Multivalent self-assembled nano string light for tumor-targeted delivery and accelerated biomarkers imaging in living cells and *in vivo*

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Table S1. Sequences of oligonucleotides used in this work.

Sequence name	Sequence (5′-3′)
S1	ACA GGA TTA ATC TTA TTA GTC GTC TCG TTA CTT AAA TGG TCA GAA ATA TGG GAT TAA CCA TGG TGT TTA TGA TAT GAA GTG TTG GAA GCT
S2	CTC GAG ATT ATT CTA ATT AGG ACA TTA ATC CCA TAT TTC TGA CCA TTT AAG TAA CGA AGC TTC CAA CAC TTC ATA TCA TAA ACA CCA TGG
F	GAC GAC TAA TAA GAT TAA TCC TGT TCA ACA TCA GTC TGA TAA GCTA-Cy5
Q	BHQ2-TAGC TTA TCA GAC TG
AS1411	TGT CCT AAT TAG AAT AAT CTC GAG-TTTT- GGT GGT GGT GGT TGT GGT GGT GGT GG
miRNA-21 (miR-21)	TAGC TTA TCA GAC TGA TGT TGA
single-base mismatch	TAGC TGA TCA GAC TGA TGT TGA
three-base mismatch	TAGC TGA TCA GGC TGA GGT TGA
let-7d	AGAG GTA GTA GGT TGC ATA GTT
miRNA-200b (miR-200b)	TAAT ACT GCC TGG TAA TGA TGA
miRNA-429 (miR-429)	TAAT ACT GTC TGG TAA AAC CGT
miRNA-375 (miR-375)	TTTG TTC GTT CGG CTC GCG TG A
anti-miRNA-21 (anti-miR-21)	TCAA CAT CAG TCT GAT AAG CTA

Table S2. The comparison of our method with other detection methods in terms of reaction time and incubation time.

Method	Probe carrier	Incubation time	Rection time	Ref.
Hybridization chain reaction	MnO_2 nanosheets	3h/10h	4h	1
Bipedal DNA nanowalker	AuNPs	3h	2.5h	2
Localized catalytic hairpin assembly reaction	DNA nanowire	3h	1h	3
Strand displacement reaction	Ru-SiO_2@Polydopamine	5h	5h	4
Strand displacement reaction	DNA nanowire	4h	0.5h	5
Catalyzed hairpin assembly-induced	MnO_2 nanosheets	6h	1h	6
DNAzyme reaction single-base mismatch	AuNPs	6h	3h	7
Strand displacement reaction	**DNA nanowire**	**3h**	**10min**	**This work**

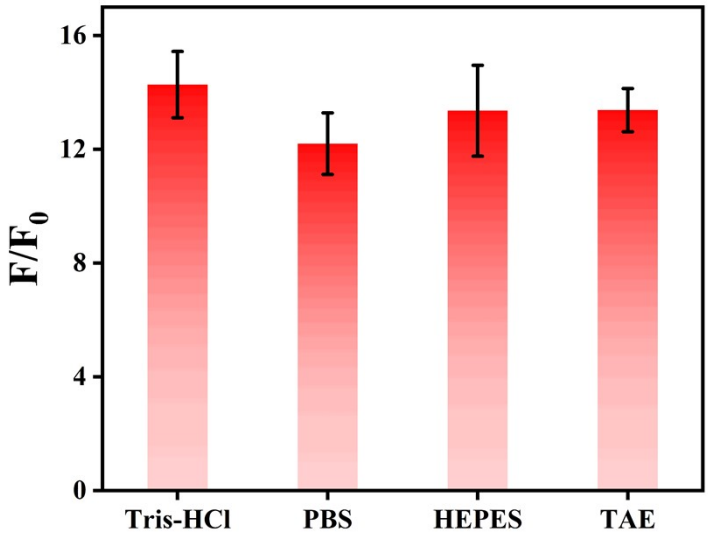

Fig. S1 Optimization of the reaction buffer. F and F_0 is the fluorescence intensity of the nanoprobe in the presence and absence of miRNA-21, respectively. 20 mM Tris-HCl (pH 7.4) containing 20 mM Tris-base, 140 mM NaCl; 10 mM PBS (pH 7.4) containing 140 mM NaCl, 2.7 mM KCl, 10 mM $NaH_2PO_4 \cdot 2H_2O$; 20 mM HEPES (pH 7.4) containing 20 mM HEPES, 140 mM NaCl; 20 mM TAE (Ph 7.4) containing 20 mM Tris-Ac, 2 mM EDTA, 12.5 mM $MgAc_2$. The error bars indicate mean ± SD (n = 3).

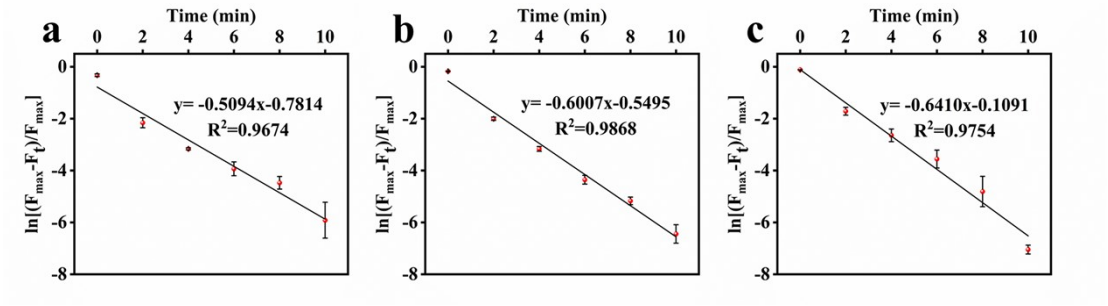

Fig. S2 Kinetic studies of the nano string light. Real-time fluorescence intensity of the nano string light treated with (a) 10 nM; (b) 20 nM and (c) 50 nM miRNA-21. The fluorescence intensity was recorded after regular interval of 2 min.

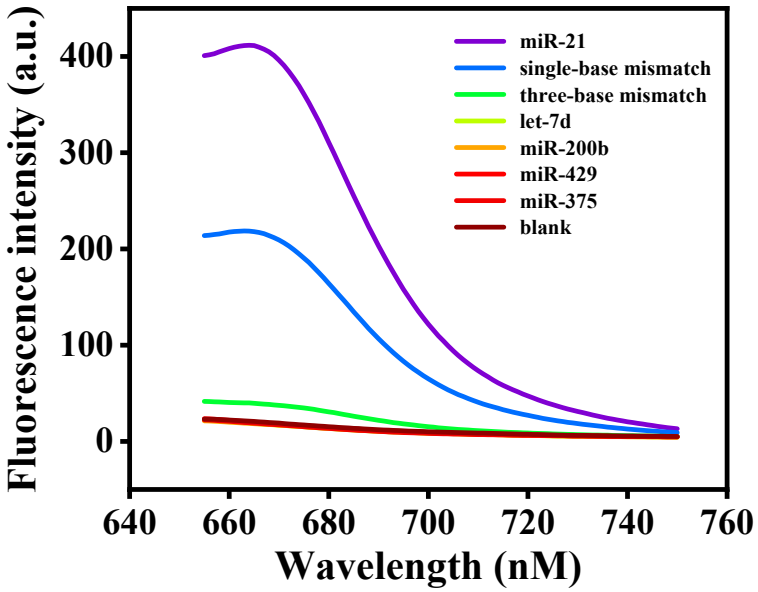

Fig. S3 Specificity of the nano string light for several miRNA targets and two miRNA-21 variants with one and three mismatched bases.

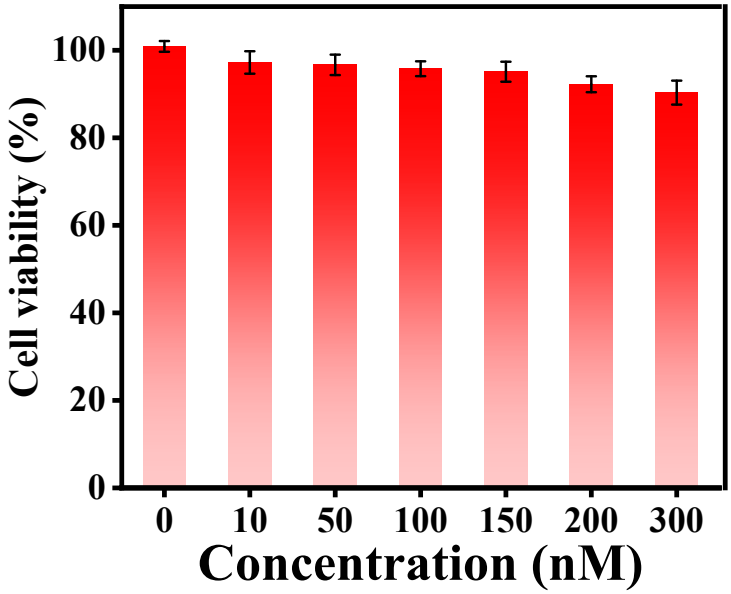

Fig. S4 Cytotoxicity assay with different concentrations of nano string light. Hela cells were incubated with various concentrations of nano string light for 24 h. The error bars indicate means ± SD (n = 3).

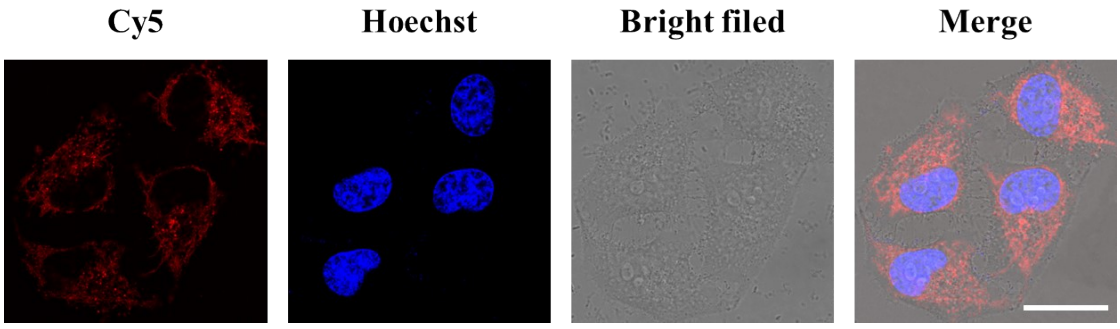

Fig. S5 Fluorescence colocalization analysis. Hela cells were incubated with 100 nM DNA nano string light (red channel), then treated with 5 mg/mL Hoechst 33342 (blue channel). Scale bar is 25 μm.

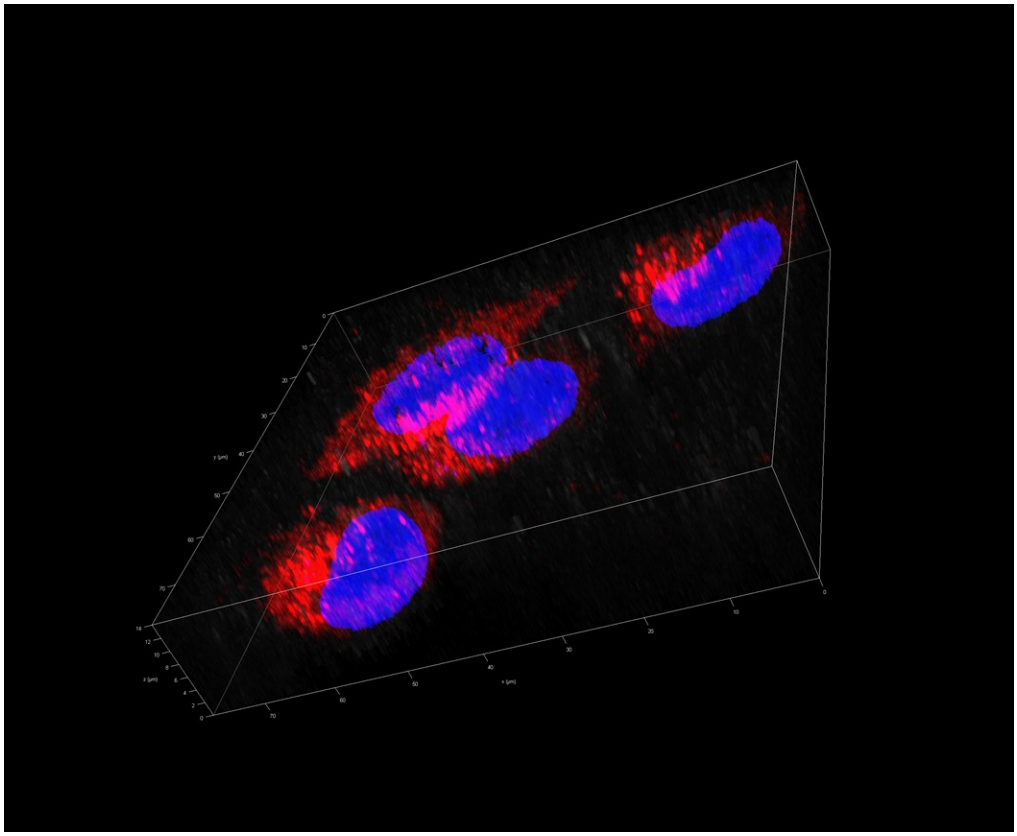

Fig. S6 Three-dimensional (3D) fluorescence imaging of miR-21 in HeLa cells. The HeLa cells were incubated with 100 nM nano string light for 3 h at 37 ℃.

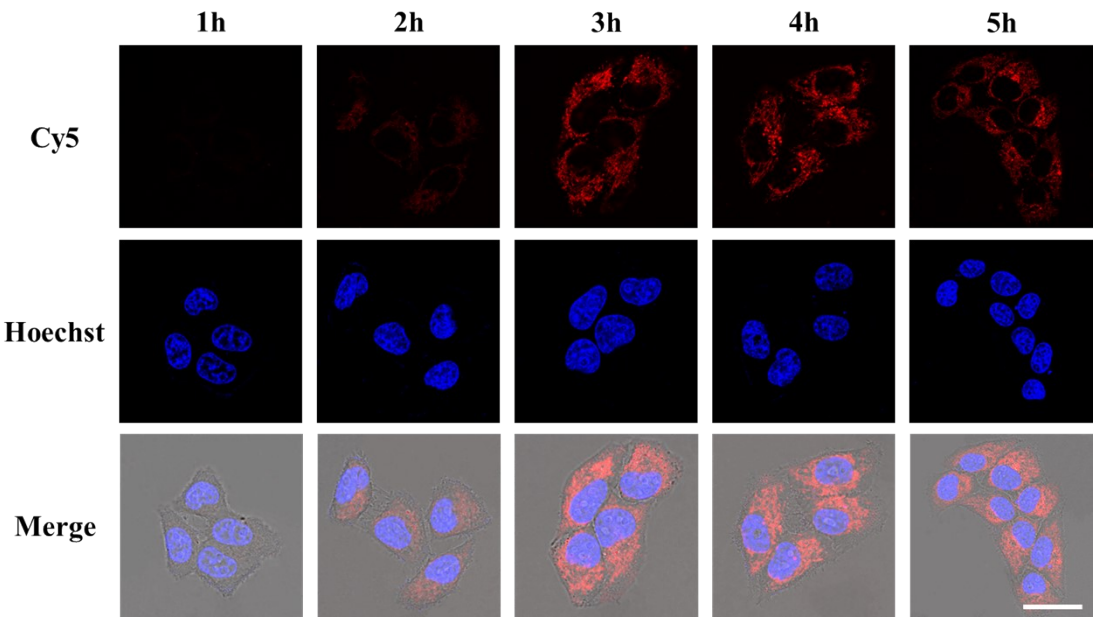

Fig. S7 Optimization of the incubation time in living cells. The Hela cells were treated with 100 nM nano string light for 1, 2, 3, 4 and 5 h at 37 ℃. The imaging was performed with 100×oil immersion objective. The Cy5 fluorescence emission was collected under an excitation of 633 nm. Scale bar is 25 μm.

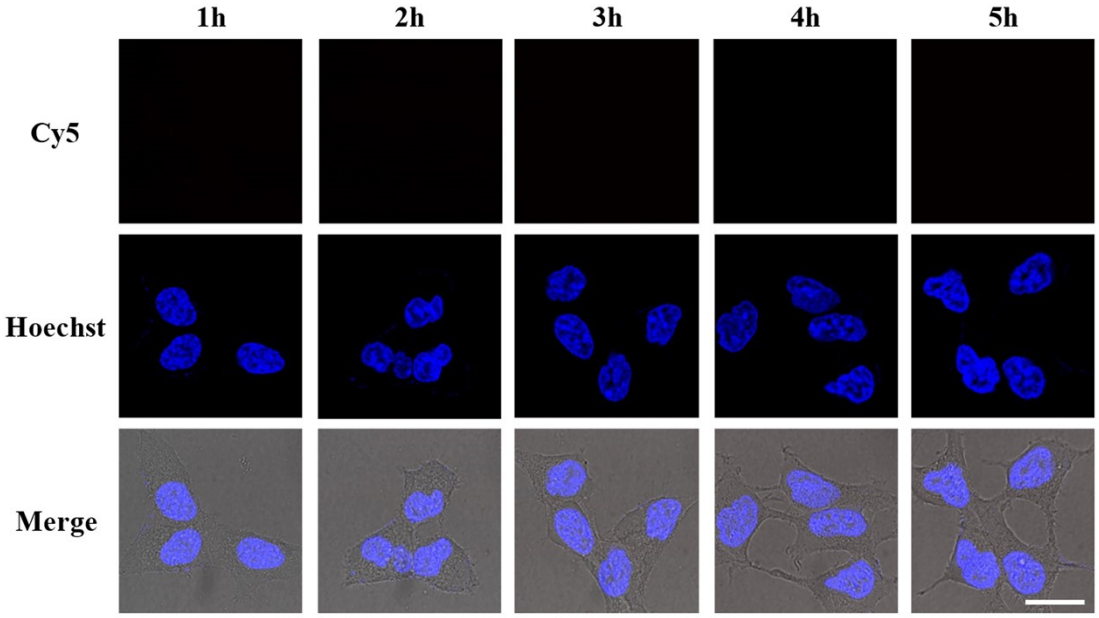

Fig. S8 Optimization of the incubation time in normal cells. The HEK-293 cells were treated with 100 nM nano string light for 1, 2, 3, 4 and 5 h at 37 ℃. The imaging was performed with 100×oil immersion objective. The Cy5 fluorescence emission was collected under an excitation of 633 nm. Scale bar is 25 μm.

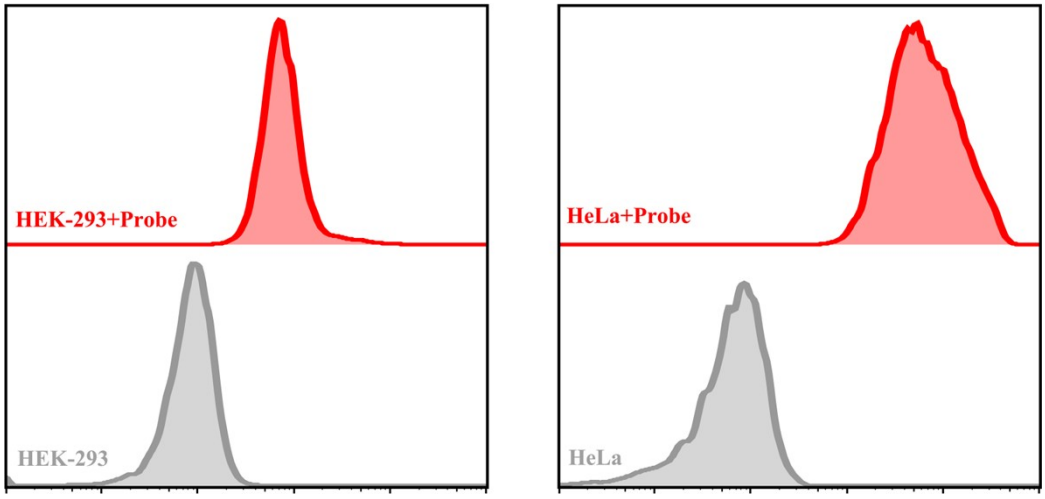

Fig. S9 The flow cytometric assay of HEK-293 cells and HeLa cells. The HEK-293 cells and HeLa cells were treated with 100 nM nano string light for 3 h at 37 ℃.

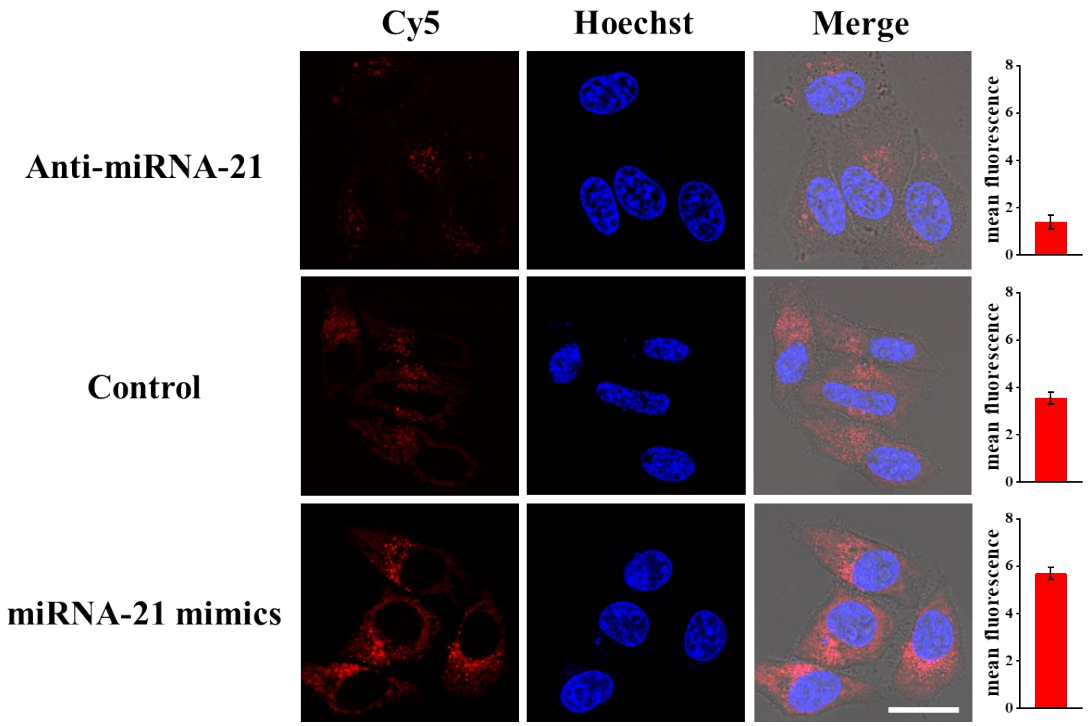

Fig. S10 Confocal images of the nano string light under different conditions. The cells were incubated with nanoprobe at 37 ℃ for 3h. Scale bar is 25 μm.

Supplementary References

1. Miao, P.; Tang, Y., Two-Dimensional Hybridization Chain Reaction Strategy for Highly Sensitive Analysis of Intracellular mRNA. *Anal. Chem.,* 2020, **92**, 12700-12709.
2. Lv, M.-M.; Liu, J.-W.; Yu, R.-Q.; Jiang, J.-H., A bipedal DNA nanowalker fueled by catalytic assembly for imaging of base-excision repairing in living cells. *Chem. Sci.,* 2020, **11**, 10361-10366.
3. Wei, Q.; Huang, J.; Li, J.; Wang, J.; Yang, X.; Liu, J.; Wang, K., A DNA nanowire based localized catalytic hairpin assembly reaction for microRNA imaging in live cells. *Chem. Sci.,* 2018, **9**, 7802-7808.
4. Deng, X.; Liu, X.; Wu, S.; Zang, S.; Lin, X.; Zhao, Y.; Duan, C., Ratiometric Fluorescence Imaging of Intracellular MicroRNA with NIR-Assisted Signal Amplification by a Ru-SiO2@Polydopamine Nanoplatform. *ACS Appl. Mater. Interfaces,* 2021, **13**, 45214-45223.
5. Wang, A.; Lin, Q.; Liu, S.; Li, J.; Wang, J.; Quan, K.; Yang, X.; Huang, J.; Wang, K., Aptamer-tethered self-assembled FRET-flares for microRNA imaging in living cancer cells. *Chem. Commun.,*2020, **56**, 2463-2466.
6. Song, X.; Ding, Q.; Zhang, J.; Sun, R.; Yin, L.; Wei, W.; Pu, Y.; Liu, S., Smart Catalyzed Hairpin Assembly-Induced DNAzyme Nanosystem for Intracellular UDG Imaging. *Anal. Chem.,* 2021, **93**, 13687-13693.
7. Li, J.; Wang, J.; Liu, S.; Xie, N.; Quan, K.; Yang, Y.; Yang, X.; Huang, J.; Wang, K., Amplified FRET Nanoflares: An Endogenous mRNA-Powered Nanomachine for Intracellular MicroRNA Imaging. *Angew. Chem., Int. Ed.,* 2020, **59**, 20104-20111.