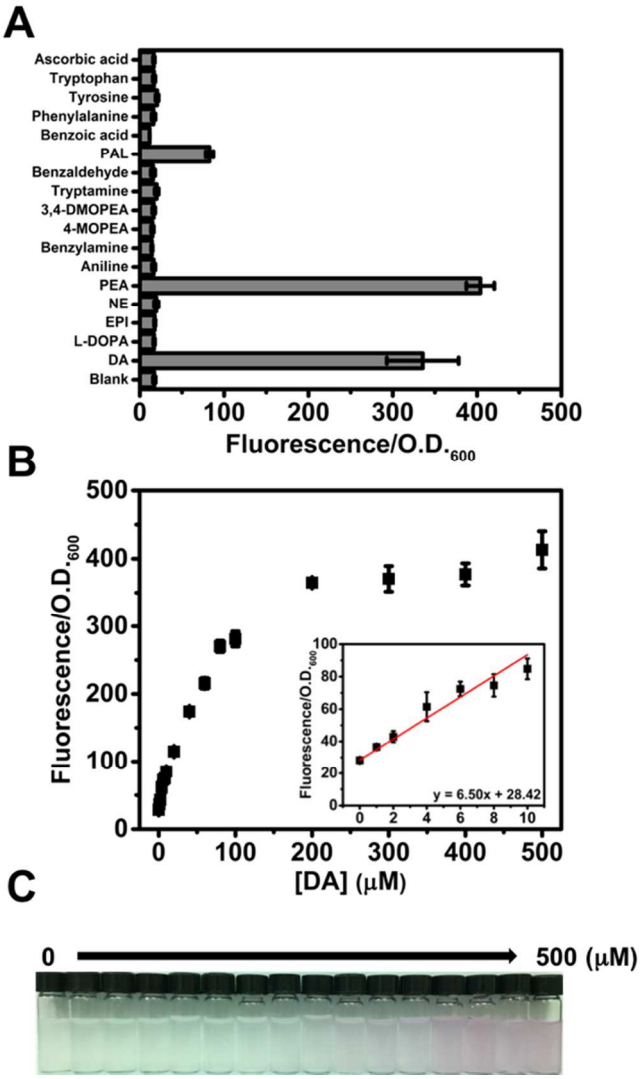


Figure 2 Expression of RFP from YCY_620 cells. (A) Quantitative analysis of the fluorescence in response to various substrates at the final concentration of 500 μM in M9 medium. Error bars represent the standard deviation from triplicate measurements. (B) Dose–response and linear calibration curves of red fluorescence intensity / OD600 ratio at different concentrations of DA. (C) Photograph of YCY_620 cells of (B).

Interestingly, in this system, L-DOPA showed significant increase of A_{432} but low RFP signals. To confirm that the tynA promoter was activated in the presence of L-DOPA, we

constructed a strain YCY_646 carrying Mjdod-his plasmid. Western immunoblotting analysis revealed that a greater quantity of His-tagged Mjdod and its proteolytic fragments were present in the cell lysates following an increase in DA concentrations (Figure S5). In contrast, we did not observe any His-tagged Mjdod in the presence of L-DOPA, which is consistent with the results of RFP analysis. Next, to rule out the possibility that L-DOPA had been converted by native DOPA dioxygenase in *E. coli*,[25] wild type cells were then incubated with L-DOPA at 500 μM for 5 h. No betaxanthin pigment was observed without the expression of Mjdod (Figure S6). Nonetheless, it is possible that trace quantities of proteins from the leaky expression of the promoter initiated the formation of betaxanthin pigment from L-DOPA.

We applied the system to the detection of L-DOPA at various concentrations. Similar to the above-mention DA detection, the induction of A_{432} was examined upon the increasing concentrations of L-DOPA (Figure 3C). The linear regression and photograph of visualized color change were shown in Figure 3D.

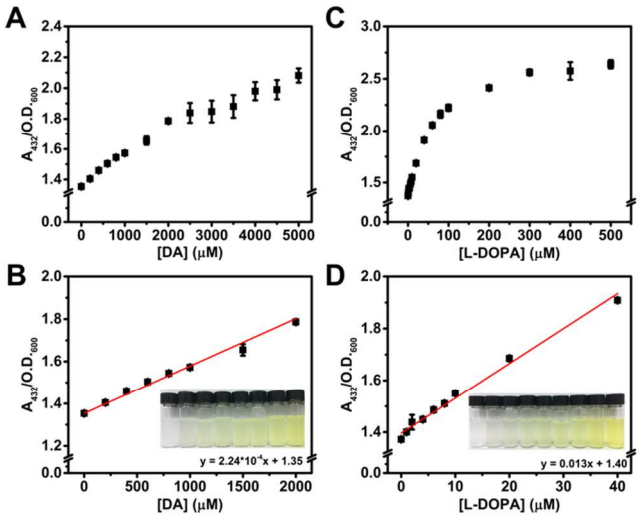


Figure 3 (Decarboxylated)betaxanthin productions and the increase in A_{432} from YCY_628 cells. (A)(C) Dose–response curves and (B)(D) linear calibration curves of A_{432} from cells were plotted against the final concentration of DA and L-DOPA. Insets: photograph of YCY_628 cells of (B) and (D).

To enable the differentiation of DA from other compounds, we designed a dual-signal biosensor that carried RFP and Mjdod-His and was driven by the TynA promoter (Figure 1). Promoter DNA sequence of YCY_637 is shown in Scheme S2. We then evaluated whether the system could generate unique outputs for common catecholamine neurotransmitters. For this, we examined the dose-response curves between fluorescence/A_{432} and various concentrations of PEA, L-DOPA, DA, and EPI. Four different compounds showed unique patterns of induction (Figure S7). PEA and L-DOPA respectively presented either RFP or A_{432} signals only, whereas DA presented both RFP and A_{432} signals, EPI presented neither fluorescence nor A_{432} signals (Figure 4A-D).

To test the interference effects between DA and L-DOPA, we examined the fluorescence intensity and A_{432} of cells that had been cultured in media supplemented with DA, L-DOPA, or a mixture of DA and L-DOPA. We found that in the coexistence of high amounts of L-DOPA (100 or 500 μM), fluorescence intensities were nearly identical to those obtained from samples that contained DA alone (Figure 4E). Interestingly however, the increase of A_{432} of DA and L-DOPA mixture had a stronger effect than did either DA or L-DOPA alone (Figure 4F). The reason could be that the DA-betaxanthin complex was produced in the mixture of DA and L-DOPA.

Many approaches have been developed with high sensitivity for the detection of DA (Table S4); however, bacterial whole-cell biosensors are simple, fast, and cost-effective alternatives to conventional analytical methods. In summary, we developed a novel whole-cell biosensor for the analysis of DA. We demonstrated that a combination of dual output signals makes it possible to differentiate among catecholamine neurotransmitters and greatly reduces the effects of interference between DA and L-DOPA. Continued investigation molecular mechanism of how FeaR responds to DA will improve the sensitivity and selectivity of the DA detection.

This study demonstrates the feasibility of designing biosensor using metabolites as precursors in the generation of output signals to enhance the specificity of the biosensors.

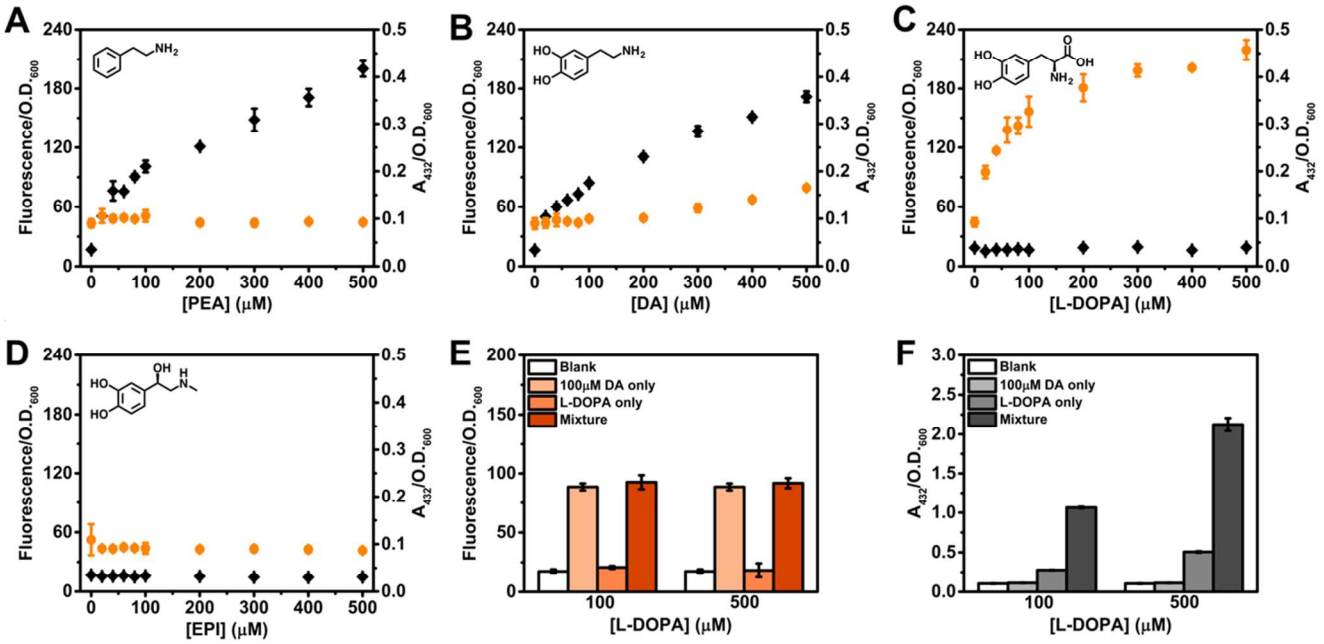


Figure 4 Dual- signals (RFP and pigment absorbance at 432 nm) from YCY_637 cells. RFP intensity (black square) and A_{432} (orange circle) were plotted against the final concentrations of (A) PEA, (B) DA, (C) L-DOPA, and (D) EPI. Interference effects between DA and L-DOPA were shown in (E) and (F). Graph shows the (E) red fluorescence intensity / OD600 ratio (F) A_{432} / OD600 of blank, DA, L-DOPA, and mixture of DA with 100 or 500 μM L-DOPA.

ASSOCIATED CONTENT

Supporting Information (SI). Experimental details, RFP and betaxanthin characterizations, western blot, and UV-Vis spectra were provided in Figure S1-S7, Scheme S1-2, and Table S1-S4. This material is available free of charge via the Internet at http://pubs.acs.org.

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REFERENCES

(1) Schwab, L. C.; Garas, S. N.; Drouin-Ouellet, J.; Mason, S. L.; Stott, S. R.; Barker, R. A. *Expert Rev. Neurother.* **2015**, 15, 445-458.
(2) Maas, R.; Wassenberg, T.; Lin, J. P.; van de Warrenburg, B. P. C.; Willemsen, M. *Neurology* **2017**, 88, 1865-1871.
(3) Rangel-Barajas, C.; Coronel, I.; Floran, B. *Aging Dis.* **2015**, 6, 349-368.
(4) Stocchi, F.; Torti, M.; Fossati, C. *Expert Opin. Pharmacother.* **2016**, 17, 1889-1902.
(5) Jiang, K.; Wang, Y.; Thakur, G.; Kotsuchibashi, Y.; Naicker, S.; Narain, R.; Thundat, T. *ACS Appl. Mater. Interfaces* **2017**, 9, 15225-15231.
(6) Alvarez-Martos, I.; Ferapontova, E. E. *Anal. Chem.* **2016**, 88, 3608-3616.
(7) Fu, X.; Tan, X.; Yuan, R.; Chen, S. *Biosens. Bioelectron.* **2017**, 90, 61-68.
(8) Cai, J.; Huang, J.; Ge, M.; Iocozzia, J.; Lin, Z.; Zhang, K. Q.; Lai, Y. *Small* **2017**, 13.
(9) Baron, R.; Zayats, M.; Willner, I. *Anal. Chem.* **2005**, 77, 1566-1571.
(10) Rabinovich, M. L.; Vasil'chenko, L. G.; Karapetyan, K. N.; Shumakovich, G. P.; Yershevich, O. P.; Ludwig, R.; Haltrich, D.; Hadar, Y.; Kozlov, Y. P.; Yaropolov, A. I. *Biotechnol. J.* **2007**, 2, 546-558.
(11) Hu, R.; Zhang, X.; Xu, Q.; Lu, D. Q.; Yang, Y. H.; Xu, Q. Q.; Ruan, Q.; Mo, L. T.; Zhang, X. B. *Biosens. Bioelectron.* **2017**, 92, 40-46.
(12) Yildirim, A.; Bayindir, M. *Anal. Chem.* **2014**, 86, 5508-5512.
(13) Zhang, X.; Chen, X.; Kai, S.; Wang, H.-Y.; Yang, J.; Wu, F.-G.; Chen, Z. *Anal. Chem.* **2015**, 87, 3360-3365.
(14) Li, J.; Yang, J.; Yang, Z.; Li, Y.; Yu, S.; Xu, Q.; Hu, X. *Anal. Methods* **2012**, 4, 1725-1728.
(15) Gu, H.; Varner, E. L.; Groskreutz, S. R.; Michael, A. C.; Weber, S. G. *Anal. Chem.* **2015**, 87, 6088-6094.
(16) Sasaki, N.; Abe, Y.; Goda, Y.; Adachi, T.; Kasahara, K.; Ozeki, Y. *Plant Cell Physiol.* **2009**, 50, 1012-1016.
(17) Nakatsuka, T.; Yamada, E.; Takahashi, H.; Imamura, T.; Suzuki, M.; Ozeki, Y.; Tsujimura, I.; Saito, M.; Sakamoto, Y.; Sasaki, N.; Nishihara, M. *Sci. Rep.* **2013**, 3, 1970.
(18) Sekiguchi, H.; Ozeki, Y.; Sasaki, N. *J. Agric. Food Chem.* **2010**, 58, 12504-12509.
(19) DeLoache, W. C.; Russ, Z. N.; Narcross, L.; Gonzales, A. M.; Martin, V. J.; Dueber, J. E. *Nat. Chem. Biol.* **2015**, 11, 465-471.
(20) Chen, P.-H.; Lin, C.; Guo, K.-H.; Yeh, Y.-C. *RSC Adv.* **2017**, 7, 29302-29305.
(21) Prieto, M. A.; Galan, B.; Torres, B.; Ferrandez, A.; Fernandez, C.; Minambres, B.; Garcia, J. L.; Diaz, E. *FEMS Microbiol. Rev.* **2004**, 28, 503-518.
(22) Zeng, J.; Spiro, S. *J. Bacteriol.* **2013**, 195, 5141-5150.

(23) Diaz, E.; Ferrandez, A.; Prieto, M. A.; Garcia, J. L. *Microbiol. Mol. Biol. Rev.* **2001**, 65, 523-569.
(24) Zhang, M.; Chen, X.; Way, N.; Yoshikawa, H.; Deng, H.; Ke, X.; Yu, W.; Chen, P.; He, C.; Chi, X.; Lu, Z. *Dev. Sci.* **2011**, 14, 1059-1065.
(25) Gandia-Herrero, F.; Garcia-Carmona, F. *Appl. Microbiol. Biotechnol.* **2014**, 98, 1165-1174.

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