Supporting Information

Stable, polyvalent aptamer-conjugated near infrared fluorescent nanocomposite for high-performance cancer cell-targeted imaging and therapy

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1. Materials and reagents

DNA oligonucleotides (Table S1) were synthesized and purified by Sangon Biotech. Co. Ltd (Shanghai, China). The concentrations of the oligonucleotides were represented as single-stranded concentrations, which were determined by measuring the absorbance at 260 nm. The molar extinction coefficient was determined using a nearest neighbor approximation (http://www.idtdan.com/analyzer/Applications/OligoAnalyzer). $AgNO_3$ was obtained from Alfa Aesar Corporation. Sodium borohydride ($NaBH_4$), NaH_2PO_4 and Na_2HPO_4 were purchased from Heowns Biochem Technologies. LLC. (Tianjin, China). Hydrogen tetrachloroaurate(III) ($HAuCl_4 \cdot 4H_2O$, 99.99%), Trisodium citrate ($C_6H_5Na_3O_7 \cdot 2H_2O$) were purchased from China National Pharmaceutical Group Corporation (Shanghai, China). 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) was all obtained from Sigma-Aldrich (St. Louis, MO, USA). Deoxyribonucleases I (DNase I) was obtained from New England Biolabs Beijing Ltd. Thioflavin T (3,6-dimethyl-2-(4-dimethylaminophenyl)-benzothiazolium cation, ThT) was obtained from Sigma-Aldrich. 4′,6-diamidino-2-phenylindole (DAPI) was obtained from Beyotime Institute of Biotechnology (Shanghai, China). Deionized and sterilized water (resistance > 18 MΩ/cm) was used throughout the experiments. All of the chemicals were of analytical grade and used without further purification.

Table S1. The oligonucleotides used in this work

Oligonucleotides	Sequence (5′→3′)[a,b]	Probe preparation
aDNA1	HS-AAAAAAAAAAAAA**AGCCTAAGAGTTGAGCAT**TGGTGGTGGTGGTTGTGGTGGTGGTGG	AuNP@(AS1411-AgNCs)$_n$ AuNP@(AS1411)$_n$
aDNA-control	HS-AAAAAAAAAAAAA**AGCCTAAGAGTTGAGCAT**T	AuNP@(AgNCs)$_n$
aDNA1′	AAAAAAAAAAAAA**AGCCTAAGAGTTGAGCAT**TGGTGGTGGTGGTTGTGGTGGTGGTGG	AS1411-AgNCs
tDNA1	CCCCCCCCCCCCCCC**ATGCTCAACTCTTAGGCT**	AuNP@(AS1411-AgNCs)$_n$ (λ_{em} = 660 nm)
tDNA2	CCCACCCACCCGCCC**ATGCTCAACTCTTAGGCT**	AuNP@(AS1411-AgNCs)$_n$ (λ_{em} = 760 nm) AS1411-AgNCs
tDNA3	CCCACCCACCCGCCC	AgNCs (λ_{em} = 760 nm)

[a] The red bases represent AS1411 aptamer sequence.

[b] The bold and underlined bases indicate the complementary bases used for assembly of DNA-templated AgNCs on AuNPs.

2. Dynamic light scattering (DLS) analysis

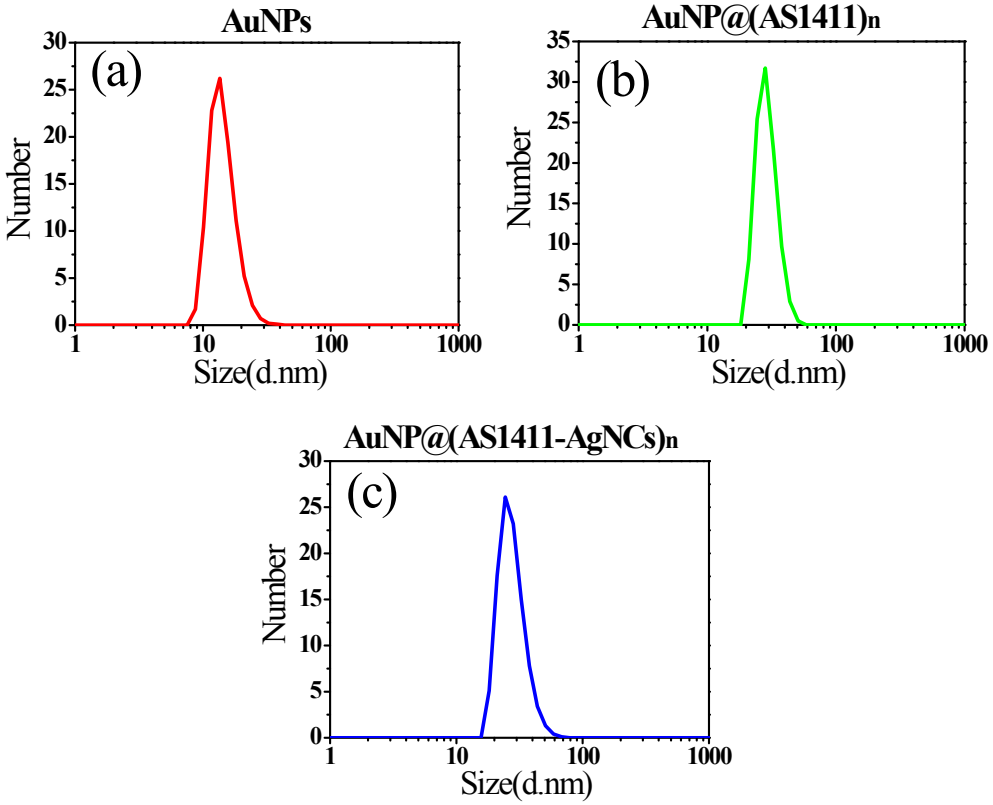

Figure S1. DLS characterization of the size distribution of AuNPs, AuNP@$(AS1411)_n$, and AuNP@$(AS1411\text{-}AgNCs)_n$.

3. DNA density on AuNP surface

The aDNA1 strands modified on 1 nM AuNPs were liberated into solution by mercaptoethanol, the liberated DNA strands were determined by using Thioflavin T (ThT，10 μM) as the fluorescent probe. Average number of aDNA1 per AuNP was calculated as:

$$\frac{[aDNA1\ on\ AuNP]}{[AuNP]} = \frac{(14.44 + 27.215)/0.398}{1} = 105$$ (aDNA1/AuNP).

Since each aDNA1 has one AS1411 unit, the AS1411 density on Au nanoparticle was also 105 AS1411/AuNP.

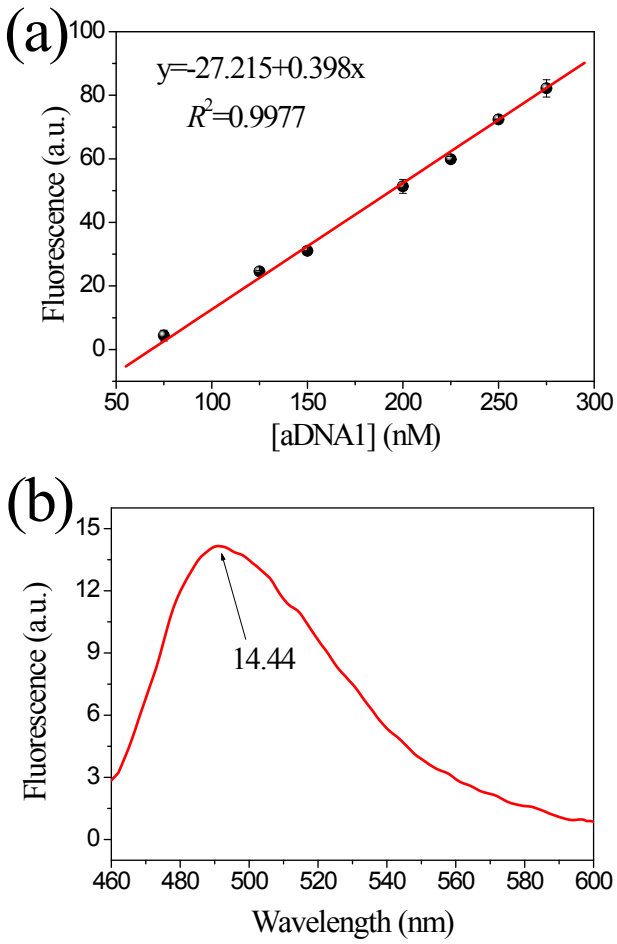

Figure S2. Determination of DNA density loaded on AuNP surface. (a) aDNA1 concentration-dependent changes of ThT fluorescence intensity at 425 nm; (b) fluorescence spectrum of ThT after incubation with the aDNA1 liberated from AuNPs.

The error bars represent the standard deviation of three repetitive measurements.

4. HRTEM of AuNP@(AS1411-AgNCs)$_n$.

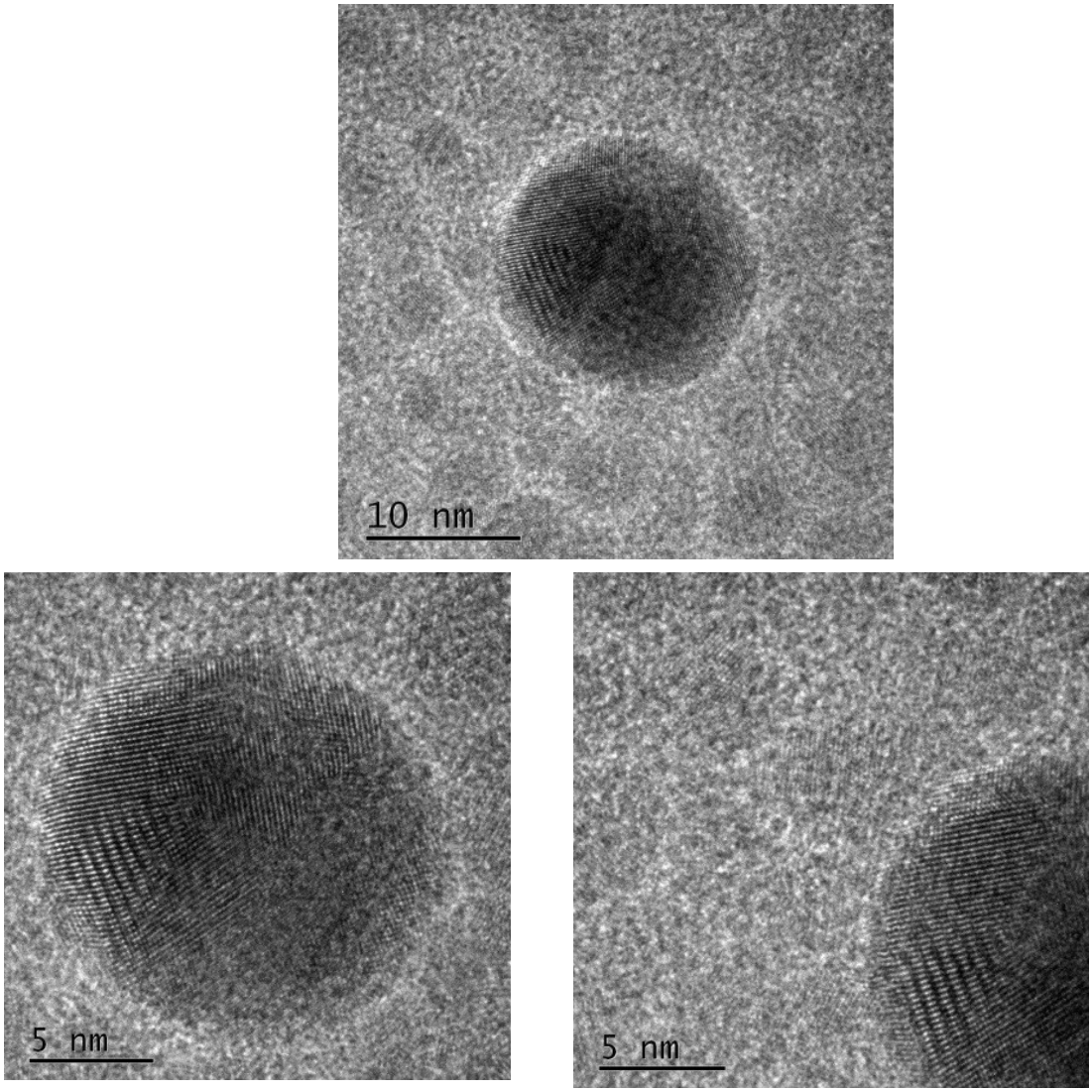

Figure S3. HRTEM of AuNP@(AS1411-AgNCs)$_n$.

5. Gel electrophoresis characterization of the assembly between DNA-templated AgNCs and DNA-modified AuNPs

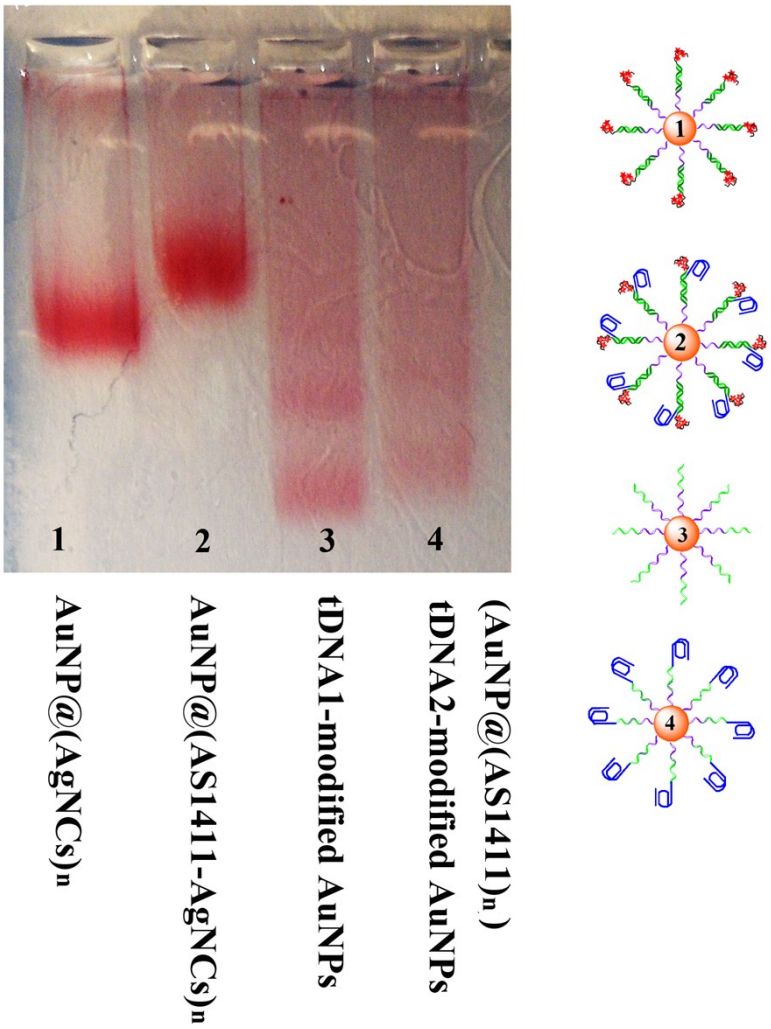

Figure S4. Agarose gel electrophoresis image of DNA-modified AuNPs before and after assembling with DNA-templated AgNCs.

6. Confocal microscopy imaging of live cells

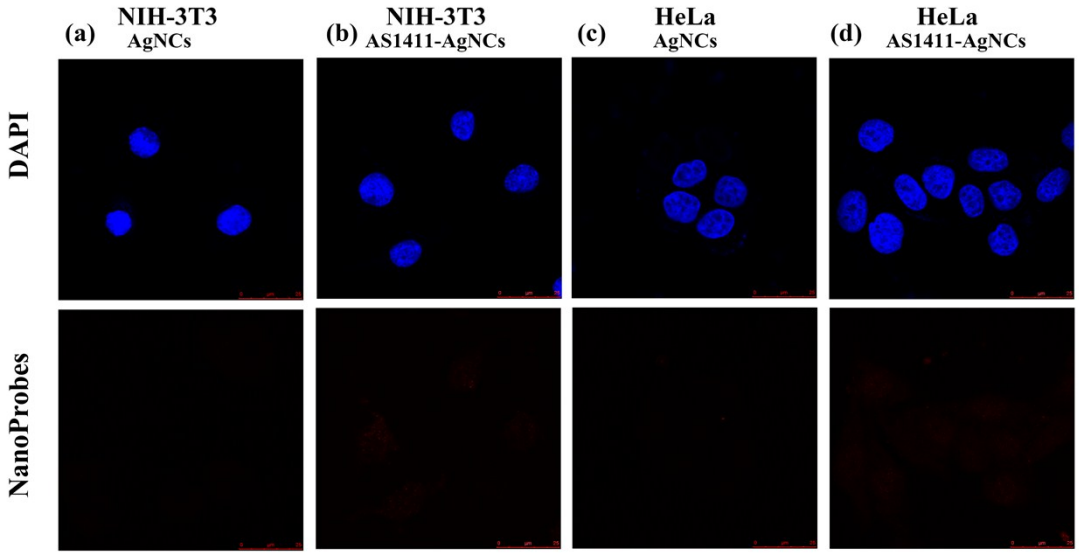

Figure S5. Confocal fluorescence microscopy images of NIH-3T3 and HeLa cells treated with AgNCs (200 nM) or AS1411-AgNCs (200 nM) for 3h. Scale bar = 25 μm

7. DOX loading in AuNP@(AS1411-AgNCs)$_n$

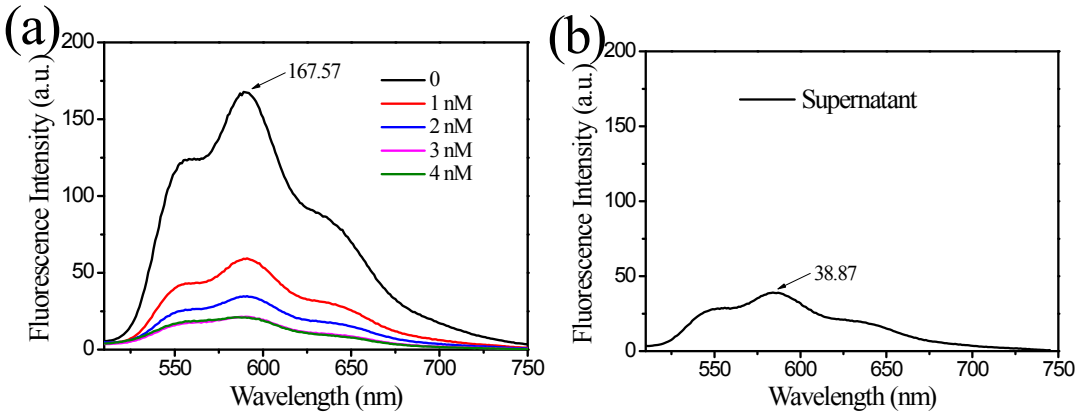

Figure S6. DOX loading in AuNP@(AS1411-AgNCs)$_n$. (a) The fluorescence signals given the mixtures of 2 μM DOX and different concentrations of AuNP@(AS1411-AgNCs)$_n$; (b) The fluorescence signal of the supernatant obtained by separating

AuNP@(AS1411-AgNCs)$_n$ from the mixture of 2 μM DOX and 3 nM AuNP@(AS1411-AgNCs)$_n$.