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Monitoring and Modulating the Catalytic Hybridization Circuit for Self-Adaptive Bioorthogonal DNA Assembly

Xue Gong†, Shizhen He†, Ruomeng Li, Yingying Chen, Kaiyue Tan, Yeqing Wan, Xiaoqing Liu, Fuan Wang*

Constructing artificial domino nanoarchitectures, especially dynamic DNA circuits associated with the actuation of biological functions inside live cells, represents a versatile and powerful strategy to regulate the behaviors and fate of various living entities. However, the stepwise operation of conventional DNA circuits always relies on freely diffusing reactants, which substantially slows down their operation rate and efficiency. Herein, a self-adaptive localized catalytic circuit (LCC) is developed to execute the self-sustained bioorthogonal assembly of DNA nanosponges within a crowded intracellular environment. The LCC-generated DNA scaffolds are utilized as versatile templates for realizing the proximity confinement of LCC reactants. Single-molecule-detecting fluorescence correlation spectroscopy (FCS) is used to explore the reaction acceleration of the catalytic circuit. This self-adaptive DNA circuit facilitates the bioorthogonal assembly of highly branched DNA networks for robust and accurate monitoring of miRNA targets. Based on its intriguing and modular design, the LCC system provides a pivotal molecular toolbox for future applications in early disease diagnosis.

Introduction

Biological systems routinely reconfigure their growth, self-repair, and transportation via the assembly of various domino nanoarchitectures. For instance, the cytoskeleton, consisting of various dynamic protein assemblies, can form intricate signal networks for ensuring cellular stability, motility, and division. For mimicking these naturally occurring structures, the integration of synthetic self-assembled nanostructures with the actuation of biological behaviors showed great potential for clinical diagnosis and therapy as well as drug delivery. The assembly of synthetic circuitry with cellular function needs the self-assembly of biocompatible architectures within crowded and complex biological environments. However, in practice, synthetic circuits have so far relied on freely diffusing reactants, which substantially slows down their operation rate and efficiency. The colocalization of synthetic circuitry reactants to enhance their effective concentration represents one charming way to realize the non-natural assemblies in living cells.

With its highly predictable base-pairing, low cost, ease of synthesis, and high biocompatibility, DNA has been recognized as a highly promising biomolecule for designing localized molecular biocomputing circuits for different aims. A wide range of localized DNA circuits has been implemented on DNA origamis, molecular scaffolds, and spherical nucleic acids (SNA). Such spatially confined arrangement plays a pivotal role in increasing the effective concentration of reactants through proximity confinement and simultaneously achieves the reduced crosstalk between these spatially separated reaction processes. However, the proximal immobilization of DNA circuitry elements on DNA origami requires time-consuming and disturbing optimization procedures to facilitate the efficient execution of various biocomputing events. Meanwhile, these circuits, immobilized on these as-synthetic or as-assembled molecular scaffolds, are sensitive to the scaffolds defects where these non- or mis-incorporated circuitry components could easily terminate the propagation of cascaded signal transaction along these different scaffolds. Besides, the complicated adjustment and optimization of these circuitry reactants and scaffolds are always confronted with the lack of scalability and modularity for the flexible design and efficient execution of these biocomputing circuits. It is expected that the circuitry-generated DNA scaffolds could be utilized as versatile self-confined proximity-guided temples to realize the facile and reliable arrangement of circuit reactants for achieving precisely localized biocomputing circuits. Thus, it remains a huge challenge for designing a self-templated and localized DNA circuit that exquisitely integrates the accelerating cross-catalytic reaction within the self-supplemented molecular matrix. The self-sustained proximity-accelerated catalytic reaction could facilitate the robust monitoring of biomolecule targets, thus making the self-adaptive localized catalytic circuits highly desirable for clinical diagnostics, yet are still unexplored.

Herein, by using endogenous microRNA (miRNA) as a model promoter, we construct a self-adaptive localized catalytic circuit (LCC) that allows the bioorthogonal assembly of DNA

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Electronic Supplementary Information (ESI) available: experimental procedure, DNA sequences, AFM characterizations, cell imaging, FCS analysis, and additional figures, schemes and tables. See DOI: 10.1039/x0xx000000x
Figure 1. Principle of the LCC system for hierarchical assembly of DNA nanosponges using miR-21-responsive localized catalytic hybridization reaction. (A) and (B) Mechanism of the spatially localized catalytic hybridization reaction using catenated DNA reactants. (C) Detailed reaction procedure of the LCC system.

nanosponges for efficiently amplified imaging of miRNA. In the LCC system, the key concept is to introduce the structural symmetry on DNA sequences to achieve interconnecting catenated DNA reactants with self-confined hybridization capability. These engineered self-supplemented catenated DNA reactants are packaged into the folate-(FA)-modified poly (D, L-lactic-co-glycolic acid) (PLGA) nanovesicles, which facilitate their efficient and selective accumulation/endocytosis in target tumour cells. Then, the endogenous miRNA-initiated an autonomous and successive catalytic DNA assembly in a self-sustaining and self-adapting manner, leading to the bioorthogonal assembly of highly branched DNA nanosponges. In the LCC system, the promoter could be recycled for multiple rounds of catalytic hybridization without being consumed, thus resulting in a remarkably amplified transduction of the corresponding promoter. The circuit modules and mechanisms are extensively investigated in vitro and in vivo by using the single-molecule-detecting fluorescence correlation spectroscopy (FCS) technique. Based on a systematic investigation, the catenated DNA probes with symmetrical fragments could increase the local concentration of circuity reactants for accelerating the localized catalytic reaction and could also reduce the cellular diffusibility of circuity products for achieving the high-contrast imaging of cellular biomarkers. Specifically, the intracellular location of biomarkers is scarcely affected during the self-adaptive hierarchical assembly of the compact DNA nanosponges, thus facilitating the fast and precise positioning of intracellular analytes with high spatial resolution. As a versatile “plug-and-play” approach, our proposed LCC system shows great potential for constructing complex cascading DNA circuits in live cells, which reveals high potential applications in early disease diagnosis and drug-delivery.

Results and discussion

The self-adaptive bioorthogonal regulation of LCC is realized via the spatially confined catalytic hybridization reaction of three elaborately designed DNA reactants (Figure 1, for details, see Figure S1). Each of the DNA reactant units, H₁, H₂, and H₃, is grafted with a symmetrical sequence at its 3’-end to form the catenated structure, therefore is prone to assembling into Y-shaped DNA units with three self-grafted building blocks, which then lead to ultimate the formation of the advanced DNA architectures by the localized catalytic hybridization reaction (Figure 1A). MiR-21 (T), a new oncogene biomarker, is selected as the model promoter to demonstrate the stimulus-responsive self-sustained DNA assembly (Figure 1B). The promoter T opens the catenated H₁ through
stranded-displacement mechanism. The activated $H_3$ immediately hybridizes with the toehold of the catenated $H_2$, generating the transition-state intermediate $T$-$H_2$-$H_3$ triplex that was attached to the other two metastable hairpin building blocks. As a result, the opened $H_2$ hybridizes and opens the catenated $H_3$ to regenerate the promoter and produce the intermediate Y-shaped $H_1$-$H_2$-$H_3$ triplex tethering with three metastable hairpin building blocks under the self-confined configuration. The released promoter is able to hybridize with the nearby tethered $H_3$ of the as-assembled Y-shaped $H_1$-$H_2$-$H_3$ triplex for substantially promoting the localized catalytic hybridization reaction of LCC reactants, thus realizing the self-sustained hierarchical assembly of DNA nanosponges (Figure 1C).

Noteworthy, a large number of Y-shaped DNAs are thus cross-hierarchical assembly of DNA nanosponges (Figure 2A). The kinetic monitoring, (C) normalized FCS curves, and (D) PAGE characterization of the different LCC system outlined in (A): (a) miR-21+$H_1$/mutant LCC; (a') $H_1$/mutant LCC; (b) miR-21+$H_2$/mutant LCC; (b') $H_2$/mutant LCC; (c) miR-21+$H_3$/mutant LCC; (c') $H_3$/mutant LCC; (d) miR-21+intact LCC; (d') intact LCC. The "+" and "-" denote the presence and absence of the corresponding nucleic acid components, respectively. (E) AFM image and cross-sectional analysis of the LCC products. (F) Rheology test of the LCC-generated DNA hydrogels.

miR-21 could effectively catalyze the assembly of DNA nanostructures. To investigate whether the developed LCC system could accelerate the reaction kinetics, the non-localized hybridization procedure was established by altering the symmetrical domain. For consistency, here the symmetrical domain of $H_1$ and $H_3$ was substituted with a random sequence to generate $H_{1C}$ and $H_{3C}$ that could not form catenated conformation (Figure 2A, for a detailed reaction process, see Figure S3A). The kinetic characterizations of the LCC system at different conditions were investigated. As shown in Figure 2B, the introduction of miR-21 into the LCC system induced a rapid fluorescence increase (curve d), while a relatively slow and weak fluorescence emission enhancement was obtained in the defective localized system (curves a, b and c, for details, see Figures S3B and S3C), indicating that the constrain of DNA reactants into self-adaptive structure could enhance the overall reaction performance. The higher reaction rate of the LCC system was ascribed to the self-adaptive reactant-to-template design and the accompanying successive localization of reactants, which maintains structural integrity and increases the relative concentration of the self-constraint DNA reactants.

As a powerful single-molecule-detecting technique, FCS allows the measurement of the fluorescence fluctuations caused by the Brownian motion of a single molecule. Therefore, FCS was further utilized to explore the performance of the LCC system at the single-molecule level. An extremely fast molecular diffusion was observed in the catenated...
were observed (catalytic hybridization reaction proceeded as anticipated. The crosslinked hydrogel networks with a height of ~1.5 nm were investigated by atomic force microscopy (AFM). As expected, the morphological characterization of the LCC system was formed macroscopic DNA hydrogel under high concentration. After overnight incubation, a small amount of promoter (50 nM) could induce the obvious sol-gel transition. Even after a three-day incubation at room temperature, the formation of gelation was stable enough without fluidity (Figure S6). The rheology measurement was further performed to characterize the mechanical strength (Figure 2F). Even with 2.0 wt% DNA, the storage modulus (G*) was significantly higher than the loss modulus (G’’), demonstrating the solid property of the DNA hydrogel sample. These results confirmed that the self-confined LCC system is encoded with a self-accelerated characterization and a substantially higher signal amplification performance, thus providing a useful platform in bioanalytical fields.

The highly crosslinked self-assembly of DNA hydrogel and the efficient amplification features of the LCC system encouraged us to further apply this strategy for detecting miRNA (miR-21). Obviously, the fluorescence intensity increased with the gradually elevated concentration of miR-21 (Figure 3A), indicating that the promoter-responsive LCC system was highly dependent on the dosage of the analyte. The limit of detection (LOD) was acquired to be 17.5 pM according to the conventional 3σ calculation method (Figure 3B). The selectivity of the LCC system was then evaluated by testing its capacity for discrimination toward these mutant miR-21 sequences. According to Figure 3C, only intact miR-21 could generate a remarkable fluorescence signal, while the other control counterparts showed a comparably lower fluorescence readout, indicating the remarkable specificity of the present LCC system. In addition, the sensing performance could be maintained even in DMEM with 10% serum (Figure 3D), suggesting high stability and acceptable accuracy of the present LCC system in complex biological fluids.

To efficiently deliver the self-templated DNA reactants, the FA coupled PLGA copolymers (FA-PEG-PLGA) were synthesized for encapsulating these LCC reactants (Figure 4A). The synthesis and conjugation of these copolymers were demonstrated by 1H-NMR spectra (Figures 5 and S8) and UV-vis absorption spectra (Figure 4B). The DNA-loaded FA-PEG-PLGA nanovesicles (FA-nanovesicles) were synthesized using the double emulsion-solvent evaporation method. The DNA reactants were encapsulated into the nanovesicles by complexing the DNA with cationic poly L-lysine (PLL), and the encapsulation efficiency was determined to be 45%. The average diameter of the as-achieved FA-nanovesicles was 172 nm with a polydispersity index (PDI) of 0.15 (Figure 4C). The external surface morphology of the FA-nanovesicles was analyzed by scanning electron microscopy (SEM) and transmission electron microscopy (TEM) (Figures 4D and 4E, respectively), which showed a spherical shape with a smooth surface. The as-prepared FA-nanovesicles could be redispersed in DMEM with 10% serum without obvious aggregation.

Figure 4. Demonstration and morphological characterization of the LCC-encapsulated FA-nanovesicles. (A) Schematic representation of the LCC-packaged FA-decorated nanovesicles. (B) UV spectrum of PLGA, FA, amine-terminated PEGylated PLGA, and FA-conjugated di-block copolymer. (C) Hydrodynamic diameter measurement, (D) SEM, and (E) TEM analysis of the LCC-packaged FA-nanovesicles.

**Figure 2**

**Figure 2C**

**Figure 2D**

**Figure 2E**

**Figure 2F**

**Figure 3A**

**Figure 3B**

**Figure 3C**

**Figure 3D**

**Figure 4A**

**Figure 4B**

**Figure 4C**

**Figure 4D**

**Figure 4E**

**Figure 5**

**Figure S6**

**Figure S7**

**Figure S8**

H1/H2-mutant system while a comparatively slower signal attenuation emerged in catenated H1 or H2-mutant system (Figure 2C). In contrast, the slowest molecular diffusion signal appeared in the intact LCC system, indicating a substantial amount of polymer nanoassembly was achieved to constrain the single molecular diffusion. The characteristic diffusion coefficient (D) assay showed that the H1/H2-mutant LCC system observed 14.23-fold faster molecular diffusion than the intact LCC system, 1.25-fold faster fluorescence fluctuation than the mere H1-mutant LCC system, or 3.56-fold higher molecular diffusion than the mere H2-mutant LCC system (Figure S4). These single molecular detection results demonstrated that the self-supplementing assembly of the localized catalytic circuit structure was equipped with faster reaction profiles. The native polyacrylamide gel electrophoresis (PAGE) analysis also confirmed that the miR-21-initiated LCC resulted in the formation of high-molecular-weight products (Figure 2D). The morphological characterization of the LCC system was investigated by atomic force microscopy (AFM). As expected, the crosslinked hydrogel networks with a height of ~1.5 nm were observed (Figure 2E), suggesting that the localized catalytic hybridization reaction proceeded as anticipated. Compared to the tiny spots of the LCC system without the promoter, the H1-mutant LCC system generated copolymer dsDNA nanowires (Figure S5), further confirming the correctness of our localized catalytic hybridization reaction mechanism. These results indicated that interconnecting catenated DNA reactants with self-confined capability could enhance the reaction rate for guaranteeing an effective signal amplification.

Then, we examined whether the promoter-responsive self-adaptive reactant-to-template hybridization reaction could form macroscopic DNA hydrogel under high concentration. After overnight incubation, a small amount of promoter (50 nM) could induce the obvious sol-gel transition. Even after a three-day incubation at room temperature, the formation of gelation was stable enough without fluidity (Figure S6). The rheology measurement was further performed to characterize the mechanical strength (Figure 2F). Even with 2.0 wt% DNA, the storage modulus (G*) was significantly higher than the loss modulus (G’’), demonstrating the solid property of the DNA hydrogel sample. These results confirmed that the self-confined LCC system is encoded with a self-accelerated characterization and a substantially higher signal amplification performance, thus providing a useful platform in bioanalytical fields.

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Figure 5. Demonstration of the LCC amplifier in MCF-7 cells. (A) Confocal microscopy analysis, (B) Flow cytometric evaluation, and (C) normalized FCS curves assay of miR-21 in MCF-7 cells that were respectively incubated with anti-miR-21-pretreated LCC (a), H₁/H₃-mutant LCC (b), H₁-mutant LCC (c), H₃-mutant LCC (d) and intact LCC (e). The cell nuclei were stained with Hoechst 33342 (blue). Scale bar: 20 μm. Data represent the mean ± s.d. of five independent experiments. (Figure S9), suggesting the high stability of our FA-nanovesicles.

To identify whether these FA-nanovesicles could effectively target specific cancer cells, their cellular uptake was evaluated by confocal laser scanning microscopy (CLSM) and flow cytometry. With different expressions of folate receptor (FR) on the cell membrane, human breast cancer (MCF-7) and normal MCF-10A cells were used as models to investigate the living-cell imaging.36-39 Noteworthy, here the nanovesicles only contained random FAM-modified DNA to evaluate the FR-mediated endocytosis. As shown in Figure S11, FA-nanovesicles exhibited an enhanced fluorescence signal as compared to that of the unmodified nanovesicles after 3 h of incubation in MCF-7 cells. These enhancement phenomena were abrogated by a free FA competition inhibition assay for validating the specific interaction between FA and FR. In contrast, CLSM and flow cytometry analysis revealed that the cellular uptake of FA-nanovesicles by MCF-10A breast cancer (MCF-7) and normal MCF-10A cells was not affected by the competition of free FA (Figure S12). The specific interaction between FA and FR was further demonstrated using a competitive inhibition assay in FR-positive human cervical cancer cells (HeLa) (Figure S13), thus confirming the specifically FA-promoted receptor-mediated endocytosis into FR-positive cells. The endocytosis inhibition experiments in MCF-7 cells showed that the FA-decorated nanovesicles internalization was predominant via the clathrin-mediated endocytosis and micropinocytosis processes (Figure S14).

Having demonstrated the reliable receptor-mediated endocytosis, we then explored the LCC system for imaging low-abundance miRNA. Herein, the miR-21-overexpressed MCF-7 cell was chosen as a model to verify the LCC amplifier. Under the optimized incubation time (Figure S15), a bright fluorescence signal was obtained in MCF-7 cells that were incubated with the intact LCC-packaged FA-nanovesicle (sample e in Figure 5A), suggesting that the stimulus-responsive catalytic hybridization reaction could proceed in the complicated intracellular environment for the sensitive monitor of low-abundance miR-21. By contrast, the H₁/H₃-mutant FA-nanovesicle observed a relatively weaker fluorescence signal (sample b), which was consistent with the quantitative flow cytometry assay (Figures 5B and S16). A remarkably enhanced fluorescence signal was observed in the intact LCC amplifier as compared to that of the H₁ or H₃-mutant LCC amplifier (samples c and d). This enhanced fluorescence signal of the intact LCC system originated from the localized hybridization with increased DNA concentration, thus providing accelerated reaction kinetics. Furthermore, a substantially decreased fluorescence signal was observed in MCF-7 cells that were pre-treated with anti-miR-21 inhibitor (sample a), confirming that the miR-21 molecule was indeed presented in MCF-7 cells for initiating the self-adaptive catalytic assembly of DNA.

Moreover, the accuracy and robustness of the designed LCC system were further evaluated using FCS to differentiate the varied expressions of miR-21 in single MCF-7 cells. As shown in Figure 5C, the most intense signal attenuation was observed in MCF-7 cells that were treated with H₁/H₃-mutant system,
followed by H3 or H2-mutant system, while a minimal diffusion signal was exhibited in MCF-7 cells treated with an intact LCC system, implying that constraining interactions of spatially proximal circuit elements could enhance the reaction kinetics in the complexed intracellular environment. As expected, a remarkable diffusion signal appeared in anti-miR-21 inhibitor pre-treated cells, implying the specific miR-21-activated the LCC system. Quantitative analysis showed that the $H_3/H_3$-mutant LCC displayed 2.40- and 31.39-fold higher fluorescence diffusion than the $H_2$-mutant and intact LCC amplifier (Figure S17), respectively. The depressed diffusion of fluorescence of the intact LCC system was attributed to the self-adaptive reactant-to-template design and the accompanying successive localization of reactants that accelerated the entire reaction process and constrained the single molecular diffusion. Furthermore, the diffusion coefficient in the miR-21-suppressed LCC amplifier was 45.28-fold higher than the non-pretreated LCC system, suggesting the specific miR-21-activated LCC amplifier in single live cells. These results furtherly manifested that our LCC-guided cellular assembly of DNA nanosponges could reliably differentiate varied expressions of miR-21.

The precise in situ localization of miR-21 of the LCC system encouraged us to implement our system for discriminating different cell types with distinct miR-21 expressions. As shown in Figure 6A, an extremely intense fluorescence signal was exhibited in MCF-7 cells while a comparatively weaker fluorescence activation emerged in HeLa cells. In contrast, nearly no detectable fluorescence response appeared in normal human lung fibroblast (MRC-5) and MCF-10A cells, implying a relatively higher expression of miR-21 in tumour cells than in normal cells, which was consistent with previous research. Meanwhile, the performance of our designed LCC system to distinguish the varied miR-21 expression levels in different cells was furtherly verified by a quantitative flow cytometry assay (Figure 6B). In addition, the shortest characteristic diffusion time was exhibited in MCF-10A and MRC-5 cells, followed by HeLa cells, while the slowest molecular diffusion signal was shown in MCF-7 cells (Figure 6C). The characteristic diffusion coefficient assay indicated that the normal MCF-10A cells achieved 6.45-fold higher signal diffusion than the tumour MCF-7 cells, and 4.37-fold higher fluorescence diffusion than the tumour HeLa cells (Figure 6D). Meanwhile, the noncancerous MRC-5 cells observed 5.42-fold faster fluorescence fluctuation than the MCF-7 cells and 3.62-fold faster molecular diffusion in HeLa cells. These results demonstrated that the designed LCC system could reliably differentiate the varied expression levels of miR-21 in different cells, suggesting a great potential application in single-cell analysis.

The satisfactory intracellular stimulus-responsive assembly performance of the LCC amplifier was confirmed in vitro, which inspired us to explore a more challenging in vivo application of the intelligent DNA self-assembly. Mice-bearing orthotropic MCF-7 breast tumour was chosen as a model to evaluate the stimulus-responsive performance of our LCC system. To achieve a reliable and accurate comparison of the miR-21-guided assembly of DNA nanostructures, these different LCC systems were firstly intratumourally injected to ensure comparable and constant DNA reactants (Figure 7A). For the enhanced light penetration ability, $H_2$ was functionalized with a Cy5/quencher pair for in vivo bioimaging. As shown in Figure 7B, the intact LCC
Figure 7. In vivo miRNA imaging by using the LCC amplifier in orthotopic MCF-7 tumour-bearing nude mice. (A) Illustration of the LCC amplifier for in vivo miRNA imaging by intratumour injection. (B) Whole-body imaging of tumour-bearing mice after intratumourally injection of various nanoagents at different time points. (C) Quantification of the fluorescence intensity at the tumour sites in (B). Data represent the mean ± s.d. of three independent replicates. (D) Western blot analysis of PDCD4 and PTEN proteins in mice tumour after intratumoural injection of PBS, anti-miR21, H2-expelled LCC, and intact LCC amplifier, respectively. (E) Illustration of the LCC amplifier for in vivo miRNA imaging by tail vein injection. (F) Whole-body fluorescence imaging of tumour-bearing mice at the indicated time points after injection of free DNA, LCC-packaged nanovesicles, and LCC-packaged FA-decorated nanovesicles, respectively. (G) Quantification of the fluorescence intensity at the tumour sites in (F). Data represent the mean ± s.d. of three independent replicates. (H) Ex vivo imaging of the major organs and tumours with different treatments in (F). H, heart; Li, Liver; S, spleen; Lu, lung; K, kidney; T, tumor.

system presented a remarkably enhanced fluorescence signal in tumour region than the H2-mutant system, indicating the localized hybridization characteristics of the LCC that accelerated the entire self-assembly procedure and simultaneously promoted the generation of an enhanced fluorescence signal. As a control, the H2-expelled tumour observed a negligible change of intratumoural fluorescence with prolonged incubation time. Quantitative analysis showed that intact LCC-treated mice exhibited a 1.8-fold higher intratumoural fluorescence signal than that treated with the H2-mutant LCC system at 6 h (Figure 7C). In addition, nearly no enhanced Cy5 signal was observed in the anti-miR-21-pretreated tumour. These results demonstrated that the miR-21-responsive assembly could indeed proceed in vivo. In our design, the activation of the LCC was based on the displacement of the promoter, in which the content of the initiator would not be influenced as well as the function of the living cells. According to the western blot analysis (Figure 7D), the designed LCC system observed little effect on the expressions of downstream PDCD4 and PTEN proteins in mice-bearing MCF-7 tumour, demonstrating the bioorthogonal assembly of DNA nanosponges in vivo.

In addition, the miR-21-activated LCC-amplified bioorthogonal assembly of DNA nanosponges was furtherly evaluated in mice-bearing orthotopic MCF-7 tumour via intravenous administration (Figure 7E). As shown in Figure 7F, an intravenously injected free LCC system was quickly eliminated without fluorescence in the tumour site. By contrast, the LCC-packaged nanovesicles could gradually be accumulated in the tumour site. Quantitative analysis showed that the FA-nanovesicles exhibited 1.7-fold higher intratumoural fluorescence signal than bare nanovesicles at 12 h post-injection (Figure 7G). The enhanced Cy5 signal of FA-nanovesicles in the tumour site was attributed to the FA-favoured cellular uptake that promoted the miR-21-catalytic assembly performance. In addition, the fluorescence signal of harvested tumours and major organs at 16 h injection was evaluated. The intratumoural Cy5 signal in FA-nanovesicle-treated mice was 1.5-fold higher than that of the bare nanovesicle-treated mice group (Figures 7H and S18). These
results significantly demonstrated FA-promoted tumour-addressable characters and miR-21-responsive DNA assembly. Furthermore, haematoxylin and eosin (H&E) staining (Figure S19) and haematology assay (Figure S20) demonstrated the satisfactory systemic biocompatibility of these functional nanovesicles.

Conclusions

In summary, the stimulus-responsive localized catalytic circuitry system was facilely engineered by the rationally designed interconnecting catenated DNA reactants for self-adaptive catalytic DNA assembly. The autonomous and cascade bioorthogonal assembly was realized for in situ generating the three-dimensional hyper-branched DNA architectures of nanoscale. By accurately monitoring the assembly of DNA reactants and products under the self-confined environment, the crucial miRNA biomarker was robustly detected in complex intracellular environments and even in vivo conditions. The real-time assembly of DNA nanospines can be monitored by the single-molecule-detecting FCS technique. The key concept of the bioorthogonally regulated circuits is to utilize the sequence/structural symmetry of DNA probes to achieve the catenated geometry conformation for realizing the self-adaptive catalytic acceleration hybridization. The promoter could continue to initiate the self-supplemented acceleration reaction to assemble DNA nanospines, thus enabling a more reliable in situ localization of miRNAs. The self-adaptive catalytic circuit paves a new avenue for developing high-performance DNA computing circuits and nanorobots, thus exhibiting promising potential in clinical diagnosis and therapeutic evaluation.

Author Contributions
X.G. and F.W conceived and designed the experiment. X.G, S.H, and R.L performed the experiment and analysed the data. Y.C and K.T synthesized FA-PLGA. S.H and Y.W collected fluorescence spectra. X.G, X.L and F.W wrote the manuscript. All authors have given approval to the final version of the manuscript.

Conflicts of interest

There are no conflicts to declare.

Acknowledgements

This work was supported by the National Natural Science Foundation of China (21874103 and 22074112), the Fundamental Research Funds for the Central Universities (2042022kf1175), and the Central Funds Guiding the Local Science and Technology Development of Shenzhen (2021szvxup101).

All animal experimental protocols were approved by the Institutional Animal Care and Use Committee of the Animal Experiment Center of Wuhan University (Wuhan, China). Four-to-six-week-old female BALB/c nude mice were purchased from Charles River Company and raised in a specific pathogen-free grade laboratory according to guidelines for laboratory animals established by the Wuhan University Center for Animal Experiment/A3-Lab. All animals were housed with a 12 h light/dark cycle at 22 °C, 40% relative humidity, and food and water ad libitum.

Notes and references

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