Supporting Information

Preparing Liposomes through Frame Guided Assembly with High-Loading Functional Nucleic Acids

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1. Materials

All chemicals were purchased from Aldrich or Alfa Aesar (Tianjing, China) and used as received unless otherwise mentioned. The DNA and RNA sequences were purchased from Xianghong Biotechnology (Beijing, China). DOPC, DOPG and PE-PEG-2000 were purchased from Avanti Lipid. Azide-modified peptide was purchased from Hefei Keshengjing Peptide Biotechnology. The MCF-7 cells (Human breast cancer cell line) and 293T•EGFP cells (Human embryonic kidney cell) were purchased from National Infrastructure of Cell Line Resource (NCR, China). The DMEM medium (Gibco, 11965092), FBS (Gibco, 10100147) and penicillin-streptomycin (Gibco, 15140122) were purchased from ThermoFisher Scientific (USA). The MTS solution (Promega G3582) was purchased from ThermoFisher Scientific. The Trizol (R0016) was purchased from Beyotime Biotechnology. The RevertAid First Strand cDNA Synthesis Kit (K1621) was purchased from ThermoFisher Scientific. The water used in all the experiments was Milli-Q deionized (18.2 MΩ.cm). TEM was performed using a HT 7700 TEM (Japan) at 100 kV. The PAGE analysis and agarose gel electrophoresis imaging was conducted on the UVP GelStudio PLUS Imaging System (USA).

2. Supplementary Results

2.1 Results of AuNPs Modified with Various Ratios of D23/D12

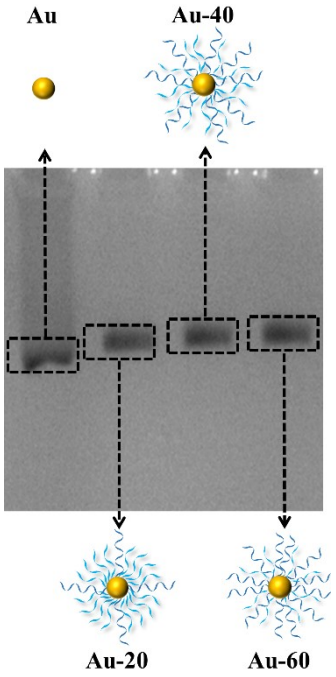

Fig. S1 The 1% agarose gel electrophoresis result of AuNPs modified with various molar ratios of D23/D12.

2.2 Measurement Results of LHG Number on AuNPs

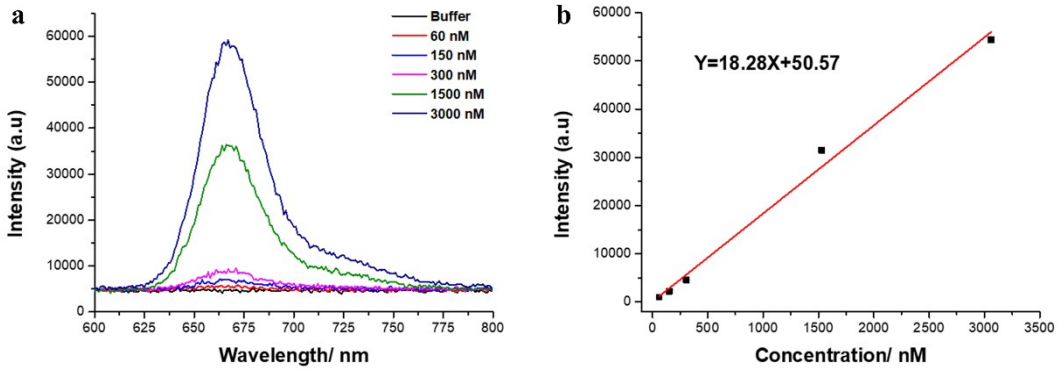

Fig. S2 The fluoresecent results of the cD23-Cy5. (a) The fluorescence spectra of the cD23-Cy5 with different concentration. (b) The calibration curve plotted based on the known concentration of probes.

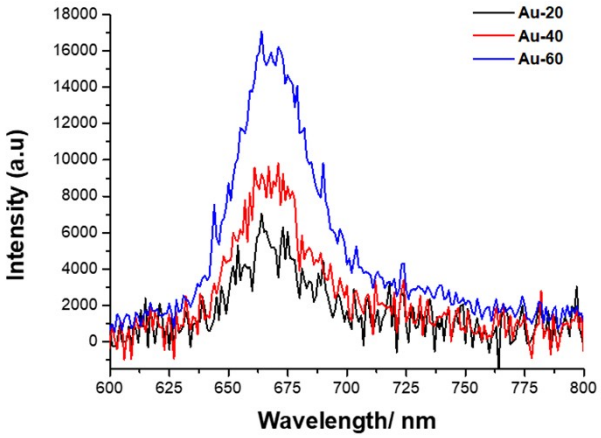

Fig. S3 The fluorescence spectra of Cy5-cD23 released from the AuNPs with various ratios of D23 (anchoring strand) after denaturing. It could be observed that with the reducing D23 decoration on AuNPs, the decreased fluorescent density of Cy5-cD23 has been recorded, which was consistent with previous results.

Table S1 Measurement Results of LHG Number on AuNPs

	Au-20	**Au-40**	**Au-60**
UV-Vis of AuNPs after urea-denaturing (A_{450})	1.76	1.59	1.77
Concentration of AuNPs (nM)	12.6	11.4	12.7
Intensity of released Cy5 (a.u) at λ_{667}	5575	9618	14802
Converted molar concentration of Cy5 (nM)	301	475	806
Ratios of DNA-to-AuNPs	24	42	63

2.3 Results of Hybridization of Various AuNP Scaffolds with Peptide Conjugates

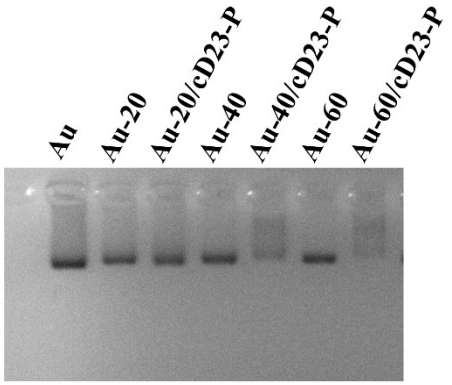

Fig. S4 The 10% native PAGE analysis of the hybridization of the various AuNP scaffolds with peptide conjugates.

2.4 FGA Assembly Results of the Frames with Lower LHG Density (F-25 to F-10)

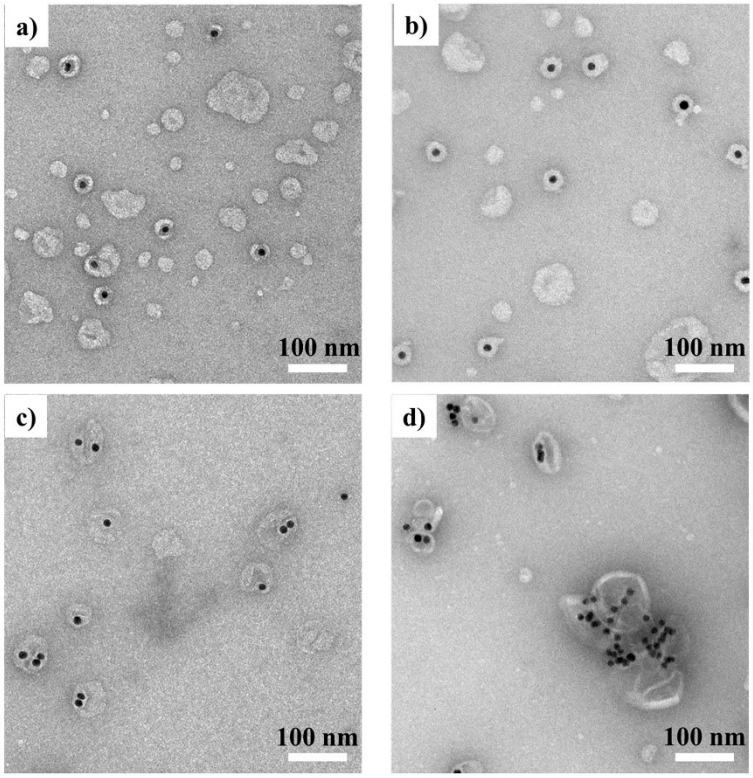

Fig S5 The TEM images of assembly results of frames with lower LHG density under 2% OG. (a) TEM image of the assembly result of F-25. (b) TEM image of the assembly result of F-20. (c) TEM image of the assembly result of F-15. (d) TEM image of the assembly result of F-10. The scale bar is 100 nm.

2.5 Assembly Efficiency of Various Frames under Different OG Concentration

After dialysis, the solution was collected and allowed to stand undisturbed for a duration of 8 h. During this interval, when the frames could be well guided, the resulting well dispersed FGA liposomes. On the other hand, AuNPs containing incomplete liposomes or frames would tend to aggregate. Therefore, the assembly efficiency of the FGA could be calculated by determining the recovery of AuNPs in the supernatant and the concentration of AuNPs was estimated by calculating the absorbance value at 450 nm according to the reported literature.[S1]

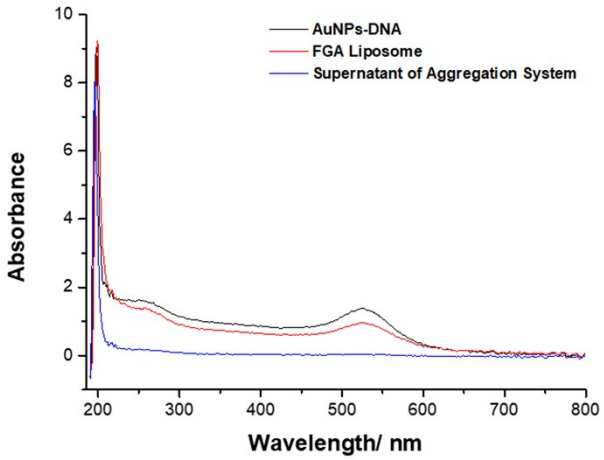

Fig. S6 The UV spectra of AuNPs-ASO, ASO FGA Liposome and supernatant of the aggregation system.

Table S2 Results of assembly efficiency

Frame \ OG	5%	4%	3%	2%	1%
F-60	**11%**, (0.08/120)	**18%**, (0.17/90)	**15%**, (0.10/113)	**10%**, (0.09/90)	**13%**, (0.11/110)
F-40	**30%**, (0.27/90)	**22%**, (0.14/120)	**15%**, (0.11/114)	**13%**, (0.11/90)	**8%**, (0.06/114)
F-20	**72%**, (0.67/96)	**74%**, (0.64/96)	**85%**, (0.67/110)	**83%**, (0.76/90)	**79%**, (0.64/120)

Note: The average absorbance of the AuNPs-DNA before dialysis was measured to be about 0.83 at the wavelength of 450 nm. The UV-Vis data details and the final volume of the samples after dialysis were shown in the bracket.

2.6 Results of Orthogonal Effects of LHG Density and Detergent Concentration

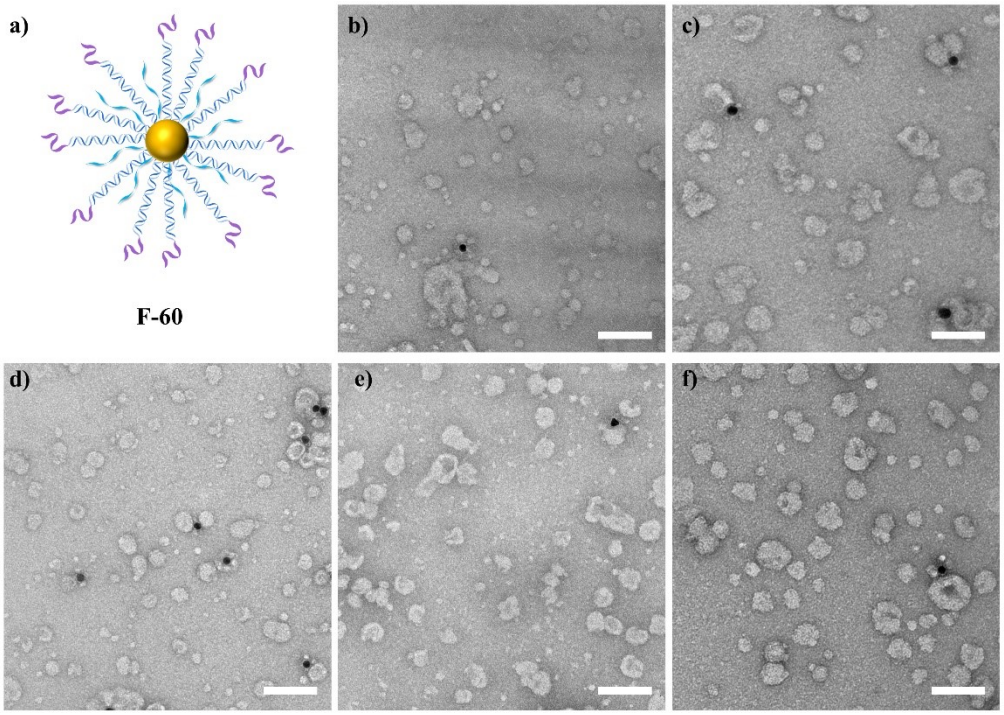

Fig. S7a Supplementary assembly results of F-60 under various OG concentration (5% to 1%) in **Fig. 2**.

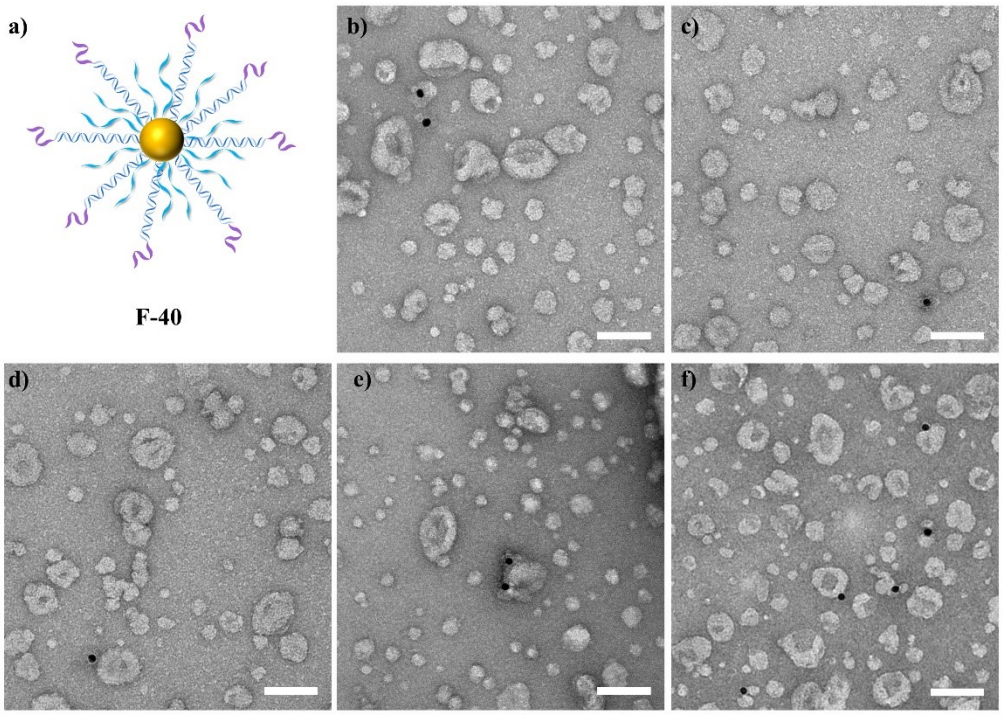

Fig. S7b Supplementary assembly results of F-40 under various OG concentration (5% to 1%) in **Fig. 2**.

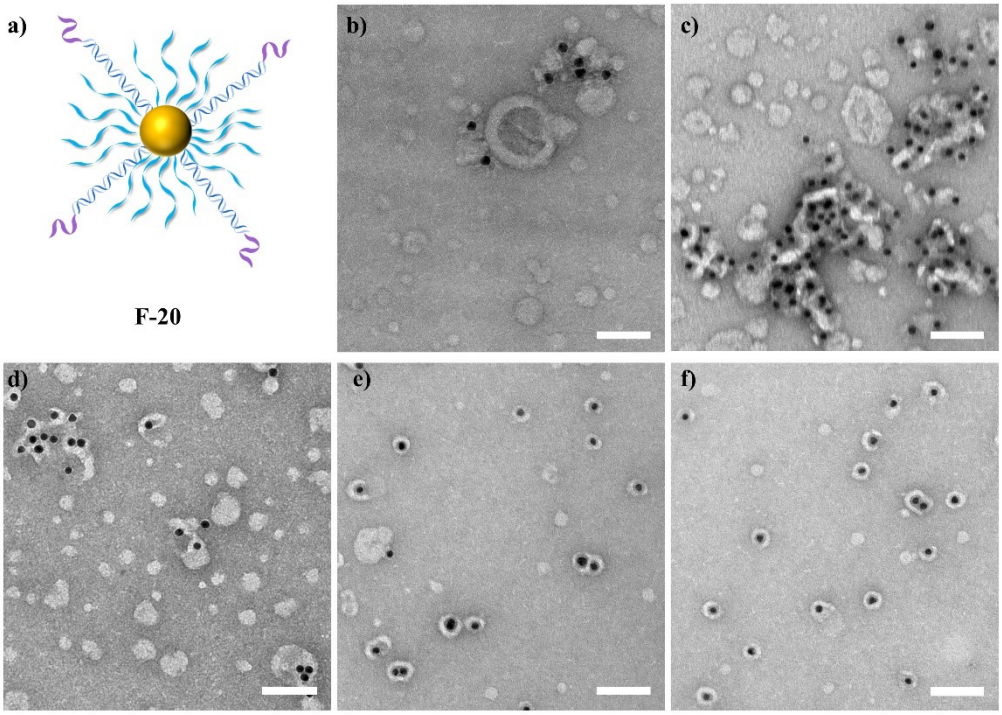

Fig. S7c Supplementary assembly results of F-20 under various OG concentration (5% to 1%) in **Fig. 2**.

2.7 Agarose Gel Electrophoresis Results of Incerased ASO Loading on AuNPs

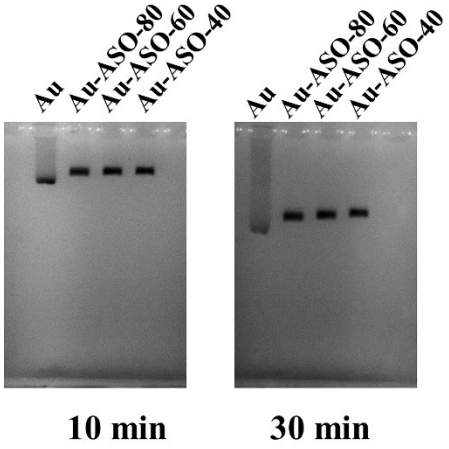

Fig. S8 The agarose gel electrophoresis results of AuNPs modified with fixed ratio of D23/ASO. It could be observed that when the AuNPs was decorated with higher ratios of the ASO, the reduced migration could be observed, which agreed well with the previous results and indicated that the increased loading of ASO has been achieved.

2.8 Measurement Results of ASO Number on AuNPs

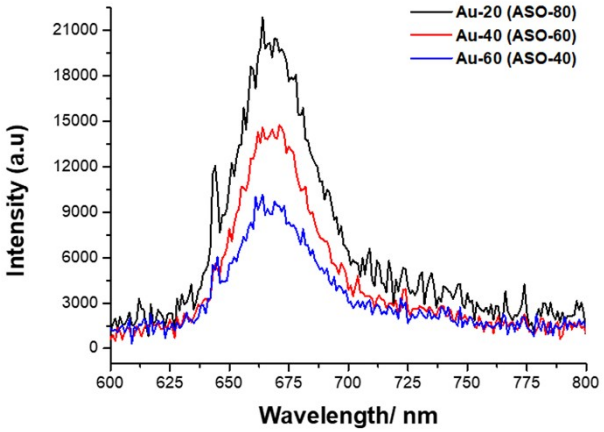

Fig. S9 The fluorescence spectra of Cy5-cASO released from the AuNPs with increased ASO (ASO-40 to ASO-80) loading after urea-based denaturing. According to the fluorescence spectra, it could be observed that with the increased modification ratio of the ASO (Au-60 to Au-20 or ASO-40 to ASO-80), the increased fluorescence intensity has been recorded, which indicated that the increased loading of nucleic acid drug could be achieved through reducing the LHG density.

Table S3 Measurement Results of ASO Number on AuNPs

	ASO-80 (Au-20)	ASO-60 (Au-40)	ASO-40 (Au-60)
UV-Vis of AuNPs after urea-denaturing (A_{450})	1.53	1.62	1.48
Concentration of AuNPs (nM)	11.0	11.6	10.6
Intensity of released Cy5 (a.u) at λ_{667}	19220	14080	8890
Converted molar concentration of Cy5 (nM)	1050	767	494
Ratios of DNA-to-AuNPs	95	66	45

2.9 Assembly Results of F-ASO under 1% OG

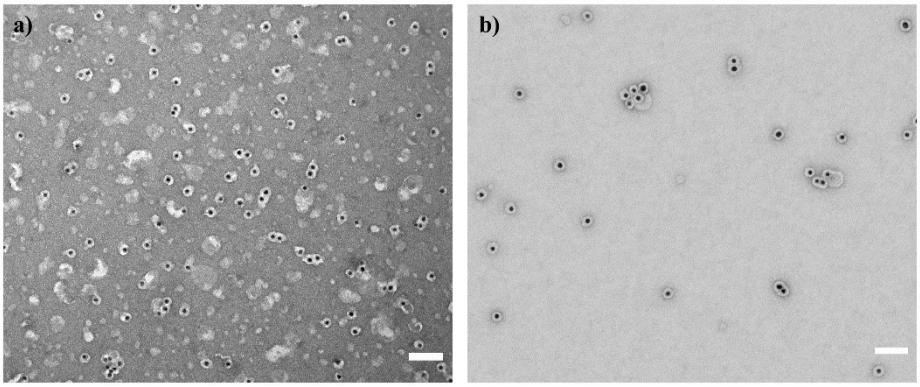

Fig. S10 The TEM images of assembly results of Au-ASO under 1% OG. (a) TEM image of the FGA-ASO-Lipo without purification. (b) TEM image of the FGA-ASO-Lipo with purification. The scale bar is 100 nm.

2.10 TEM Result of Extrusion-based Liposome

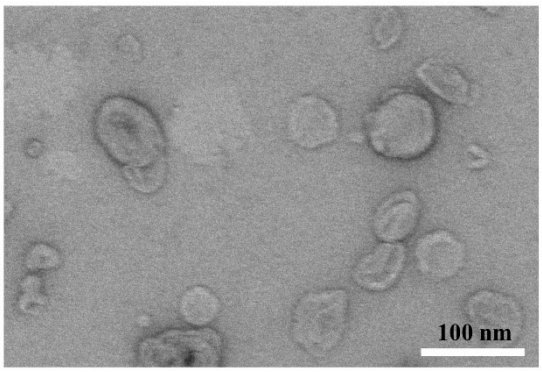

Fig. S11 The TEM image of assembly result of extrusion-based liposome. The scale bar is 100 nm.

2.11 Cell Growth Curves of MCF-7 Treated with Different Fomulations

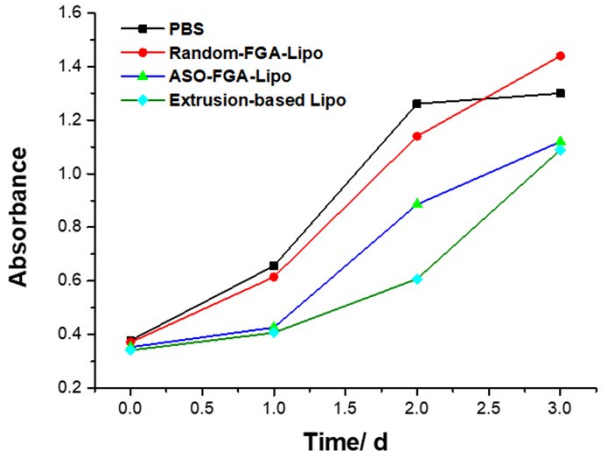

Fig. S12 The cell growth curves of the MCF-7 cell treated with different fumulations (PBS, Random-FGA-Lipo, ASO-FGA-Lipo and Extrusion-based-Lipo, respectively) from 1 d to 3 d. The loading of the ASO delivered by extrusion-based liposomes was conducted as following: the same amount ASO was added to the solution of DOPC/DOPG/PE-PEG-2000 with a final concentration as the FGA process. After extrusion, the free ASO strands were removed through an ultrafiltration tube (100 K, JUNLIBO) by centrifugation at 4500 rpm for 15 min and the procedure was repeated 4 times.

2.12 Assembly Result of Den-F under 5% OG

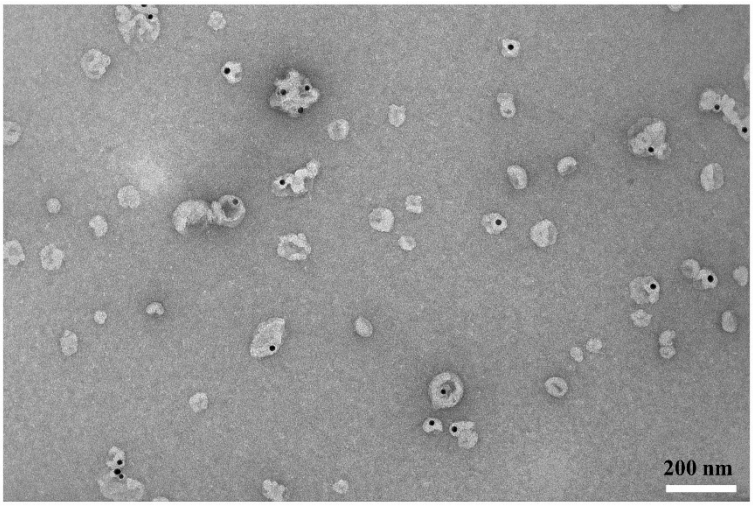

Fig. S13 The TEM image of assembly result of Den-F under 5% OG. The scale bar is 200 nm.

2.13 Related Sequences Information

Table S4 Peptide, DNA and RNA sequences used in this study

Name	Sequences
Azide-Peptide	Azide-GWARYAEWLFTTPLLLLALALLVEAAEG
D23	Thiol-TATCTGTAGGCATAAATTGGTAT
D12	Thiol-TTGACCTGTGAA
ASO	Thiol-TCTCCCAGCGTGCGCCAT
cD23	Alkynyl-ATACCAATTTATGCCTACAGATA
L20-Sense-siEGFP	**rUrArUrCrUrGrUrArGrGrCrArUrArArArUrUrGrGrGrArC rGrUrArArArCrGrGrCrCrArCrArArGrUdTdC**-thiol
Anti-siEGFP	**rArCrUrUrGrUrGrGrCrCrGrUrUrUrArCrGrUrCdGdC**-
Random sense siRNA	**rUrArUrArCrCrUrCrUrGrCrCrUrArArUrCrArUrCrUdAdT**
Random Anti-siRNA	**dTrArGrArUrGrArUrUrArGrGrCrArGrArGrGrUdTdT**
Y1c-L20	**CCAATTTATGCCTACAGATA**AGGCTGATTCGGTTCATGC GGATCCAGAAGCCACTCTGA
Y1b	TGGATCCGCATGACATTCGCCGTAAGGAAGCCACTCTGA
Y1a	CTTACGGCGAATGACCGAATCAGCCT
cD13	Alkynyl-TCAGAGTGGCTTC

2.14 Synthesis and Characterization of cD23-P

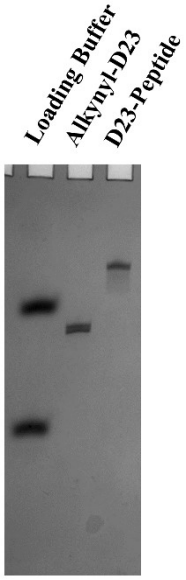

Fig. S14a Characterization of cD23-Alkynyl and cD23-Peptide strands by 20% denaturing PAGE.

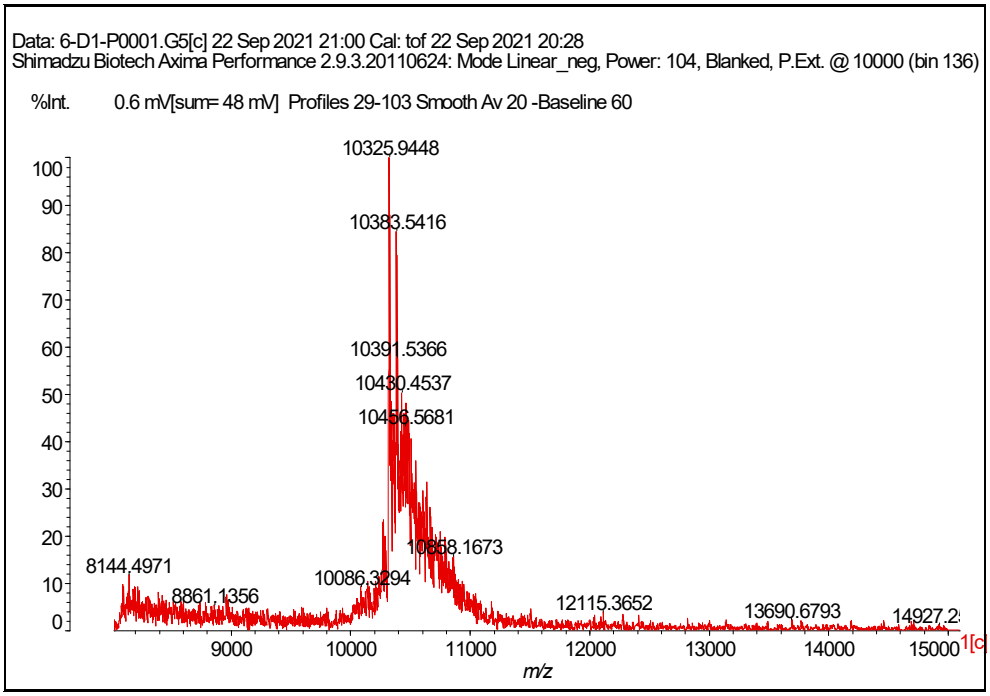

Fig. S14b MALDI-TOF characterization of cD23-Peptide conjugate. Based on the mass spectrometry results, it is evident that the observed molecular weight is 10325.9, whereas the anticipated molecular weight is 10321.7, with a minute deviation of 0.4 ‰. This deviation is well below the acceptable minimum error threshold of 5 ‰, thereby confirming our triumph in synthesizing the intended product with a precise molecular weight.

2.15 Synthesis and Characterization of cD13-P

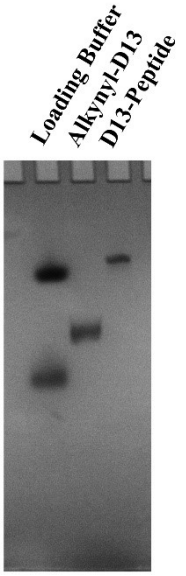

Fig. S15a Characterization of cD13-Alkynyl and cD23-Peptide strands by 20% denaturing PAGE.

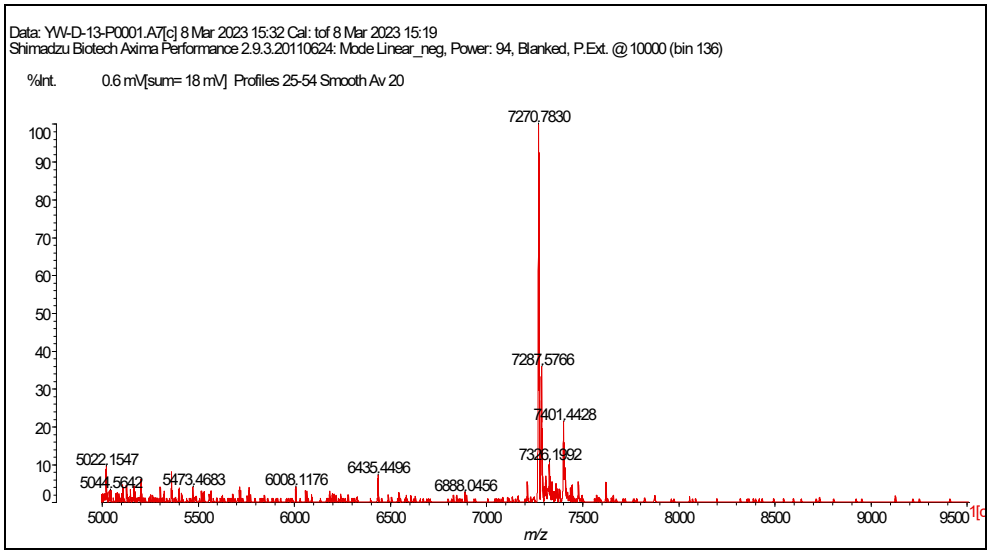

Fig. S15b MALDI-TOF characterization of cD13-Peptide conjugate. Based on the mass spectrometry results, it is evident that the observed molecular weight is 7270.8, whereas the anticipated molecular weight is 7298.3, with a minute deviation of 2.8 ‰. This deviation is well below the acceptable minimum error threshold of 5 ‰, thereby confirming our triumph in synthesizing the intended product with a precise molecular weight.

2.16 Results of hybridization of ds-siEGFP and Y1-dendrimer

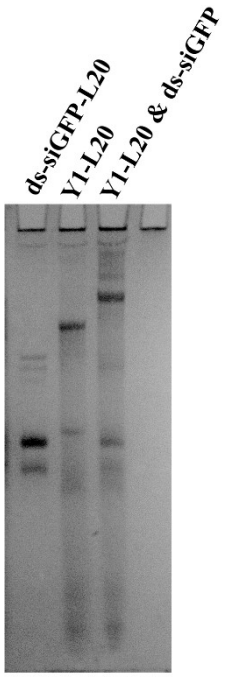

Fig. S16 The 10% native PAGE analysis of the hybridization of the ds-siEGFP and Y1-dendrimer.

2.17 Results of AuNPs modified with Y1-dendrimer

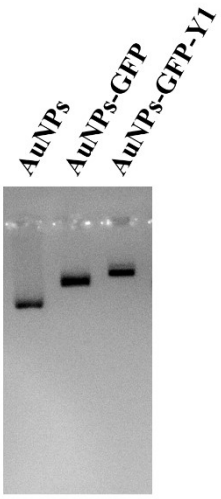

Fig. S17 The agarose gel electrophoresis results of AuNPs modified with siRNA (siEGFP) and further hybridized with the Y1-dendrimer.

Reference

S1 W. Haiss, N. T. Thanh, J. Aveyard and D. G. Fernig, *Anal. Chem.*, 2007, **79**, 4215.