Electronic Supplementary Information

Short Intrinsically Disordered Polypeptide-oligonucleotide Conjugates for Programmed Self-assembly of Nanospheres with Temperature-dependent Size Controllability

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Experimental Section

Materials: Sodium L-ascorbate (99.0%), Copper(II) sulfate pentahydrate ($CuSO_4 \cdot 5H_2O$, 99.9%) and doxorubicin hydrochloride (DOX·HCl, 98.0%) were purchased from J&K China Chemical Co. Ltd. Sodium citrate (99.0%) and chloroauric acid ($HAuCl_4$, 98.0%) were purchased from Energy Chemical. Tert- butyl alcohol and Nile Red (98.0%) was purchased from Aladdin Industrial Corporation. Chlorpromazine hydrochloride (CPZ, 98.0%) and methyl-β-cyclodextrin (MβCD, 98.0%) were purchased from Shanghai yuanye Bio-Technology Co., Ltd. Paraformaldehyde solution (4%), DAPI staining solution (10 ug/mL), Lysosome localization probe (Lyso Tracker Green DND-26), mitochondria localization probe (MitoTracker Red CMXRos), endoplasmic reticulum localization probe (ER-Green[DiOC6(3)]) and dictyosome localization probe (GolgiGreen) were purchased from Yeasen biotech Co., Ltd. Deoxyribonuclease I (Dnase I, 50 U/μL) was purchased from Beyotime Biotechnology. The alkyne functionalized ELP and all oligonucleotides were purchased from KareBayBiochem.co. Ltd. All aqueous solutions were prepared with ultrapure water (18.25 MΩ·cm) supplied by Milli-Q Advantage A10 (Merck Millipore). All chemicals were used as received without further purification.

Table S1. Oligonucleotides sequences used in this study

Oligonucleotides	**Sequences**
ON-1	5’-**azido**-CCCGCGAAATTAATACGACTCAAGATTACGGTGAAGAGA-3’
ON-2	5’-GAGTCGTATTAATTTCGCGGG-**azido**-3’
ON-3	5’-**azido**-CCCGCGAAATTAATACGACTCACTATAGGGGA-3’
ON-4	5’-TCCCCTATAGTGAGTCGTATTAATTTCGCGGG-**azido**-3’
ON-5	5’-**azido**-ATTATATTAGATGTTTTTCTA-3’
ON-SH	5’-AGTTAAACATCGCATGTGCTTTTTTTTTTT-**SH**-3’

Linear DNA linker	5'-GCACATGCGATGTTTAACTTCTCTTCACCGTAATCTT-3'
ON-FAM	5'-**FAM**-TCTCTTCACCGTAATCTT-3'
ON-TAMRA	5'-**TAMRA**-TCTCTTCACCGTAATCTT-3'

Synthesis of ELP-ONs Conjugate: The synthesis of the conjugate was performed via copper(I)-catalyzed alkyne-azide cycloaddition (CuAAC) "click" reaction. Solutions of alkynyl-ELP (1 mg/mL, 600 µL) in Millipore water/tert-butanol mixed solution, aqueous solution of azido-oligonucleotides (100 µM, 60 µL), aqueous solution of copper(II) sulfate pentahydrate (1 mM, 10 µL) and fresh aqueous solution of sodium L-ascorbate (2 mM, 10 µL) were mixed in a 1.5 mL eppendorf tube under nitrogen and stirred at 25 °C for 6 hours. After reaction, the resulting hybrid copolymer was purified using 10 % polyacrylamide gel electrophoresis (PAGE). After excision the bands were dialyzed against water for 24 hours. Subsequently, the ELP-ON conjugates were concentrated using Amicon filtering tubes 3 kDa (Amicon bioseparations-Millipore U.S.A.). Concentration of ELP-ON conjugate was measured using Nanodrop 2000C (Thermo Fisher Scientific, U.S.A.) and adjusted to 10 µM using buffer solution (pH=7.6, 1 mM EDTA, 20 mM Tris-HCl, containing 8 mM Mg^{2+}). Molar masses of ELP-ON conjugates were confirmed by ESI LC-MS (Thermo LCQ DECA XP and Thermo LTQ, Thermo Fisher Scientific, U.S.A.).

Synthesis of NSs: NSs were assembled by mixing complementary ELP-ONs in equal quantities as well as annealing from 85 ^{o}C to 25 ^{o}C at a rate of 1 $^{o}C \cdot min^{-1}$ and then incubated at 25 ^{o}C for 2 hours. NS-1 was assembled by ELP-ON1 and ELP-ON2, NS-2 was assembled by ELP-ON3 and ELP-ON4.

Synthesis of AuNP: Citrate-capped AuNP (14 nm) was prepared based on a literature reported method.[1] Loaded 148.5 mL of Millipore water into a two-neck flask and then added 1.5 mL of $HAuCl_4$ solution (1%, w%). Connected the condenser to one neck of the flask and placed the stopper in the other neck. Put the flask on the hot plate to reflux while stirring. When the solution began to reflux, removed the stopper. Quickly added 2.9 mL of 38.8 mM sodium citrate and replace the stopper. The color of the solution was gradually blackened, and then turned red slowly. After the color no longer changed, allowed the system to reflux for another 15 min. Turned off heating and cooled the

system to to room temperature under stirring. The diameter of such prepared AuNP was about 14 nm.

Synthesis of Au-SNA: Oligonucleotide functionalized AuNP was prepared based on a literature reported method.[2] Thiolated oligonucleotide ON-SH (100 μM, 5 μL) was mixed with citrate-AuNPs (14 nm, 4 nM, 500 μL). The mixture was then placed in a -40 ^{o}C refrigerator for 2 h, followed by thawing at room temperature. The solution containing the functionalized particles was centrifuged (13,000 rpm, 20 min) and resuspended in phosphate buffered saline (PBS; pH 7.4, 137 mM NaCl, 10 mM phosphate, 2.7 mM KCl, containing 0.05% Tween 20) three times to produce the purified Au-SNA used in all subsequent experiments. At the end, purified Au-SNA resuspended in buffer solution (pH=7.6, 1 mM EDTA, 20 mM Tris-HCl, 8 mM Mg^{2+}) at a concentration of 5 nM.

Transmission Electron Microscopy (TEM): Samples for TEM were prepared on carbon-coated copper grids (CF-230, Beijing Zhongjingkeyi Technology Co., Ltd). NSs and ELP-ON samples were all prepared at concentration of 0.1 μM. 10 μL of the sample solution was dropped cast on the grid and blotted after 15 min. For staining, 2% phosphotungstic acid (PPA) (pH adjusted to 7.4 using 1 M NaOH) was used for negative staining. 10 μL of the PPA solution was drop cast on the grid and blotted after 30 seconds. The samples were air-dried for 2 hours. All operations were performed in an isothermal oven. TEM images were taken on a JEM-2100 TEM (Japan Electronics Co., Ltd) at an acceleration voltage of 200 keV.

Dynamic Light Scattering (DLS): Analysis of particle size in solution was performed by dynamic light scattering (DLS) on a Laser Light Scattering Spectrometer (ALV/CGS-5022F, ALV Laser Vertriebsgesellschaft m.b.H) equipped with an ALV-High QE APD detector and an ALV-5000 digital correlator using a He-Ne laser (the wavelength λ = 632.8 nm, 22 mW) as the light source. The scattering angle is 90°. NS-1 sample was prepared at concentration of 0.1 μM in aqueous solution. For studying temperature-dependent particle size distribution, the NS-1 was added into standard glass bottle (diameter of 17 mm) and pre-incubated in chamber at desired temperatures (20 °C, 30 °C, 40 °C, 50 °C, 60 °C, 50 °C, 40 °C, 30 °C and 20 °C) for 30 min before measurement, respectively. The reported data represent an average of at least three measurements.

Melting Assay of Au-SNA-(NS-1): A batch of Au-SNA (5 nM, 150 μL) was hybridized

with NS-1 (5 mM, 30 μL) and ON-linker (10 mM, 15 μL) in buffer solution (pH=7.6, 1 mM EDTA, 20 mM Tris-HCl, 8 mM Mg2+). The extinction for the solution containing aggregates was measured using an ultraviolet-visible Spectrophotometer (Varian Cary 100, Varian, Inc). For melting temperature assay studies, the aggregates were incubated at 35 ºC, 40 ºC, 45 ºC, 50 ºC, 55 ºC, 60 ºC, 62 ºC, 64 ºC, 66 ºC, 68 ºC, 70 ºC, 72 ºC, 74 ºC, 78 °C and 85 ºC while monitoring the absorbance at 524 nm. Each sample was incubated for at least 15 min at the desired temperature before measurements.

Preparation and Fluorescence Characterization of Nile Red-Encapsulated NS: NS and relevant complementary oligonucleotide samples were diluted with buffer solution (pH=7.6, 1 mM EDTA, 20 mM Tris-HCl, 8 mM Mg^{2+}) to a final concentration of 1 μM (100 μL), and then 2.5 μL of Nile Red stock solution (2 mM in acetone) was added. The samples were vortexed briefly, sealed, and incubated for 24 hours at room temperature in the absence of light. Fluorescence spectra were recorded at room temperature using a Fluorospectrophotometer (Varian Cary Eclipse, Varian, Inc) with an excitation wavelength of 550 nm and monitoring emission between 570 and 750 nm, with excitation and emission slit widths both set at 5 nm.

Fluorescence Label of NS-1: NS-1 sample was diluted with buffer solution (pH=7.6, 1 mM EDTA, 20 mM Tris-HCl, 8 mM Mg^{2+}), and then 5-fold dye-labeled oligonucleotide (ON-FAM or ON-TAMRA) was added. The mixture was incubated for 4 hours at room temperature in the absence of light and the excess dye-labeled oligonucleotide was then purified three times using Amicon filtering tubes 10 kDa (Amicon bioseparations-Millipore U.S.A.).

Cultures of HepG2 cells: HepG2 cells were seeded in 35-mm glass-bottomed culture dishes in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum (FBS), 100 U/mL penicillin and 100 μg/mL streptomycin at 37 °C in a humidified atmosphere of 5% CO_2.

Cellular Uptake Analyzed by Confocal Laser Scanning Microscopy: 10,000 HepG2 cells seeded on a laser confocal culture plate and cultured overnight. Then removed culture medium, the cells were incubated with fresh medium containing FAM-labelled NS-1 (equivalent FAM concentration: 500 nM) at 37 ^{o}C for 2 hours, and then the solutions were removed, and the cells were carefully washed thrice with PBS. Confocal microscopy analysis of those cells was carried out with a Nikon A1R inverted laser-

scanning confocal microscope (Nikon Corporation).

Cytotoxicity Studies: The cell toxicity was investigated by the standard CCK-8 assay. Briefly, HepG2 cells were seeded on 96-well cell culture plates (10,000 cells per well) and incubated at 37°C overnight. After the cells adhered to the plate wall, the culture medium was replaced with fresh DMEM containing different concentrations of NS-1 , free DOX or NS-1/DOX and cultured for another 24 h before discarding the cell supernatant. The cell culture medium was replaced with fresh cell culture medium (100 μL) and CCK-8 solution (10 μL) was added to every single well in the plate which was incubated for another 1 h at 37 °C. Finally, and the absorbance was measured in a microplate reader (SpectraMax M2, Molecular Devices) at wavelength of 450 nm, and the cell survival rate was obtained in relation to that in the untreated groups.

Colocalization Study of NS-1 and Intracellular Organelles: 10,000 HepG2 cells seeded on a laser confocal culture plate and cultured overnight. Then removed culture medium, the cells were incubated with fresh medium containing dye-labelled NS-1 (FAM-labelled or TAMRA-labelled, equivalent dye concentration: 500 nM) at 37 ^{o}C for 2 hours, and then the solutions were removed and the cells were carefully washed with fresh medium. And then the cells were respectively stained with Lyso Tracker Green DND-26, MitoTracker Red CMXRos, ER-Green [DiOC6(3)] or GolgiGreen according to manufacturer's recommended protocol. After stained, the solutions were removed, and the cells were carefully washed thrice with PBS. Confocal microscopy analysis of those cells was carried out with a Nikon A1R inverted laser-scanning confocal microscope (Nikon Corporation).

Study of mechanism for the endocytosis of NS-1： Each laser confocal culture plate was seeded with 10,000 HepG2 cells and cultured overnight. HepG2 cells were divided into 4 groups: control group, energy suppression group, clathrin-pathway inhibition group, and caveolin-pathway inhibition group. For control group, removed culture medium, the cells were incubated with fresh medium containing 500 nM TAMRA-labelled NS-1 at 37 ^{o}C for 4 hours. For energy suppression group, cells were pretreated at 4 ^{o}C for 30 mins, then removed culture medium, the cells were incubated with precooled fresh medium containing 500 nM TAMRA-labelled NS-1 at 4 ^{o}C for 4 hours. For clathrin-pathway inhibition group, and caveolin-pathway inhibition group, cells were pretreated with chemical blockers at 37 ^{o}C for 30 mins, then removed culture medium, the cells were incubated with fresh medium containing chemical blockers and 500 nM TAMRA-labelled NS-1 at 37 ^{o}C for 4 hours. 5 μg/mL CPZ for clathrin-pathway

inhibition group, and 12.5 mg/mL MβCD for caveolin-pathway inhibition group. After incubation, culture medium was removed, and the cells were carefully washed with fresh medium. And then the cells were fixed with 4% paraformaldehyde solution and stain the cell nucleus with DAPI staining solution (10 ug/mL). After stained, the solutions were removed, and the cells were carefully washed thrice with PBS. Confocal microscopy analysis of those cells was carried out with a Nikon A1R inverted laser-scanning confocal microscope (Nikon Corporation). The average fluorescence intensity of cells was calculated by Image J software. The fluorescence intensity of at least 200 cells was calculated in each group.

Preparation of DOX Loaded NS: NS samples were diluted with buffer solution (pH=7.6, 1 mM EDTA, 20 mM Tris-HCl, 8 mM Mg^{2+}) to a final concentration of 1 μM (100 μL), and then 1 μL of DOX·HCl solution (10 mM in Millipore water) was added. The samples were vortexed briefly, sealed, and incubated overnight at room temperature in the absence of light, and the excess DOX·HCl was then purified three times using Amicon filtering tubes 3 kDa (Amicon bioseparations-Millipore U.S.A.).

Determination of the release kinetics of DOX from NS-1/DOX: For control group, NS-1/DOX (1 μM for NS-1, 1 mL) was added into a 3.5 kDa dialysis bag. For DNase I treated group, NS-1/DOX (1 μM for NS-1, 1 mL) and DNase I (50 U/μL, 2 μL) were added into a 3.5 kDa dialysis bag. Two dialysis bags were placed in 50 ml dialysate (pH=7.6, 20 mM Tris-HCl, 8 mM $MgCl_2$, 1 mM $CaCl_2$) and incubated at 37 ^oC. 200 μL dialysate was taken every other time and added equal volume fresh dialysate. The fluorescence intensity at 560 nm was measured in a microplate reader (SpectraMax M2, Molecular Devices).

Cellular Uptake of NS-1/DOX Analyzed by Confocal Laser Scanning Microscopy: DOX loaded NS-1 with FAM-labelled was prepared using the method described above. 10,000 HepG2 cells seeded on a laser confocal culture plate and cultured overnight. Then removed culture medium, the cells were incubated with fresh medium containing DOX-encapsulated NS-1 with FAM-labelled (equivalent FAM concentration: 500 nM) at 37 ^oC for 12 hours, and then the solutions were removed and the cells were carefully washed thrice with PBS. Confocal microscopy analysis of those cells was carried out with a Nikon A1R inverted laser-scanning confocal microscope (Nikon Corporation).

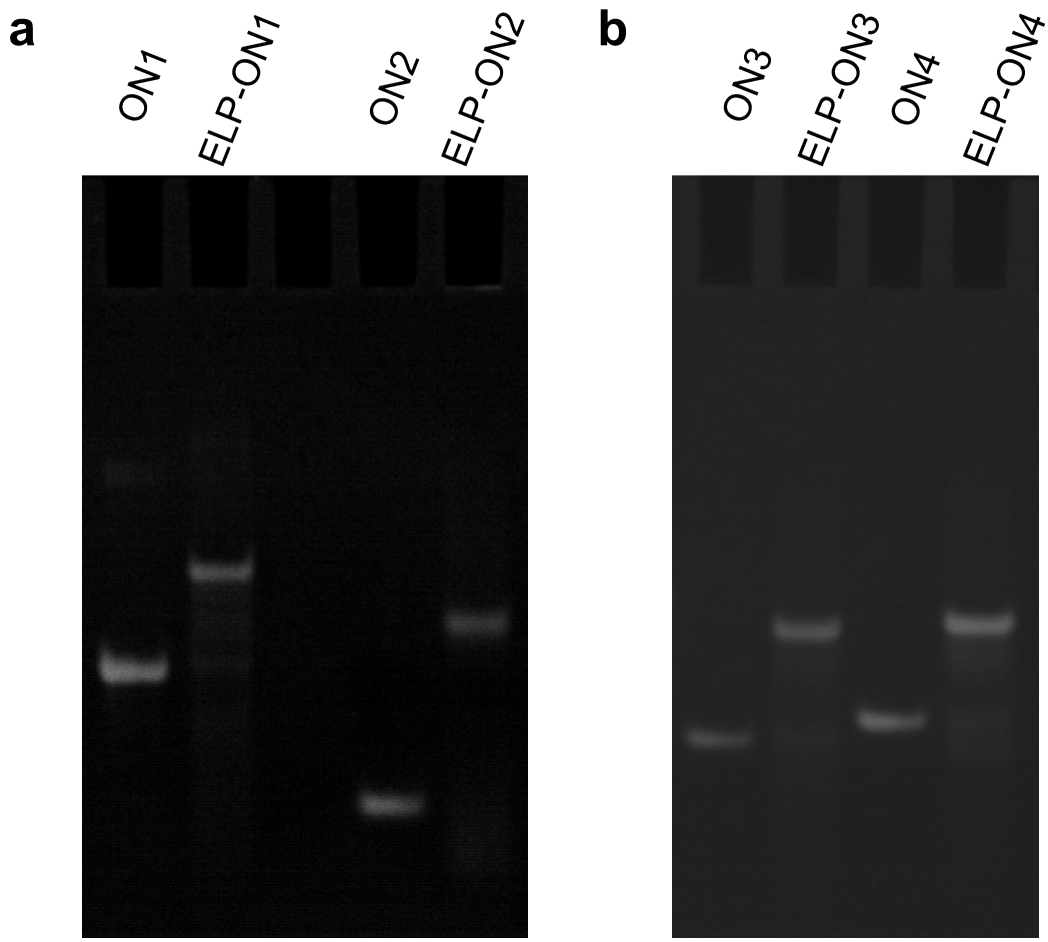

Figure S1. Analysis of oligonucleotides and relevant ELP-ON conjugates in 10 % polyacrylamide gel (PAGE).

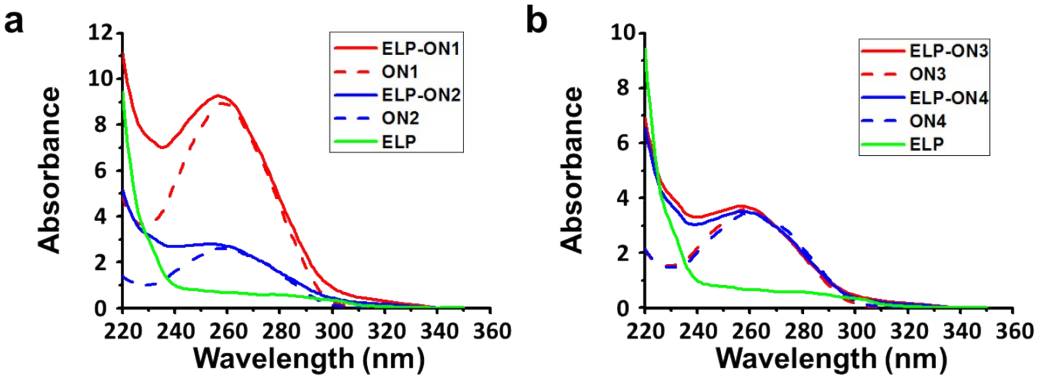

Figure S2. UV absorption spectrum of oligonucleotides and relevant ELP-ON conjugates.

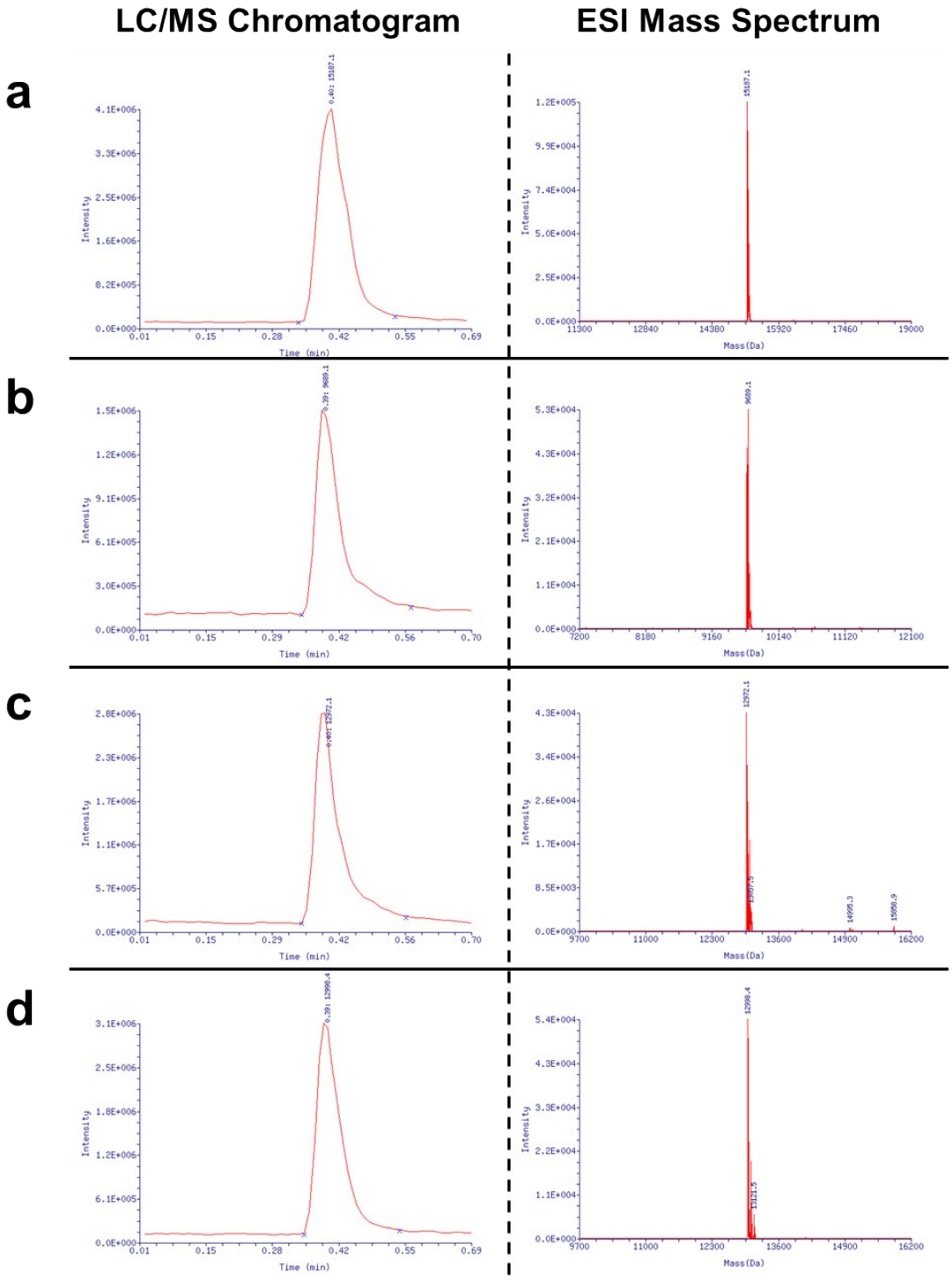

Figure S3. LC-MS date of ELP-ON conjugates. (a) ELP-ON1 (calculating mass: 15189.2, observed mass: 15187.1). (b) ELP-ON2 (calculating mass: 9668.8, observed mass: 9689.1). (c) ELP-ON3 (calculating mass: 12973.8, observed mass: 12972.1). (d) ELP-ON4 (calculating mass: 12997.8, observed mass: 12998.4).

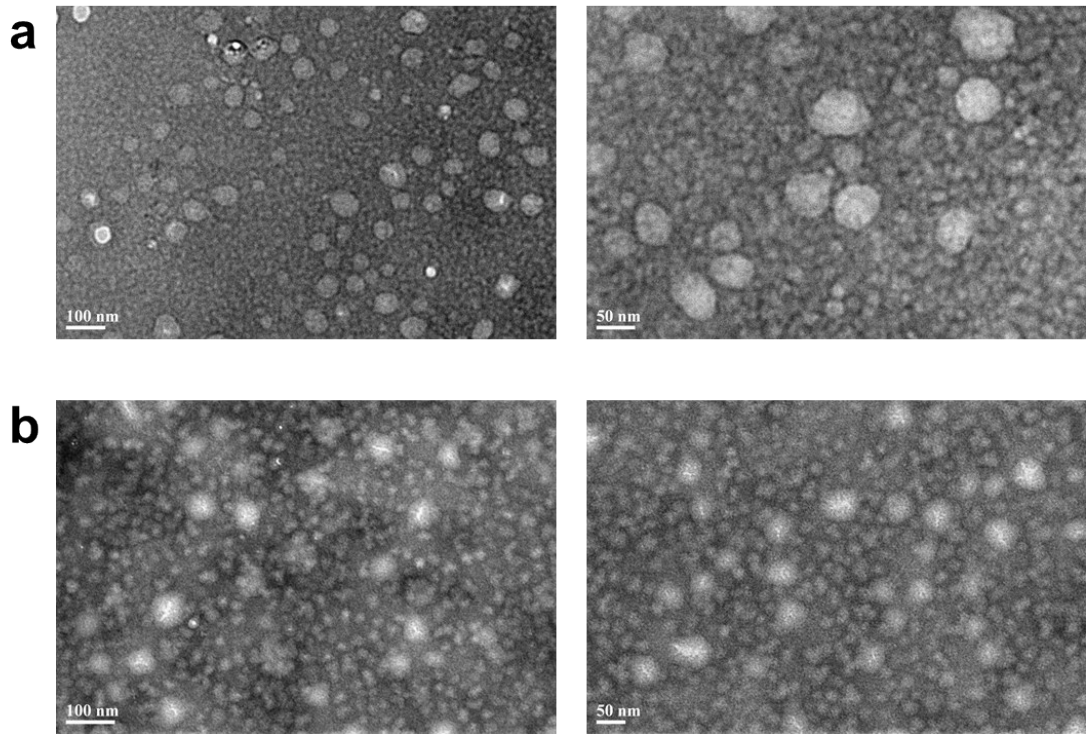

Figure S4. TEM images of NSs at room temperature after negative staining with phosphotungstic acid. (a) NS-1, (b) NS-2.

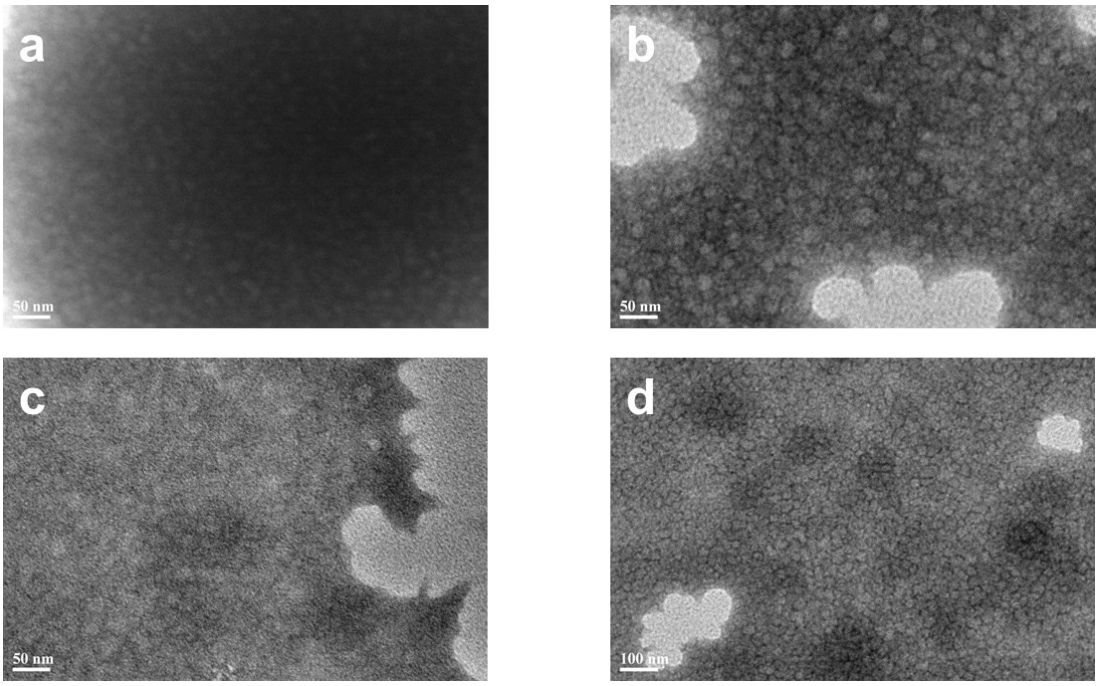

Figure S5. TEM images of single-stranded ELP-ON conjugates after negative staining with phosphotungstic acid. (a) ELP-ON1, (b) ELP-ON2, (c) ELP-ON3, (d) ELP-ON4.

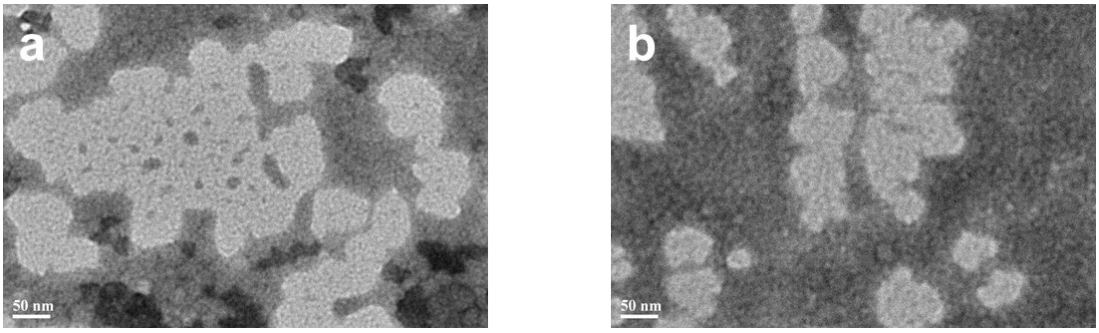

Figure S6. TEM images of non-complementary ELP-ON conjugates after negative staining with phosphotungstic acid. (a) ELP-ON5, (b) ELP(ON2-ON5) mixture.

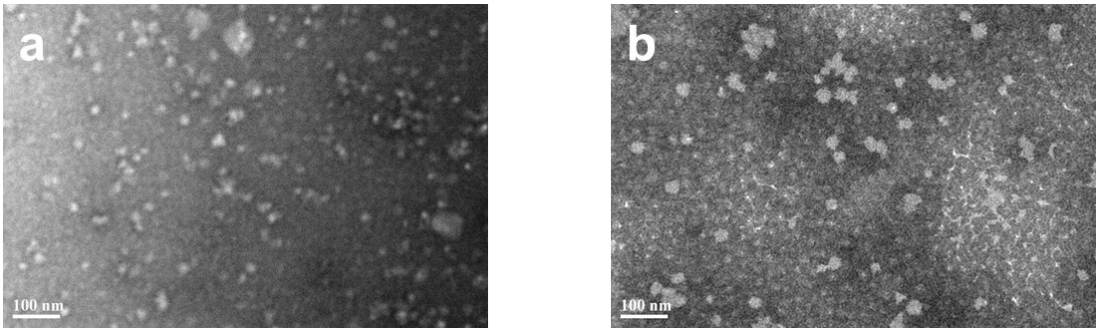

Figure S7. TEM images of NSs incubated with nucleic acid denaturant formamide after negative staining with phosphotungstic acid. (a) NS-1, (b) NS-2.

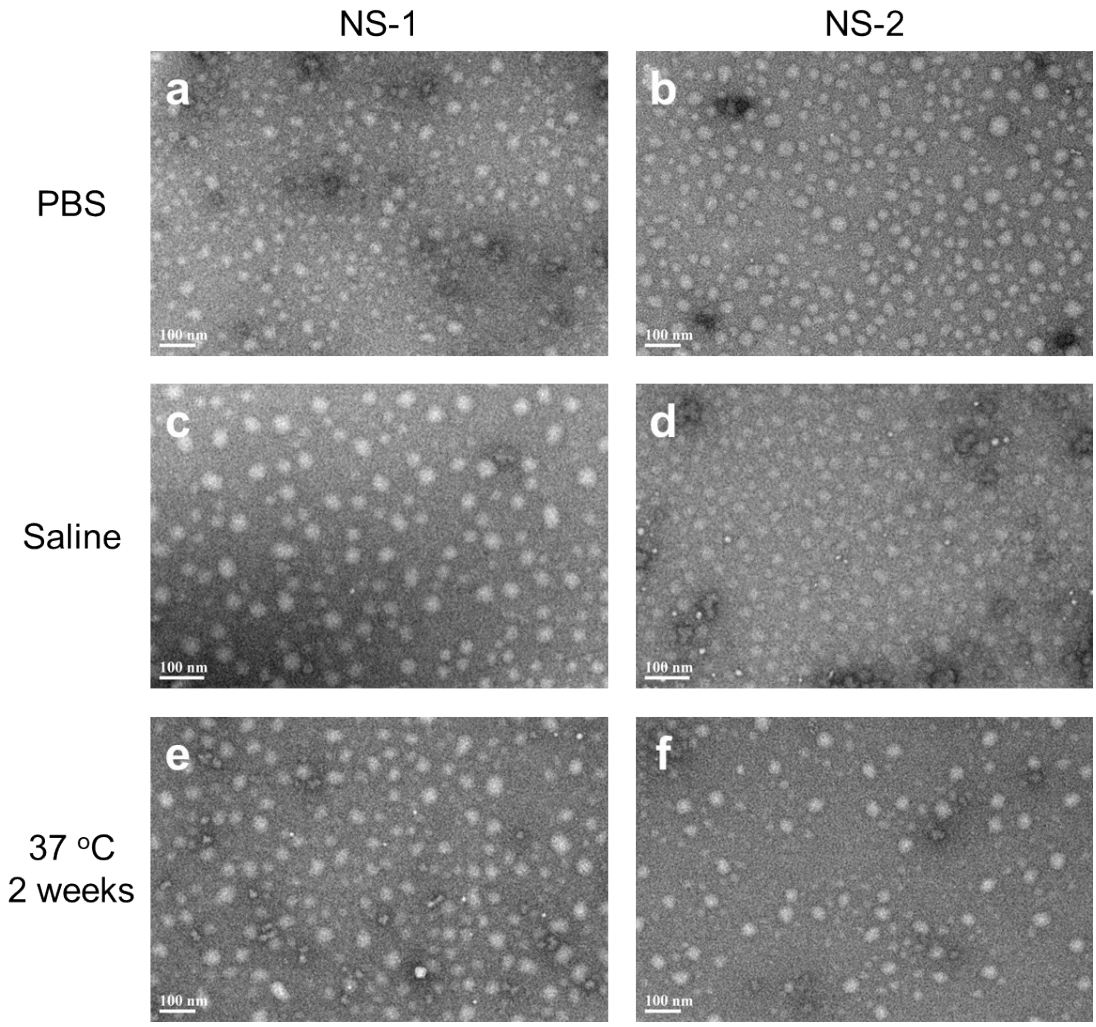

Figure S8. Stability analysis of NSs confirmed by TEM imaging. (a) NS-1 in PBS solution, (b) NS-2 in PBS solution, (c) NS-1 in physiological saline solution, (d) NS-2 in physiological saline solution, (e) NS-1 at 37 °C for 2 weeks, (f) NS-2 at 37 °C for 2 weeks.

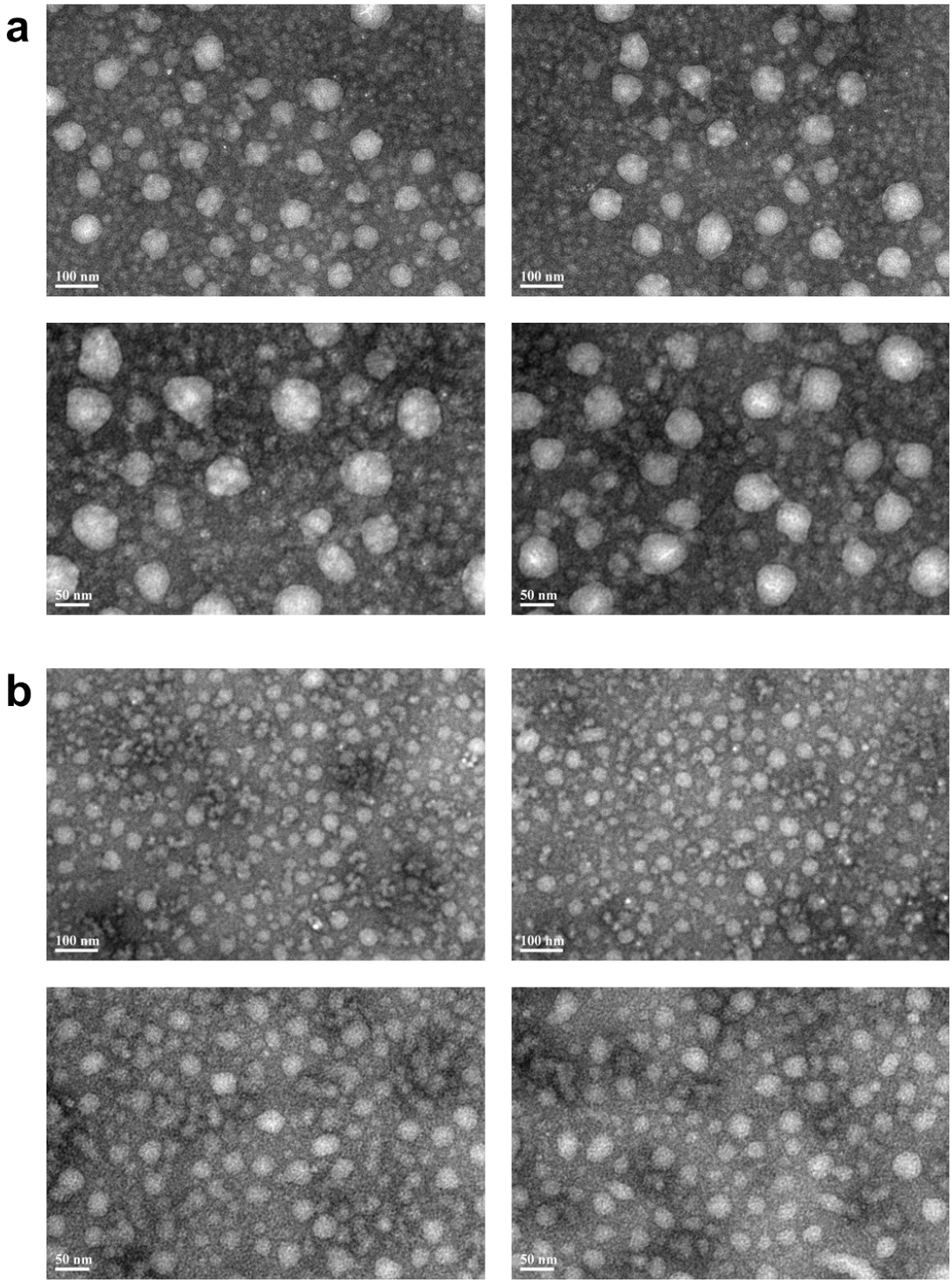

Figure S9. Additional TEM images of NS-1 at different temperatures after negative staining with phosphotungstic acid. (a) 20 ^{o}C, (b) 60 ^{o}C.

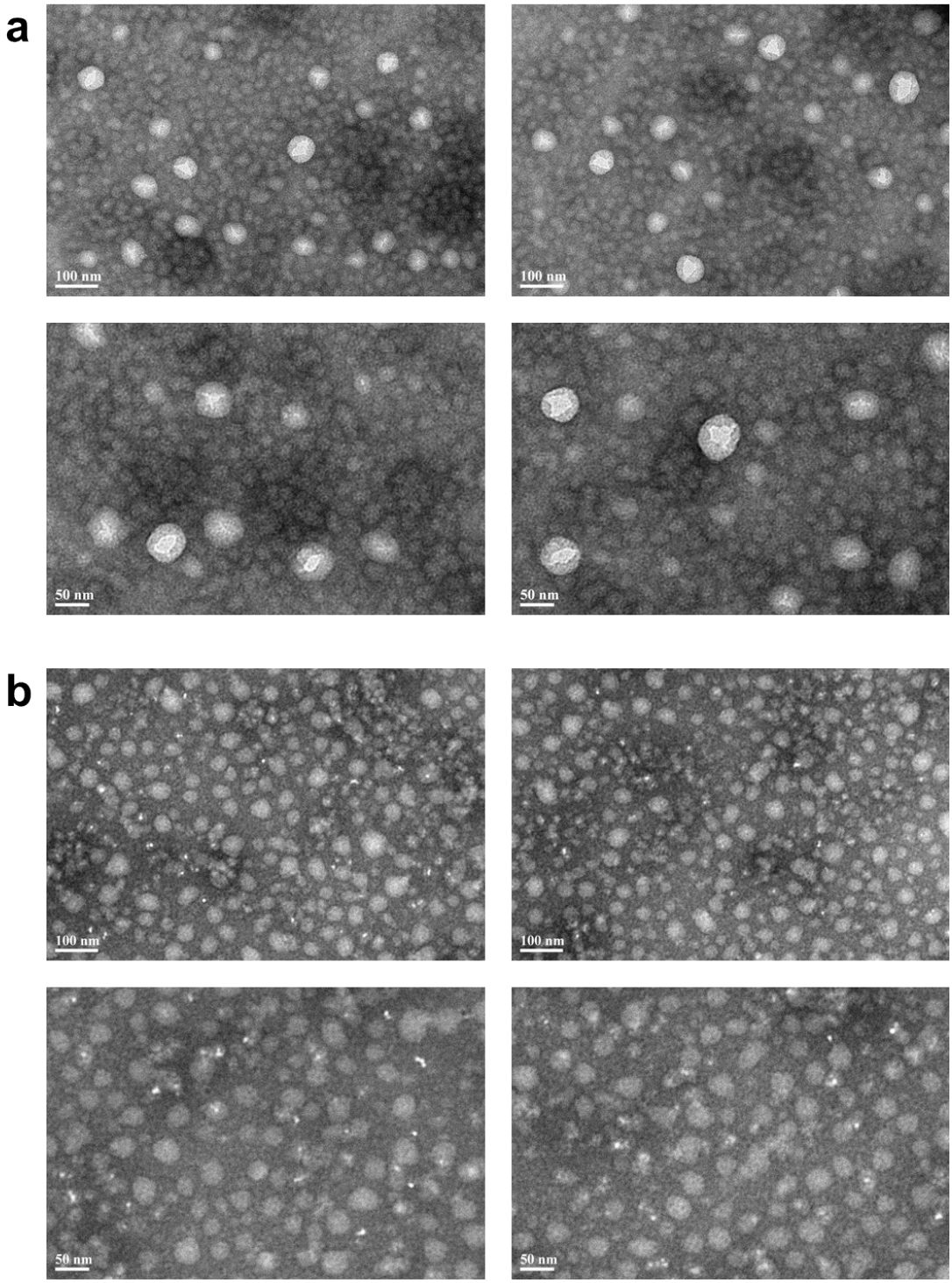

Figure S10. TEM images of NS-2 at different temperatures after negative staining with phosphotungstic acid. (a) 20 ^{o}C, (b) 60 ^{o}C.

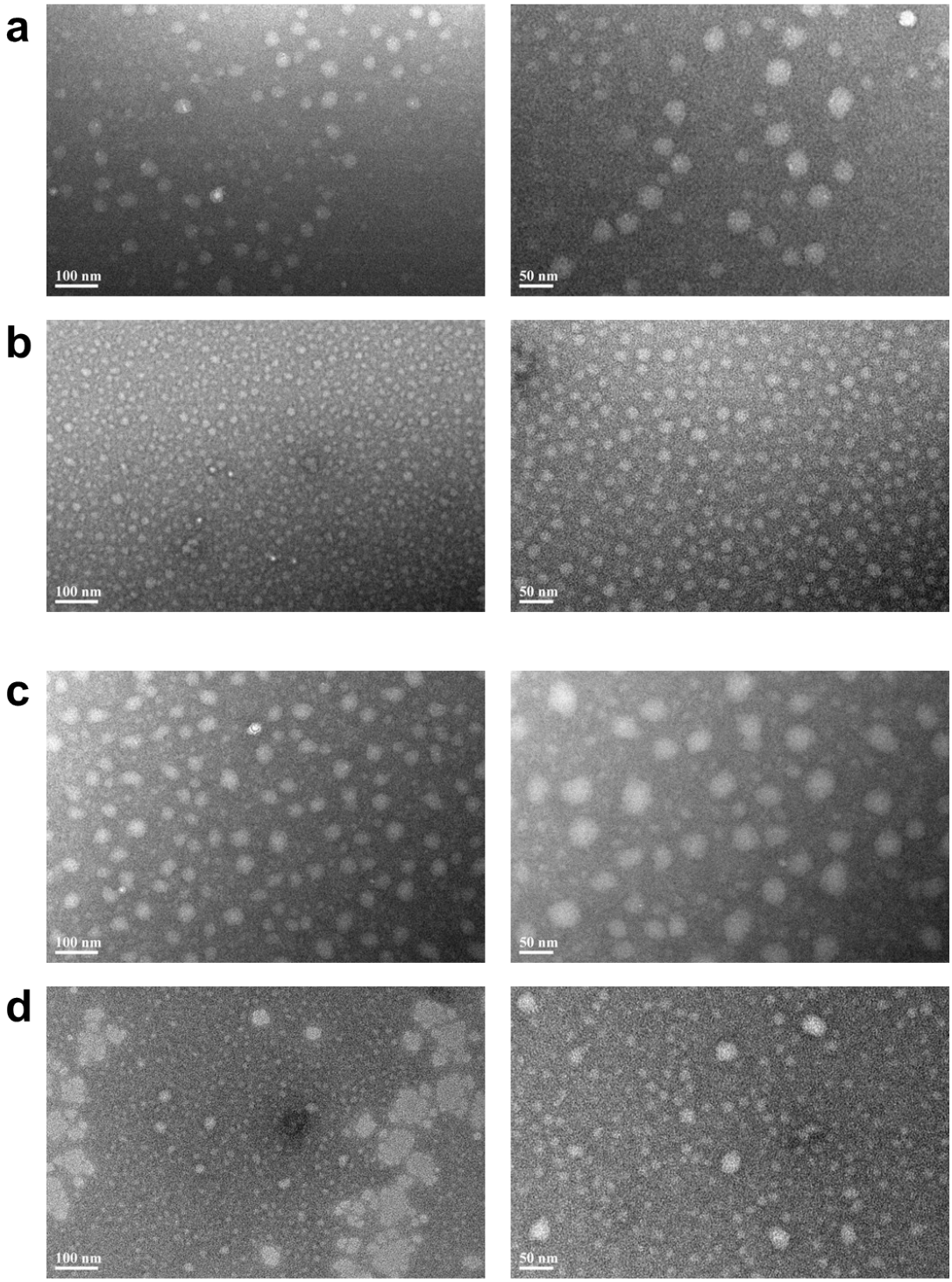

Figure S11. TEM images of NSs in 2 mM Mg^{2+} at different temperatures after negative staining with phosphotungstic acid. (a) NS-1 at 20 ^{o}C, (b) NS-1 at 60 ^{o}C, (c) NS-2 at 20 ^{o}C, (d) NS-2 at 60 ^{o}C.

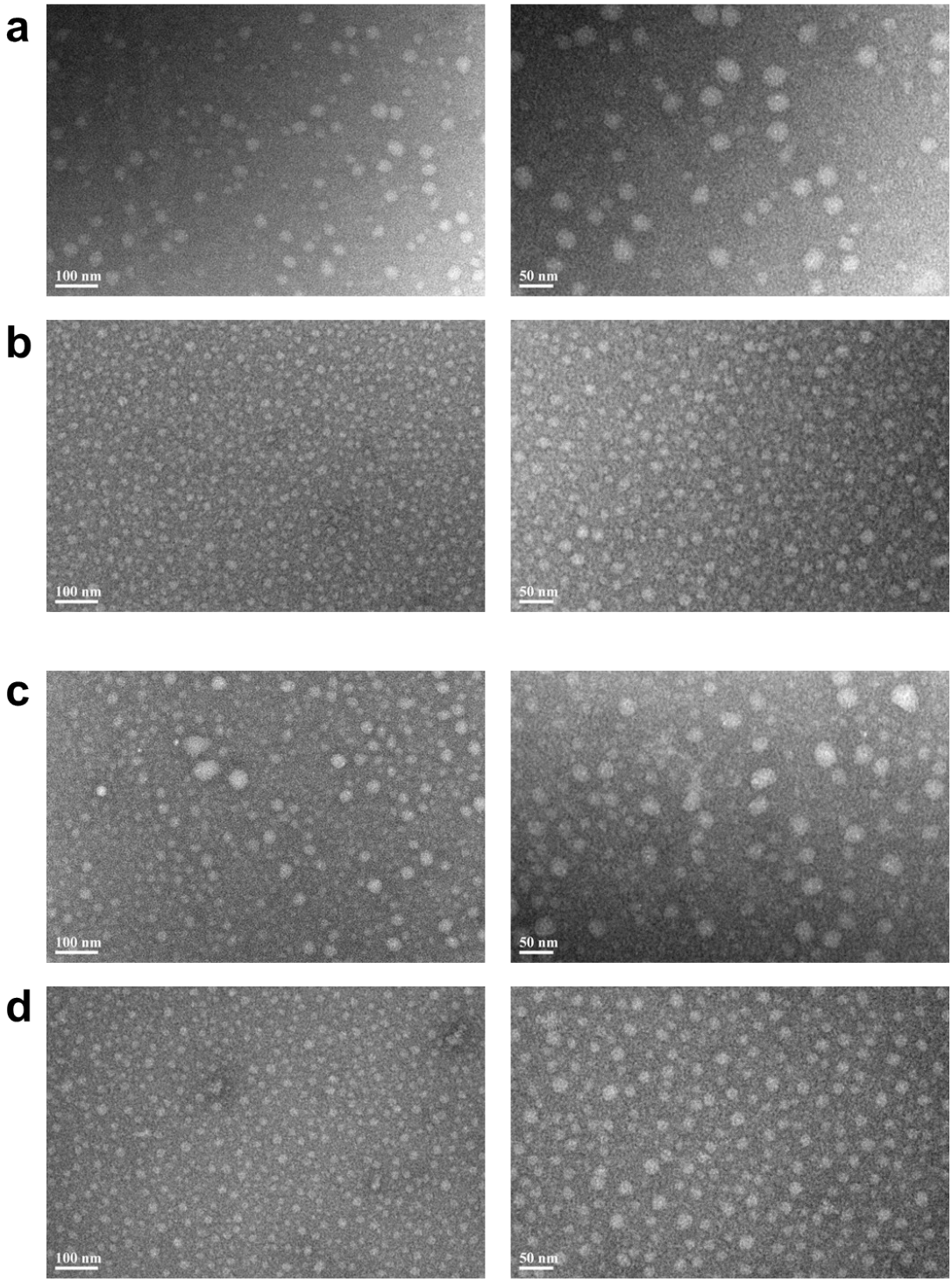

Figure S12. TEM images of NSs in 25 mM Mg^{2+} at different temperatures after negative staining with phosphotungstic acid. (a) NS-1 at 20 ^{o}C, (b) NS-1 at 60 ^{o}C, (c) NS-2 at 20 ^{o}C, (d) NS-2 at 60 ^{o}C.

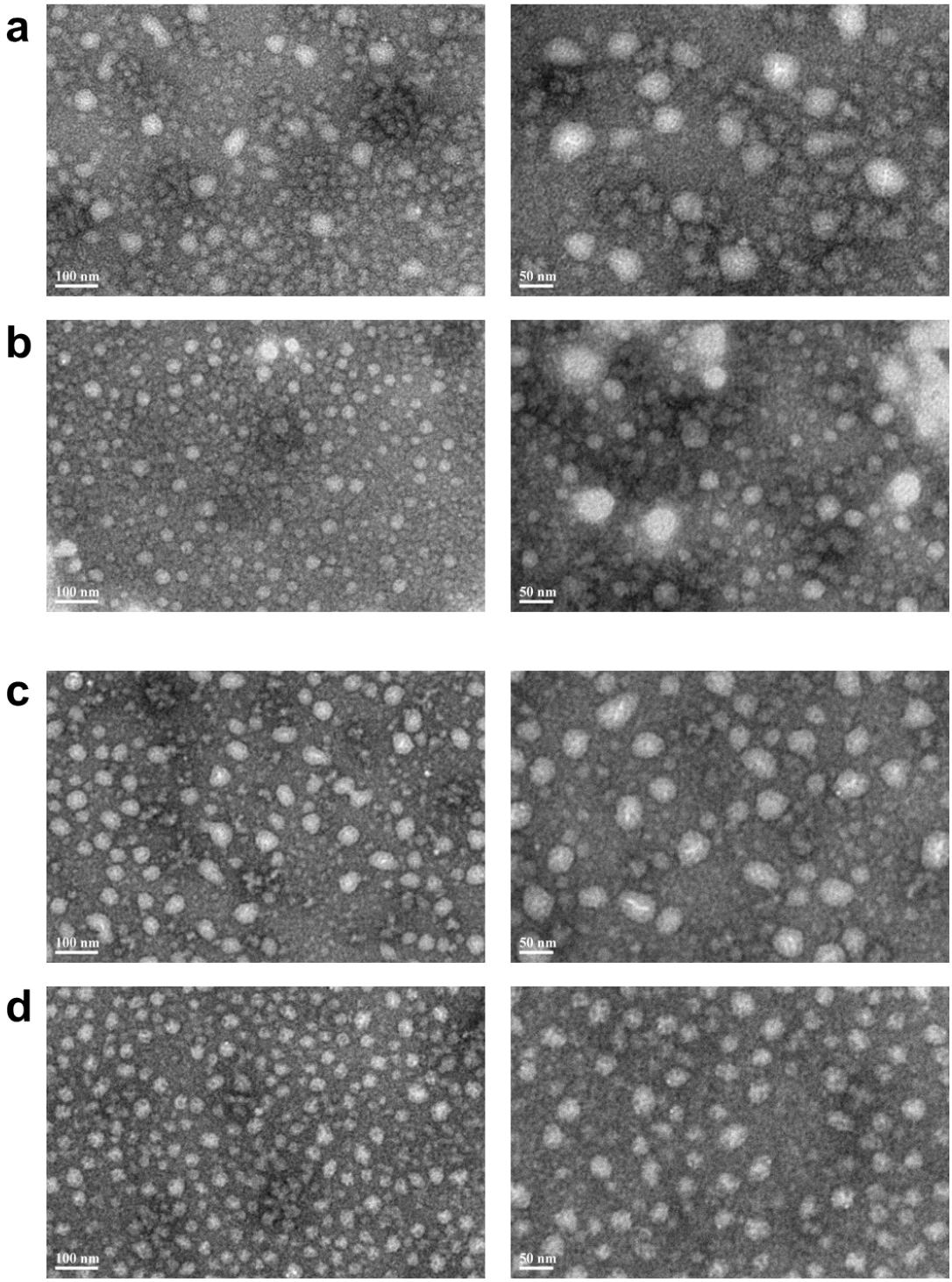

Figure S13. TEM images of NSs in the absence of Mg^{2+} at different temperatures after negative staining with phosphotungstic acid. (a) NS-1 at 20 ^{o}C, (b) NS-1 at 60 ^{o}C, (c) NS-2 at 20 ^{o}C, (d) NS-2 at 60 ^{o}C.

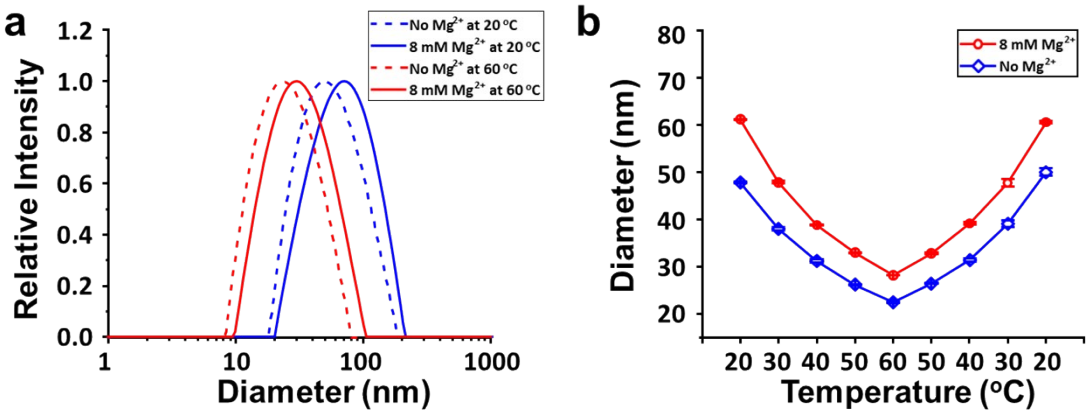

Figure S14. Temperature-dependent size regulation of NS-1 in 8 mM Mg^{2+} or no Mg^{2+}. (a) DLS characterization of NS-1 at two selected temperatures: 20 °C and 60 °C separately; (b) Analysis of hydrodynamic diameter of NS-1 as a function of temperature upon heating from 20 °C to 60 °C and then gradually decreasing from 60 °C back to 20 °C. Error bars were generated as the standard error of the mean from at least three replicates.

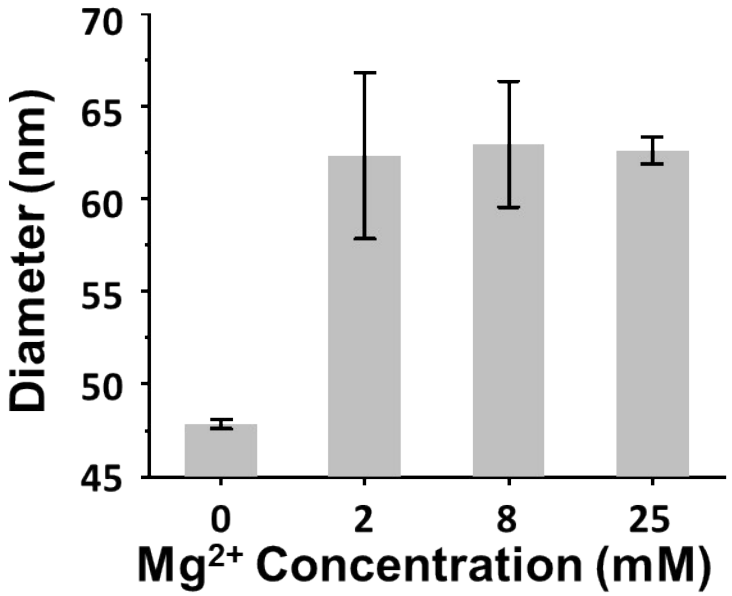

Figure S15. Analysis of hydrodynamic diameter of NS-1 in aqueous solution containing 0, 2 mM, 8 mM, 25 mM Mg^{2+}. Error bars were generated as the standard error of the mean from at least three replicates.

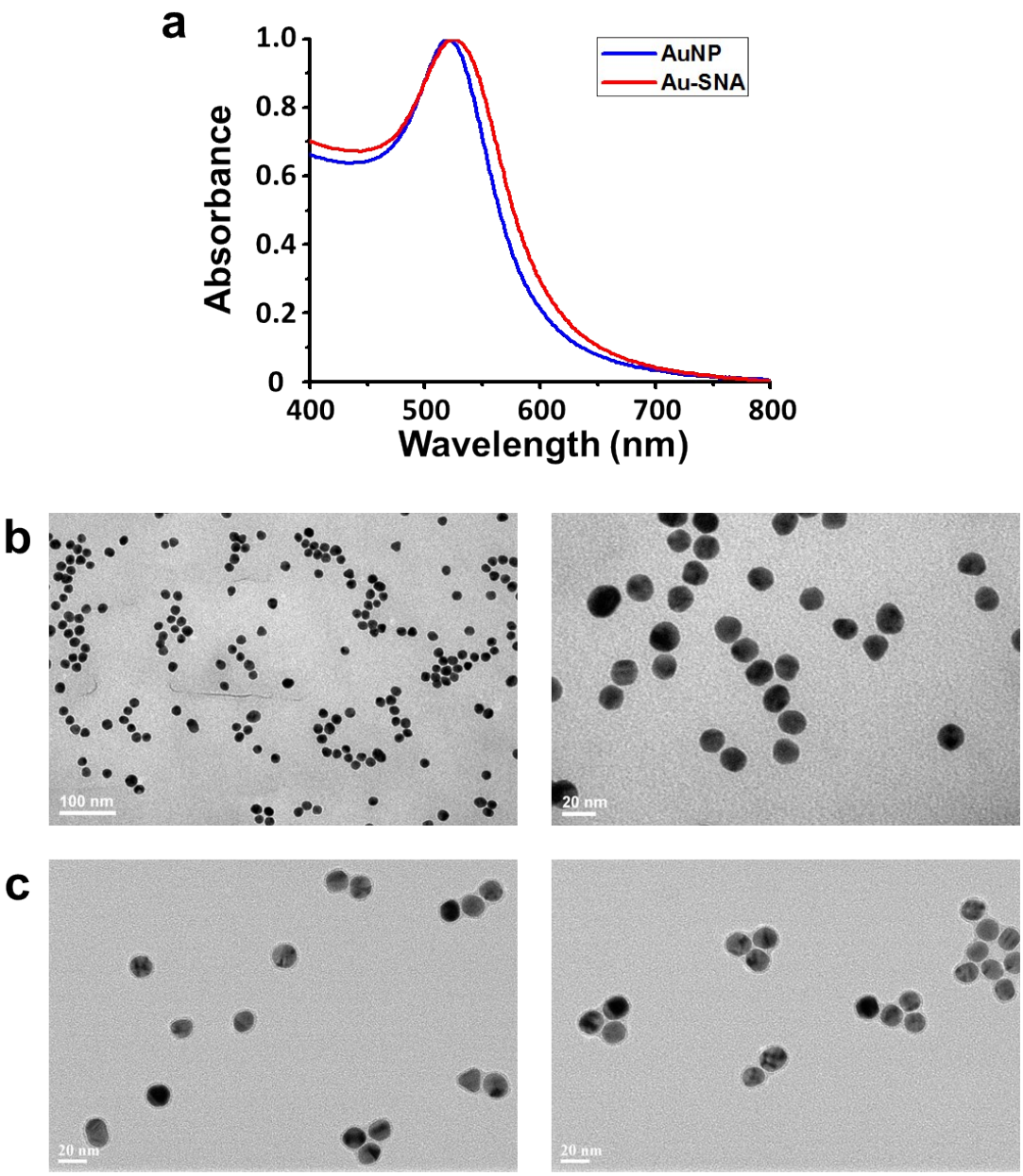

Figure S16. Characterization of AuNP and Au-SNA. (a) UV-Vis absorption spectrum of AuNP and Au-SNA. TEM images of (b) AuNP, (c) Au-SNA. Au-SNA sample was negative stained with uranyl acetat.

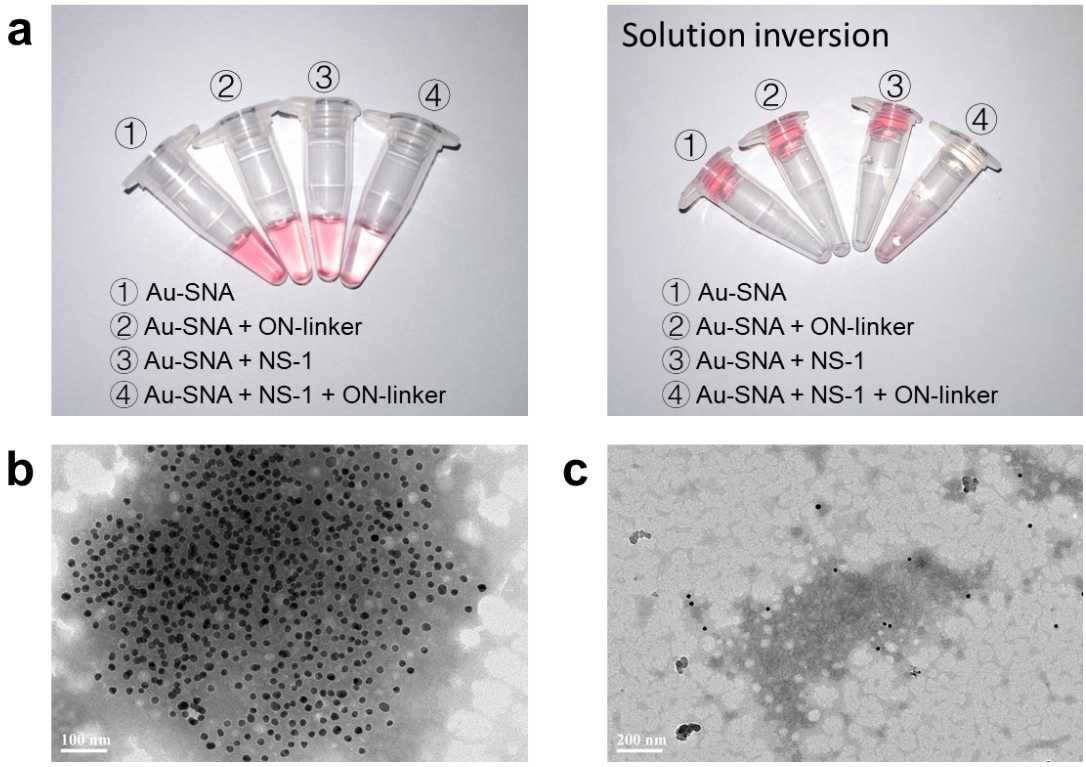

Figure S17. Characterization of Au-SNA-(NS-1). (a) Photograph of Au-SNA after incubated with NS-1 or ON-linker or NS-1 and ON-linker. (b) TEM images of AuNP-(NS-1) aggregates. (c) TEM images of NS-1 and Au-SNA mixture without ON-linker.

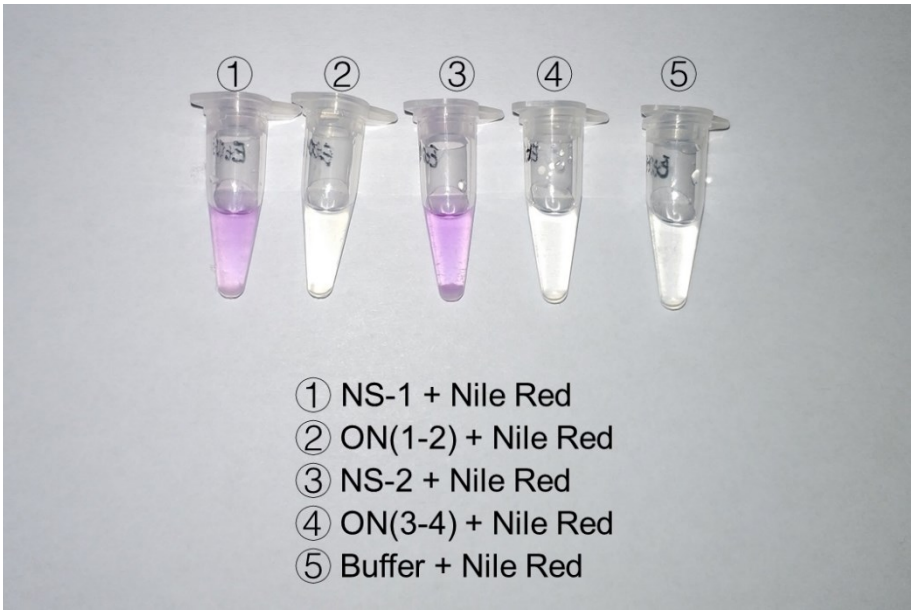

Figure S18. Photograph of NSs, relevant double stranded oligonucleotides and buffer solution after incubated with Nile Red.

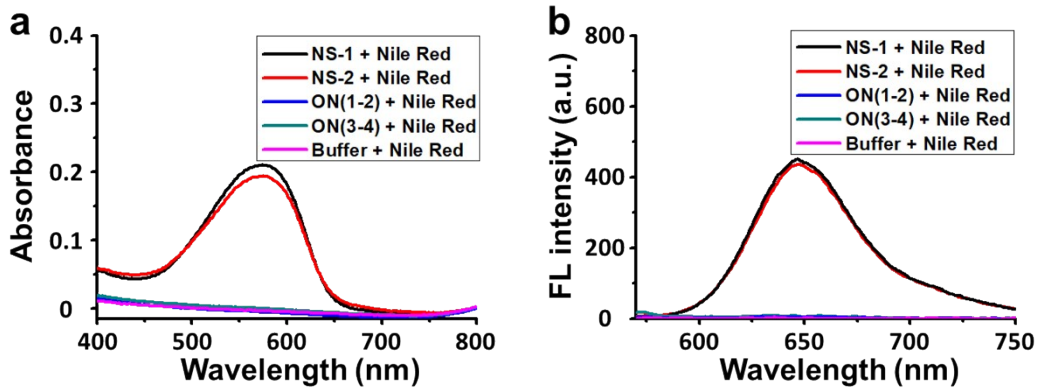

Figure S19. (a) Absorbance spectroscopy and (b) fluorescence spectroscopy of Nile Red in different solutions including NS-1, NS-2, ON(1-2), ON(3-4) and neutral buffer.

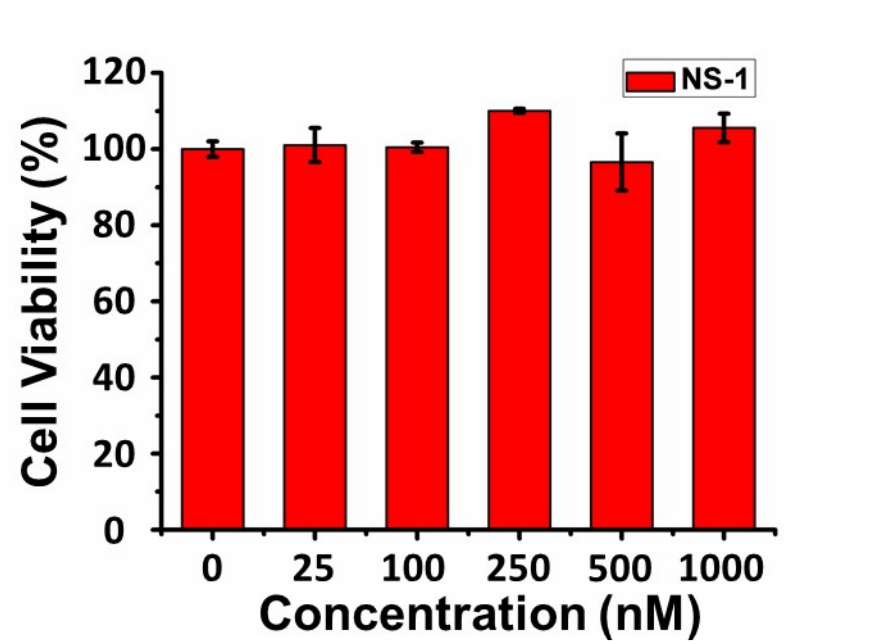

Figure S20. Cell cytotoxicity assay of HepG2 cells treated with NS-1 for 24 hours at different concentrations. Error bars were generated as the standard error of the mean from at least three replicates.

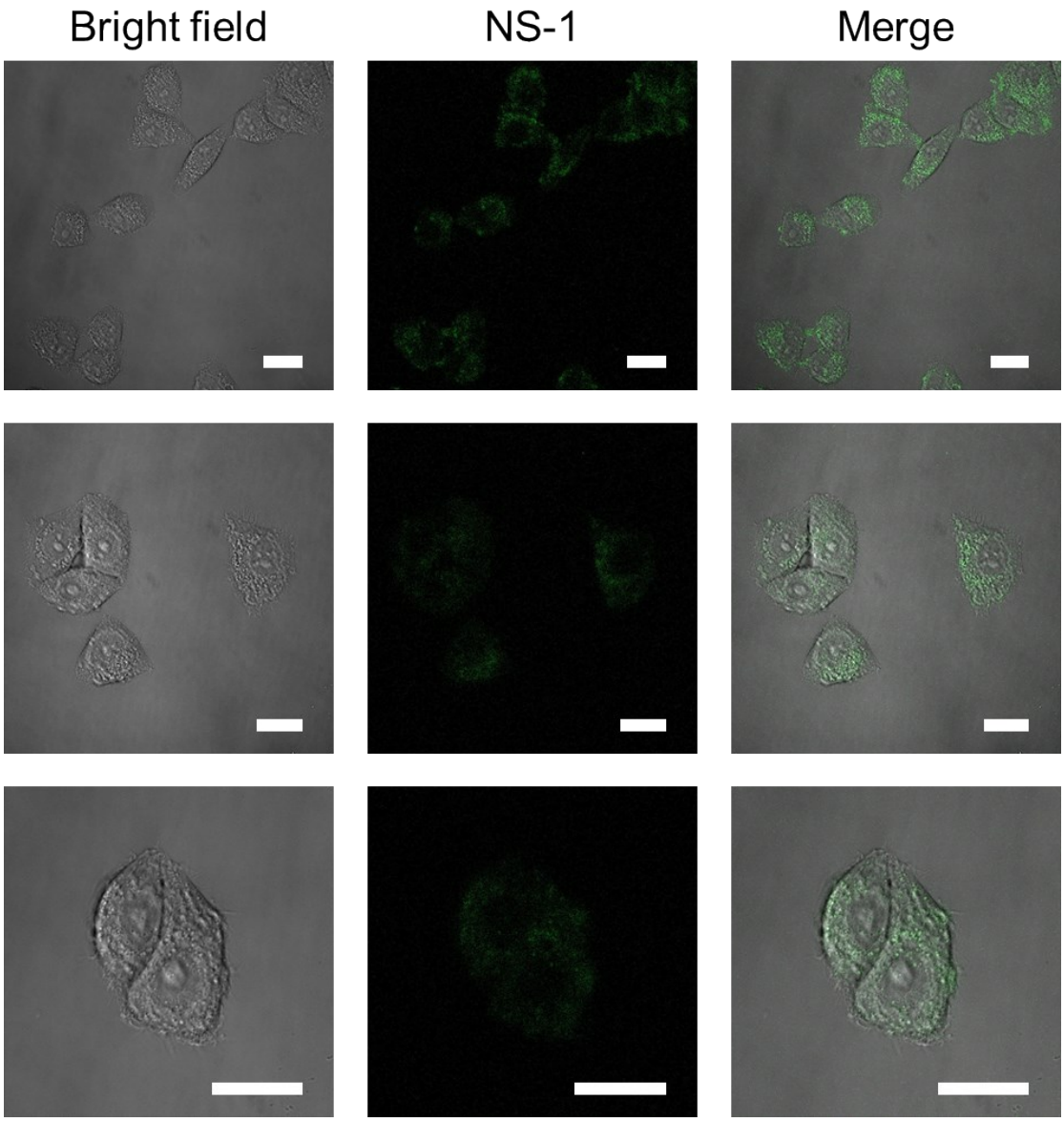

Figure S21. CLSM images of the HepG2 cells incubated with FAM-labelled NS-1 (green) for 2 hours. Scale bars are 20 μm.

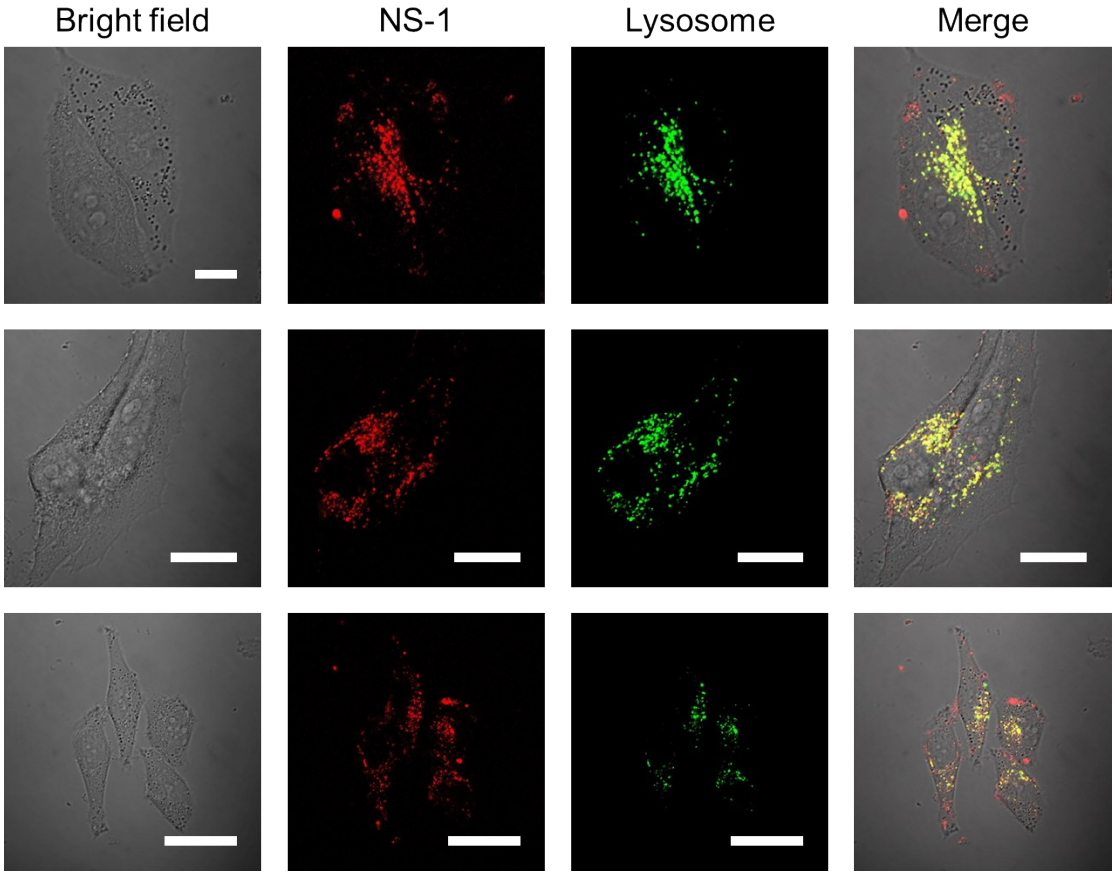

Figure S22. Colocalization study of TAMRA-labelled NS-1 (red) and lysosome (green). Scale bars are 20 μm.

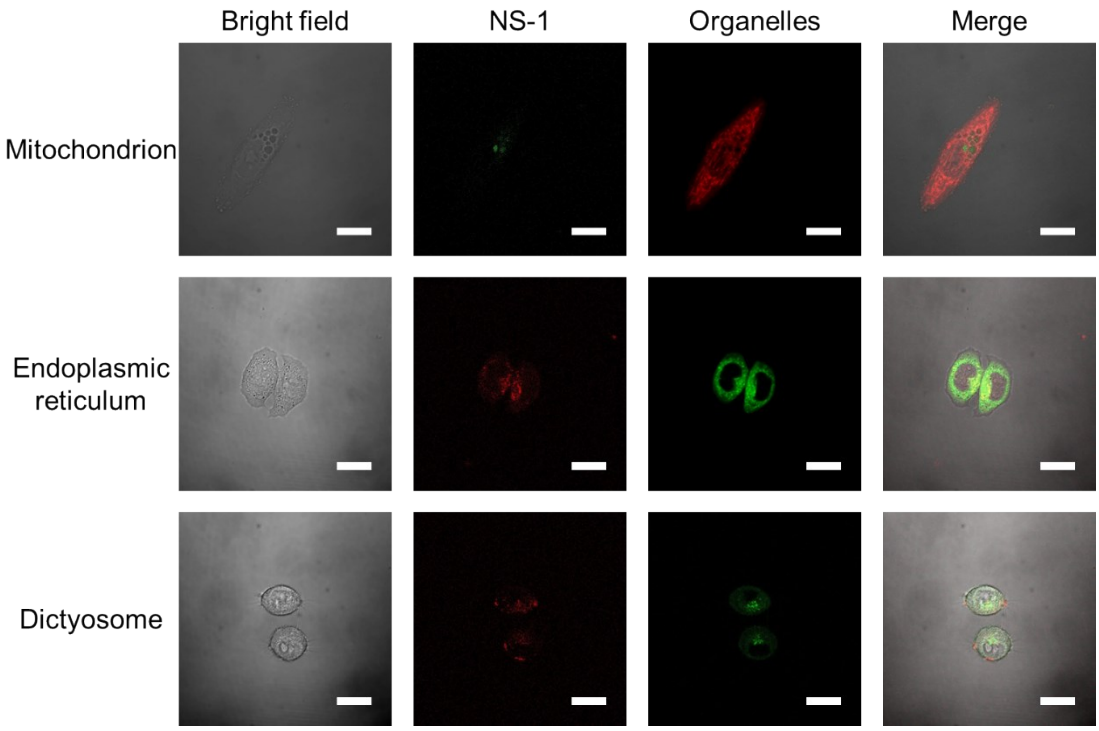

Figure S23. Colocalization study of NS-1 and other Intracellular organelles (mitochondrion, endoplasmic reticulum and dictyosome). Scale bars are 20 μm.

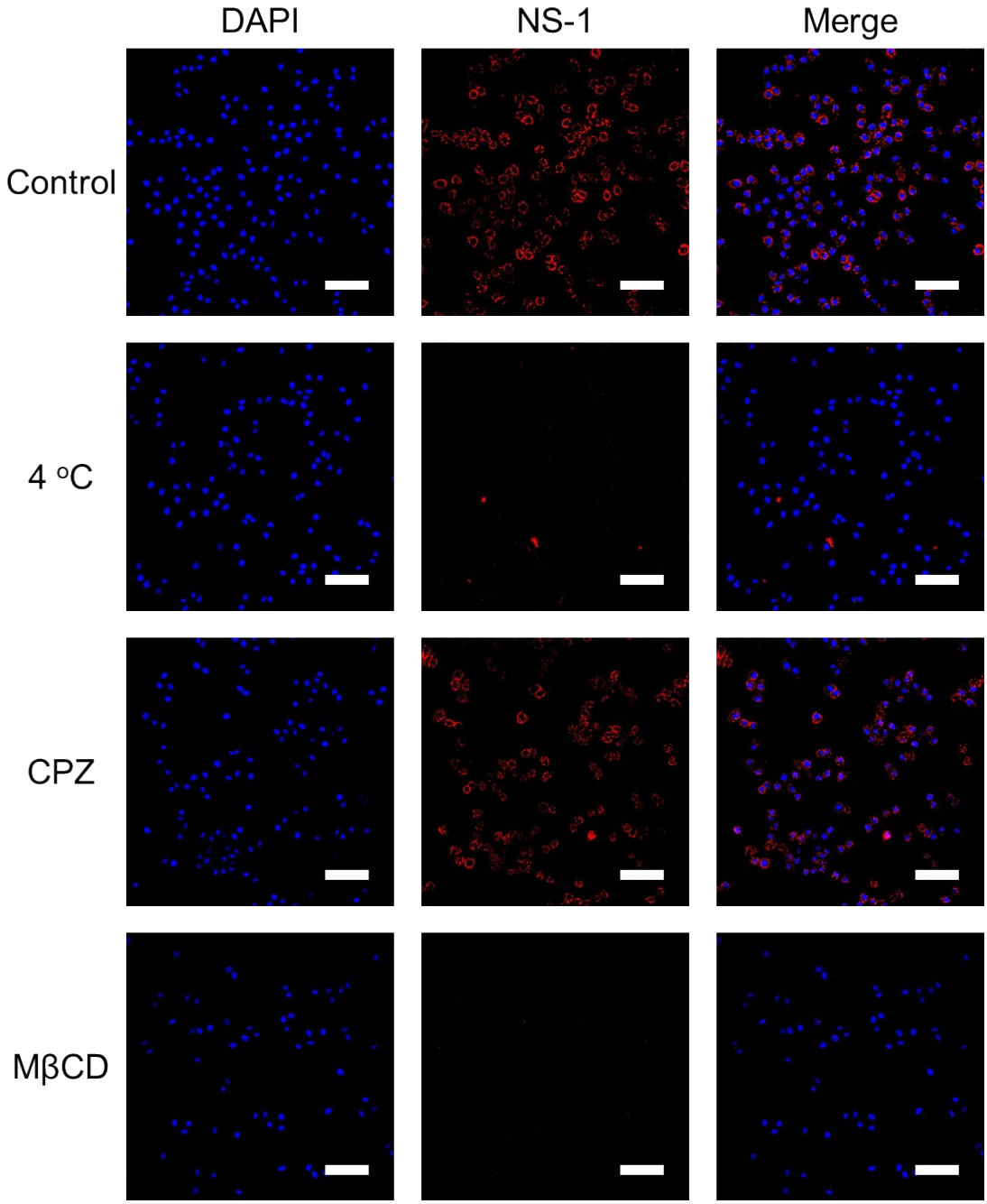

Figure S24. Additional CLSM images of endocytosis pathway of NS-1-TAMRA. HepG2 cells were treated at 4 °C, CPZ (5 μg/mL) and MβCD (12.5 mg/mL), separately. Scale bars: 100 μm.

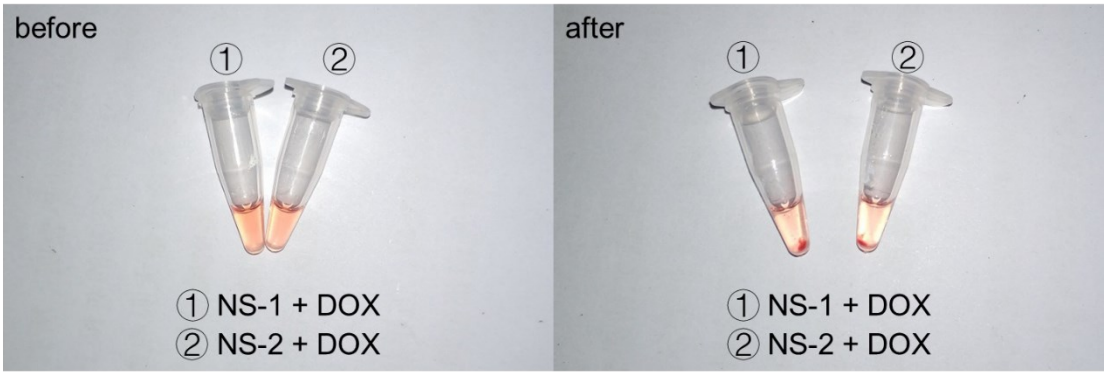

Figure S25. Photograph of NSs before and after incubated with DOX.

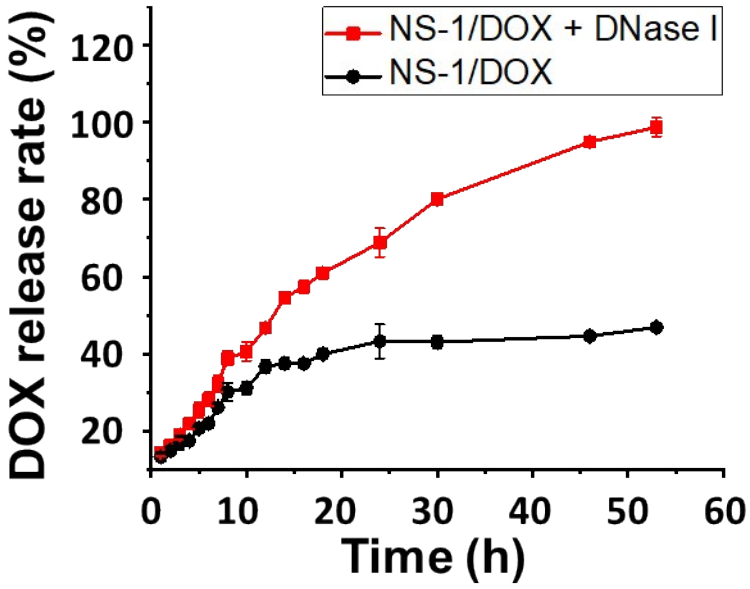

Figure S26. DOX release profiles from NS-1/DOX within 0.1 U/μL DNase I and control group.

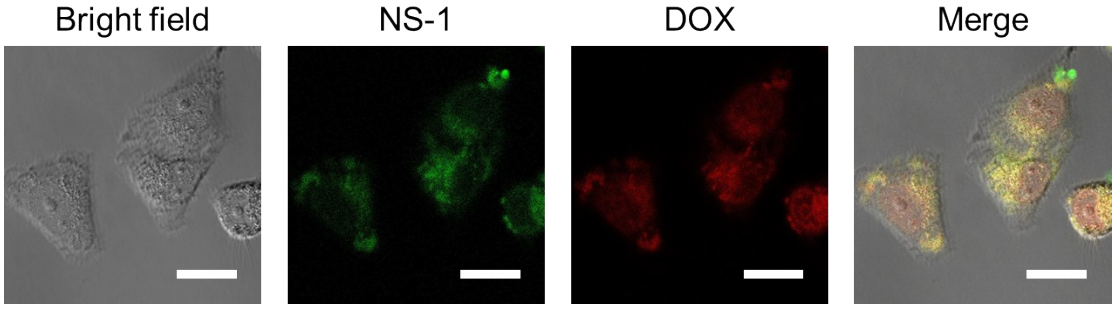

Figure S27. Additional CLSM images of the HepG2 cells incubated with DOX loaded FAM-labelled NS-1. Scale bars are 20 μm.

Additional References

1. J. Liu and Y. Lu, *Nat. Protoc.*, 2006, **1**, 246-252.

2. B. W. Liu and J. W. Liu, *J. Am. Chem. Soc.*, 2017, **139**, 9471-9474.

Table S2. Hydrodynamic diameter of NS-1 at 8 mM Mg^{2+} in different temperature

Temperature (°C)	Hydrodynamic Radius (nm)			Average Radius (nm)	Average Diameter (nm)
20	30.55	30.63	30.50	30.56	61.12
30	24.07	23.86	23.84	23.92	47.84
40	19.36	19.39	19.52	19.42	38.84
50	16.51	16.46	16.49	16.49	32.98
60	14.15	14.15	14.14	14.15	28.30
50	16.46	16.33	16.45	16.41	32.82
40	19.49	19.53	19.75	19.59	39.18
30	23.46	24.15	24.01	23.87	47.74
20	30.14	30.35	30.30	30.26	60.52

Table S3. Hydrodynamic diameter of NS-1 in the absence of Mg^{2+} in different temperature

Temperature (°C)	Hydrodynamic Radius (nm)			Average Radius (nm)	Average Diameter (nm)
20	24.06	23.86	23.83	23.92	47.84
30	19.17	18.88	19.01	19.02	38.04
40	15.62	15.78	15.52	15.64	31.28
50	13.12	13.10	13.11	13.11	26.22
60	11.43	11.22	11.22	11.29	22.58
50	13.32	13.24	13.29	13.28	26.56
40	15.56	15.83	15.87	15.75	31.50
30	19.16	19.81	19.74	19.57	39.14
20	24.68	25.41	25.00	25.03	50.06