Electronic Supplementary Information

Experimental section

Materials and apparatus

The DNA and microRNAs used in this study were synthesized and HPLC-purified by Takara Biotechnology Co., Ltd. (Dalian, China), and the sequences are listed in Table S1. Cell Counting Kit-8 (CCK-8) kit was purchased from Sangon Biotech Co., Ltd. (Shanghai, China). Doxorubicin (Dox), bovine serum albumin (BSA), hemin and luminol were obtained from Aladdin Industrial Inc. (Shanghai, China). Carboxyl-modified magnetic microparticles (MMPs) (~1 μm, 10 mg/mL), magnetic nanoparticles (MNPs) (100~200 nm, 10 mg/mL) and magnetic rack were purchased from BaseLine Chromtech Research Centre (Tianjin, China). All regents were of analytical grade and used without further purification. Ultrapure water was used in all experiments.

Table S1. Oligonucleotide sequences used in this assay

name	sequences (5'-3')
UIP	NH_2-CGCGTTAACATACAATAGATCGCG
miR-122	UGGAGUGUGACAAUGGUGUUUG
AS1 for miR-122	CAAACACCATTATGTTAAC
AS2 for miR-122	GCATCCTTATCGTGATCTATTGTGTCACACTCCATATGTAGTCGCGC
BU1	FAM-CAGTAGTCGATGGCTTCCACAACATAC-FAM
BU1'	CAGTAGTCGATGGCTTCCACAACATAC
BU2	TGGGTACGATAAGGATGCGTATGTTGTGGATCCATTGACGGAGAGGTGGGTAGGGCGGG
BU2'	ATCTAACTGCTGCGCCGCCGGGAAAATACTGTACGGTTAGAACGATAAGGATGCGTATG TTGTGGATCCATTGACGGAGAGG
BU3	TGGGTCACGATTGACGACCCTCATCGACTACTGGCGCGACTACATATGGGTAGGGCGGG
connector	GCATCCTTATCGTCCTCTCCGTCAATCCAGTCGTCAATCGTGTATGTAGTCGCGC
miR-21	UAGCUUAUCAGACUGAUGUUGA
AS1 for miR-21	TCAACATCAGTATGTTAAC
AS2 for miR-21	GCATCCTTATCGTGATCTATTGTCTGATAAGCTATATGTAGTCGCGC

CRET imaging experiments were carried out using an EC3 imaging system equipped with a thermoelectrically cooled CCD camera (UVP, USA). Scan electron microscopy (SEM) was performed on a JSM-6700F instrument (JEOL, Japan). The native polyacrylamide gel electrophoresis (PAGE) was carried out using a Beijing Liuyi WD-9413B gel imaging system (Beijing, China). Fluorescent images were recorded with a Leica fluorescence microscope (Leica, Germany). All measurements were carried out at room temperature unless stated otherwise.

Preparation of UIP-attached MMPs

Firstly, 10 μL of carboxyl-modified MMPs (10 mg/mL) was washed with imidazole-HCl solution (0.1 M, pH 6.8) twice. Then, 200 μL of EDC (0.1 M) and NHS (0.01 M) was added, followed by gently shaking at room temperature for 30 min to activate carboxyl groups of MMPs. After magnetic separation, 10 μL of 5'-end amine-modified UIP (1 μM) and 10 μL of BSA (1 μM) were introduced into the activated MMPs. After incubation at 37 ^{o}C overnight, UIP was covalently bound onto MMPs. The resultant UIP-functionalized MMP hybrids were purified by magnetic separation and rinsed with PBS buffer (10 mM, pH 7.4) three times, and then dispersed in PBS and stored at 4 ^{o}C for further use.

CRET imaging of miR-122

Previously, the building units were prepared by annealing equal volume of BU1, BU2 and BU3 (3 μM for each) at 95 ^{o}C for 5 min and gradually cooling down to 25 ^{o}C over a period of 2 h. Then, 10 μL of miR-122 with different concentrations was mixed with 10 μL of AS1 (1 nM) and 10 μL of AS2 (1 nM) and incubated with the as-synthesized UIP-MMP hybrids at 25 ^{o}C for 1 h. Subsequently, the resultant MMPs were magnetically washed with PBS buffer and then incubated with a mixture of building units (1 μM) and connectors (1 μM) in HEPES buffer (25 mM, pH 7.4) that containing NaCl (200 mM) and hemin (1 μM) at room temperature for 2 h. To assemble K^{+}-stabilized hemin/G-quadruplex, KCl solution (20 mM) was added. The obtained DNA network-caged MMPs were rinsed with PBS three times, and dispersed in 50 μL of PBS buffer. CRET imaging was monitored by addition of luminol (0.01 M) and H_2O_2 (0.3 M) using a cooled CCD camera. The spots were automatically quantified by VisionWorks LS image acquisition. The total CL intensity covering the whole emission wavelengths of each spot was calculated as the mean pixel within a circle of a given diameter around each spot center and analyzed by the own software of the chemiluminescence imager.

Drug loading and cytotoxicity studies

Firstly, aptamer-based DNA network-caged MNPs were prepared as above except that aptamer sgc8-functionalized BU2' was used to assemble the building units and the concentration of DNA that had the same sequence as miR-122 was 0.1 μM without addition of hemin. For Dox loading,

100 μL of aptamer-based DNA network-caged MNPs were incubated with Dox (1 mM) at room temperature for 24 h. And then, the unreacted Dox in supernatant was magnetically removed, followed by UV-vis measurement at the absorbance of 480 nm to determine the amount of Dox loaded in the DNA network-caged MNPs. Meanwhile, the resulting Dox-loaded sgc8-based DNA network-caged MNPs was dissolved in 100 μL of Dulbecco's PBS, which was used to cytotoxicity experiments using a CCK-8 kit. Briefly, 100 μL of cell suspension (5×10^3 cells/well) were introduced into 96-well plate and incubated for 24 h in a cell culture incubator, followed by introduction of free Dox or Dox-DNA network-caged MNPs and incubation for 2 h. Then, 10 μL of CCK-8 Solution was added to each well and incubated for another 2 h. Finally, the absorbance at 450 nm was recorded using a microplate reader. The cell viability was determined according to the manufacturer's description.

Scanning electron microscope (SEM) characterization

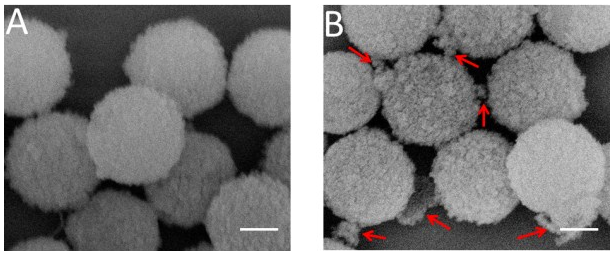
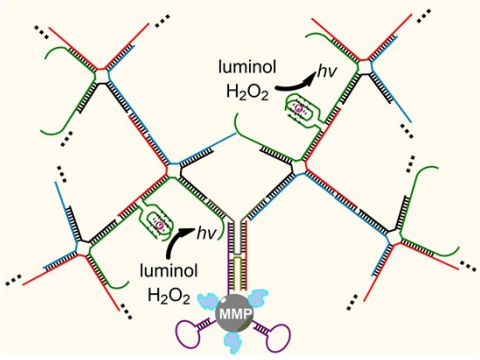


Fig. S1 SEM images of the products on MMPs in the absence (A) and in the presence (B) of target miR-122. The DNA nanostructures on MMPs are indicated by red arrows. Scale bar: 500 nm.

Control experiment



Scheme S1. Control experiment using no FAM labeled BU1 (BU1') to construct DNA-4WJ and DNA network for CL imaging of miR-122.

Analysis of miR-21 by this method

To verify the applicability of this approach to other microRNA detection, microRNA-21 (miR-21) is chosen as another target model and detected by this method. From Fig. S2, as the concentration of miR-21 increases from 10 fM to 0.1 nM, the CRET signal is intensified correspondingly, demonstrating the versatility of the present protocol for miRNA analysis.

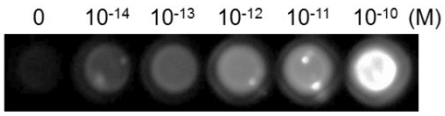


Fig. S2 CRET images of the target-initiated DNA network on MMPs for miR-21 detection.

Analysis of miR-122 in Hub7 cells

To investigate the feasibility of the proposed biosensor in complicated matrix for real point-of-care test, the amount of miR-122 in Huh7 cells (human hepatocellular carcinoma line) is quantitatively detected using this assay and leveraging a calibration curve that has been built with synthetic miR-122 (Fig. 2B in the manuscript). From Table S2, the concentration of miR-122 in one thousand Huh7 cells is 3.6 ± 0.6 pM. Thus, the amount of miR-122 in one Hub7 cell is calculated to be 21500 ± 3500 copies, which is well agreement with the results that were obtained by other quantification methods.[S1,S2] Given the expression levels of miR-122 in human serum and liver tissue,[S1,S2] this method can also be applied to the detection of miR-122 in a variety of real samples. Therefore, our proposed strategy provides a novel platform for miRNA analysis in broad applications, such as molecular disease diagnosis, biomedicine, and so on.

Table S2. Quantitation of miR-122 in Huh7 cells using the proposed strategy

sample	concentration of miR-122 in 1000 Huh7 cells (pM)	amount of miR-122 in one Huh7 cell (copies)	RSD (%)
1	4.2	25000	5.02
2	3.4	20000	7.22
3	3.0	18000	6.87

Release profiles of Dox from DNA network-caged MNPs

The release profiles of Dox from the DNA network-caged MNPs are investigated under the pHs of 5.5 and 7.4, respectively. From Fig. S3, in a physiological condition (pH 7.4), the Dox loaded in the DNA network-caged MNPs demonstrates a slow release kinetics with the maximum release less than 10% at room temperature after 24 h. By contrast, a rapid release of Dox is displayed in an acidic solution (pH 5.5), which can be attributed to the influence of low pH on DNA hybridization and the interaction between Dox and DNA.[S3] Therefore, the DNA network-caged MNPs demonstrate good stability as drug carriers under physiological conditions which thus can effectively prevent drug leakage, and facilitate drug release in an endosome-like environment.

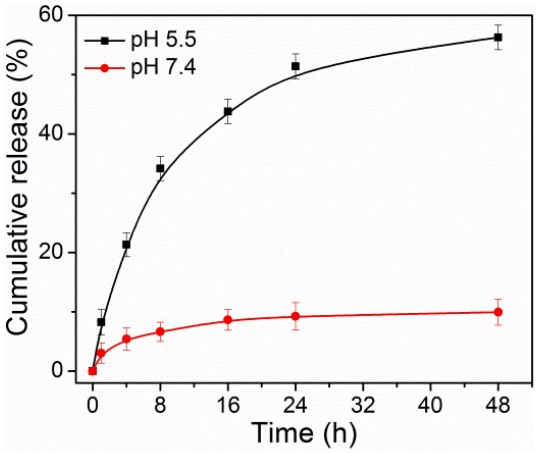


Fig. S3 Drug release behaviors of Dox-loaded AptNAs under the pHs of 5.5 and 7.4 at room temperature, respectively.

Cytotoxicity assay by treating cancer cells with free Dox

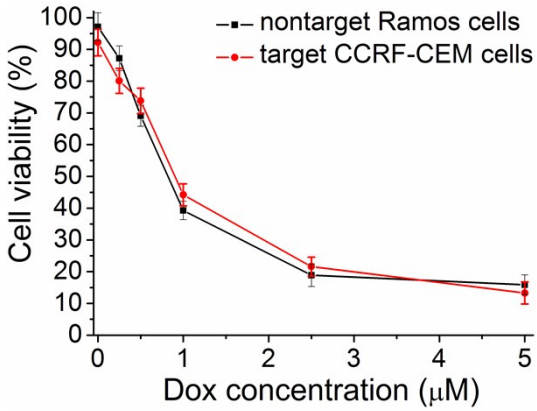


Fig. S4 Cytotoxicity of target CCRF-CEM cells and nontarget Ramos cells treated with different concentrations of free Dox by CCK-8 assay.

Confocal microscopy images

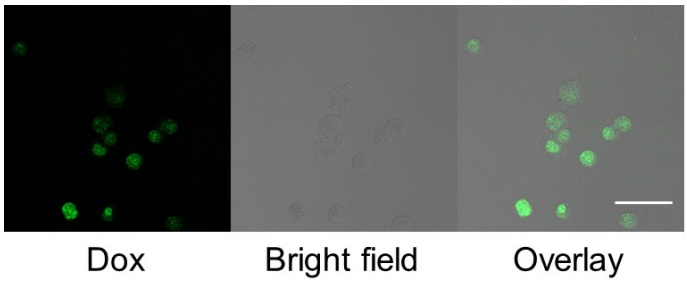


Fig. S5 Confocal microscopy images to display the internalization of the FITC-modified and sgc8-based DNA network-caged MNPs into target CCRF-CEM cells. Scale bar: 50 μm.

References

S1 A. Persat and J. G. Santiago, *Anal. Chem.*, 2011, **83**, 2310-2316.

S2 J. Chang, E. Nicolas, D. Marks, C. Sander, A. Lerro, M. A. Buendia, C. Xu, W. S. Mason, T. Moloshok, R. Bort, K. S. Zaret and J. M. Taylor, *RNA Biol.*, 2004, **1**, 106-113.

S3 C. Wu, D. Han, T. Chen, L. Peng, G. Zhu, M. You, L. Qiu, K. Sefah, X. Zhang, W. Tan, *J. Am. Chem. Soc.*, 2013, **135**, 18644-18650.