Supporting Information for

A facile method for purifying DNA-modified small particles and soft materials using aqueous two-phase system

Xiaoman Duan, and Biwu Liu*

Institute of Analytical Chemistry and Instrument for Life Science, The Key Laboratory of Biomedical Information Engineering of Ministry of Education, School of Life Science and Technology, Xi'an Jiaotong University, Xi'an, Shaanxi 710049, P. R. China

Email: biwuliu@xjtu.edu.cn

Materials and Methods

Chemicals. All DNA samples were purchased from Shanghai Sangon Biotech (Shanghai, China). The sequences and modifications of the DNAs are shown in Table S1. Sodium chloride, and 4-(2-hydroxyethyl) piperazine-1-ethanesulfonic acid (HEPES) were from Aladdin (Shanghai, China). Phosphate-buffered saline (PBS) tablets were from Solarbio (Beijing, China). Polyethylene glycol (PEG, 20 K), dextran (1K, 2K, 10K, 20K, 40K, 70K), and 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDC) were purchased from Macklin (Shanghai, China). Polystyrene microspheres (PS, 50-100 nm) were purchased from Aladdin (Shanghai, China). DNA Gel Extraction spin columns (cat.#732-6166) were from Bio-Rad. Gold nanoparticles of 13 nm and 5 nm were prepared using the citrate method[1] and the $NaBH_4$ reduction method[2], respectively. Liposome composed of 1,2-dioleoyl-sn-glycero-3-phosphocholine (DOPC) was kindly provided by Prof. Feng Wang at Hefei University of Technology. Milli-Q water was used for all experiments.

Instrumentation. The fluorescent spectra were recorded using a Fluoromax-4C-L fluorescence spectrometer (Horiba). The fluorescent intensity was measured by a Tecan microplate reader (Infinite E Plex). Photographs were taken with a cell phone in a dark room with LED excitation (Safe Imager Blue-Light Transilluminator, Invitrogen). Electrophoresis was conducted on a PowerPac Basic system (Bio-Rad, USA) and imaged by a Syngene G: BOX imaging system (Syngene System, Cambridge, UK). Aqueous two-phase separation was facilitated by a benchtop minicentrifuge (DLAB, Beijing, China). Ultracentrifugation was performed using a Beckman Coulter ultracentrifuge (Optima L100XP).

Preparation of DNA conjugates. DNA-AuNP (5 nm) conjugates were synthesized by the freezing method[3]. The received thiolated DNA samples were directly dissolved in Milli-Q water without further treatment. Typically, 2 μL of HS-9A12-FAM (100 μM) was added into 100 μL of 5 nm AuNPs (34 nM). The mixture was vortexed gently and then placed in a -20 °C freezer for 2

h. After thawing at room temperature for 10 min, 4 μL of 250 mM HEPES buffer (pH 7.6) and 5 μL of 3 M NaCl were added.

DNA-modified liposome was prepared by inserting a cholesterol-labeled DNA (Chol-9T24) into the lipid bilayers. Briefly, 100 μg/mL DOPC liposome was incubated with 100 nM Chol-9T24 and 150 nM FAM-cDNA24 in PBS (1x) in a 37 °C water bath for 10 min.

To prepare DNA-tethered tetrahedron (TDN), the mixture of TDN1-9T12, TDN2, TDN3, TDN4 (final concentration for each 100 nM), and FAM-cDNA12 (final 200 nM) were incubated in 10 mM Tris-HCl buffer (pH 8.0) containing 50 mM $MgCl_2$. After 10 min heating (95 °C), the mixture was quickly placed in a 4 °C refrigerator for 2 h.

To prepare DNA-modified polystyrene (PS), 100 μL of 500 μg/mL PS, 10 μL of 100 μM DNA24-NH_2, 50 μL of EDC (100 mM, freshly prepared) and 50 μL of NaCl (3 M) were added into 25 mM MES buffer (pH 6.0), and the total volume was 500 μL. After 3 h incubation at room temperature, 15 μL of the FAM-labeled cDNA (FAM-cDNA24, 100 μM) was introduced and the mixture was further incubated for 1 h.

To modify AuNPs (13 nm) with non-thiolated DNA, 2 μL of FAM-9A12 (100 μM) was added into 100 μL of 13 nm AuNPs (10 nM). The mixture was vortexed gently, and PBS (20x) was gradually added over 5 h with a final concentration of 1x. Finally, samples were incubated overnight at room temperature.

Purification of DNA-NP conjugates by ATPS. To remove free DNA from DNA-NP conjugates using ATPS, PEG 20K and dextran 40K were introduced to the conjugates (e.g., DNA-AuNPs (5 nm)) with designed concentrations. After centrifugation with a benchtop minicentrifuge or low speed (3500 rpm) for 3 min, 90 μL of the top PEG-rich phase (total volume 100 μL) was removed. Then 90 μL of the PEG-rich solution was introduced and the mixture was vortexed for the next round of purification. DNA-conjugated liposome, TDN, and PS were purified with similar procedures.

Confocal fluorescence microscopy. Glass coverslips were pre-cleaned with water and dried with nitrogen. A polydimethylsiloxane (PDMS) mask was applied to create a hybridization chamber (4 mm in diameter) on the slides. Then 5 μL of samples was added into the chamber. The fluorescence images were captured using a confocal microscope (Leica TCS SP8 STED 3X) with a 63x water immersion objective. The FAM was excited with a 488 nm laser line and detected with a 495–545 nm band-pass filter. The images were processed by ImageJ version 1.46r software.

Agarose gel electrophoresis and purification. The TDN was analyzed using 3% agarose gel electrophoresis in 1x TBE buffer at a constant voltage of 120 V for 45 min. After dyeing with SYBR Gold for 15 min, the gel was imaged by a Syngene G: BOX imaging system. For purification of TDN, target gel bands were excised and DNA was recovered by pestle-crushing excised bands followed by centrifugation for 25 min at 6,000 rpm at 0 °C using Freeze 'N Squeeze DNA Gel Extraction spin columns (Bio-Rad). The recovered material in the flow-through was stored at 4ºC for further use.

Table S1. The sequences of the DNA samples used in this work.

DNA names	Sequences and modifications (from 5′ to 3′)
HS-9A12	HS-AAA AAA AAA CCC AGG TTC TCT
HS-9A12-FAM	HS-AAA AAA AAA CCC AGG TTC TCT-FAM
FAM-9A12	FAM-AAA AAA AAA CCC AGG TTC TCT
Chol-9T24	Chol-TTT TTT TTT CCC AGG TTC TCT TCA CAG ATG CGT
FAM-cDNA24	FAM-ACG CAT CTG TGA AGA GAA CCT GGG
TDN1-9T12	TCA ACT GCC TGG TGA TAA AAC GAC ACT ACG TGG GAA TCT ACT ATG GCG GCT CTT CTT TTT TCC CAG GTT CTC T
TDN1-TR	TCA ACT GCC TGG TGA TAA AAC GAC ACT ACG TGG GAA TCT ACT ATG GCG GCT CTT C
TDN2	TTC AGA CTT AGG AAT GTG CTT CCC ACG TAG TGT CGT TTG TAT TGG ACC CTC GCA T
TDN3	TAT CAC CAG GCA GTT GAC AGT GTA GCA AGC TGT AAT AGA TGC GAG GGT CCA ATA C
TDN4	ACA TTC CTA AGT CTG AAA CAT TAC AGC TTG CTA CAC GAG AAG AGC CGC CAT AGT A
FAM-cDNA12	FAM- AGA GAA CCT GGG
DNA24-NH_2	CCC AGG TTC TCT TCA CAG ATG CGT TTT TTT TTT T-NH_2
cDNA24	ACG CAT CTG TGA AGA GAA CCT GGG

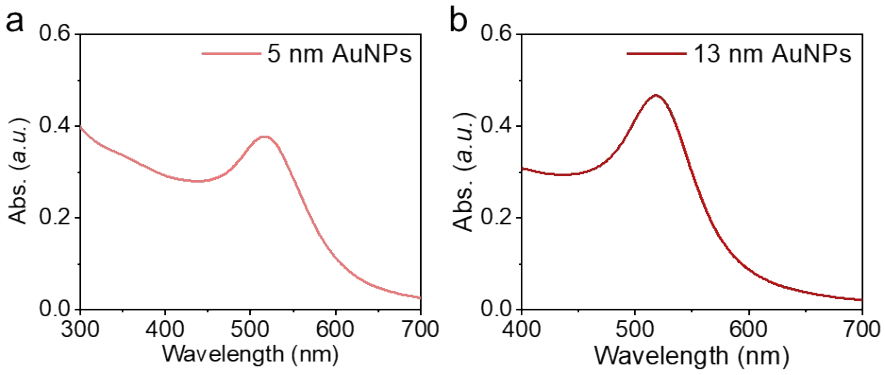

Figure S1. The absorption spectrum of (a) 5 nm and (b) 13 nm AuNPs. The absorption peak of 5 nm AuNPs is 517 nm and that of 13 nm AuNPs is 520 nm. The NP concentration was calculated to be 34 nM (5 nm) and 9 nM (13 nm).

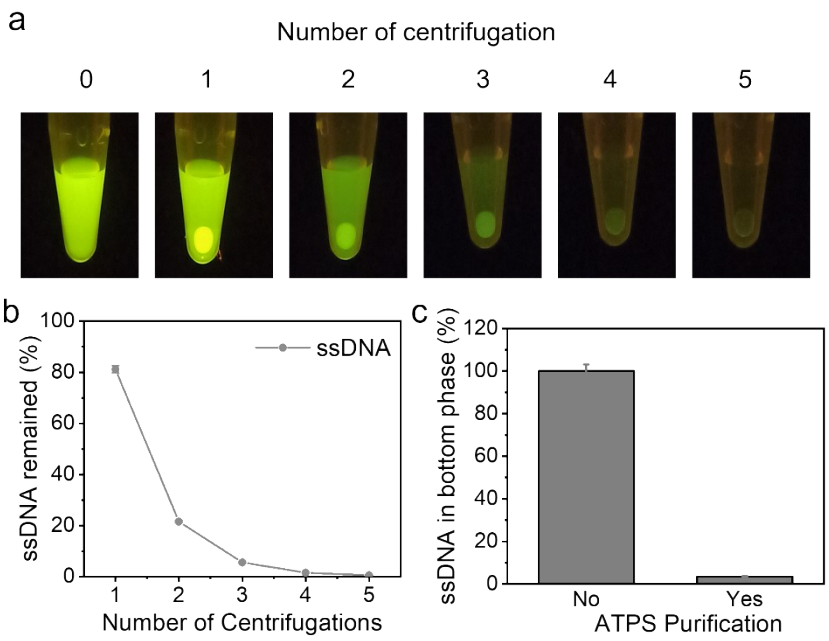

Figure S2. (a) Fluorescence images of 100 nM ssDNA (FAM-9A12) in 10 mM HEPES (pH 7.6) after each round of centrifugation with the help of ATPS. The amount of DNA that remained in the top PEG-rich phase was quantified and plotted in (b). The concentration in the bottom dextran-rich phase before ATPS and after four times washing was shown in (c). Initially, ssDNA can also accumulate in the bottom dextran-rich phase. However, after each round of washing, the partitioning would achieve a new equilibrium with less ssDNA in the bottom dextran-rich phase. Overall, after four times washing no ssDNA was left.

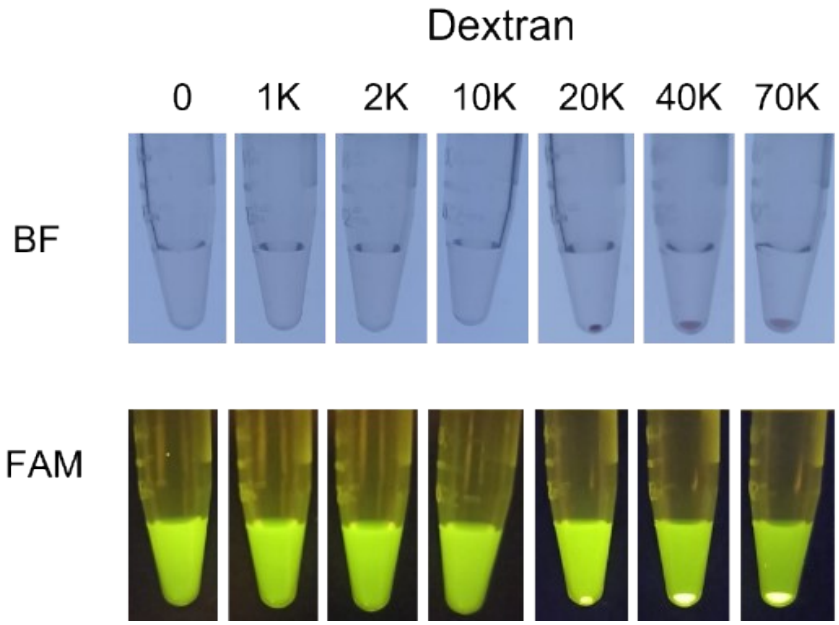

Figure S3. Images of DNA-AuNPs in 10 mM HEPES (pH 7.6, 150 mM NaCl) in the presence of PEG 20K (10%, w/v) and dextran (1%, w/v) of different molecular weights. For low MW dextran (from 1K to 10K), no phase separation occurred. For higher MW dextran (e.g., dextran 20K), there were two phases. The bottom dextran-rich phase had a red color with a stronger fluorescence, indicating the accumulation of DNA-AuNPs in the bottom phase.

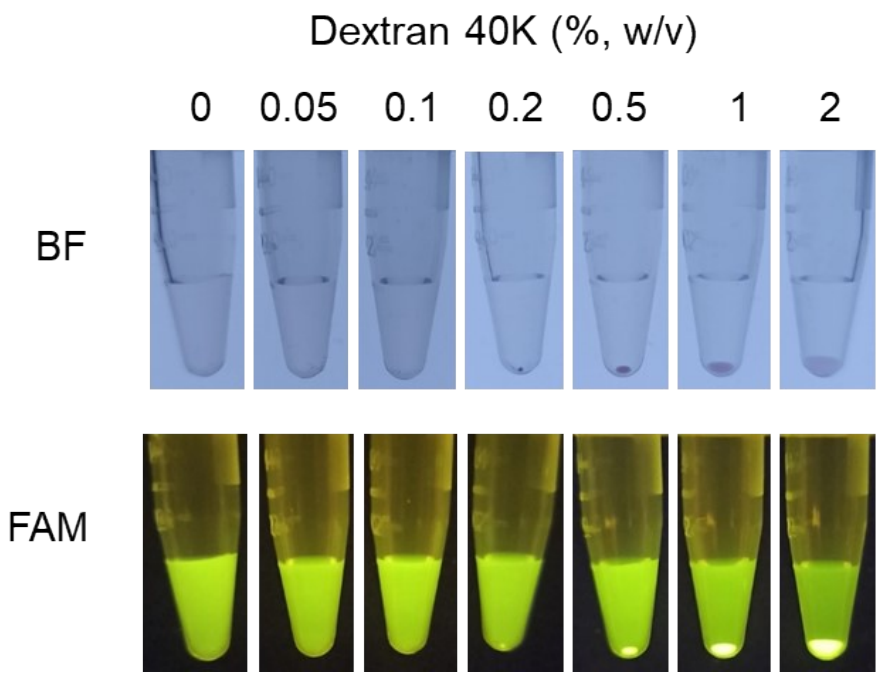

Figure S4. Effect of dextran concentration on the separation of ssDNA (HS-9A12-FAM) with DNA-AuNPs (5 nm) conjugates. No obvious phase separation was observed when dextran concentration was lower than 0.2%.

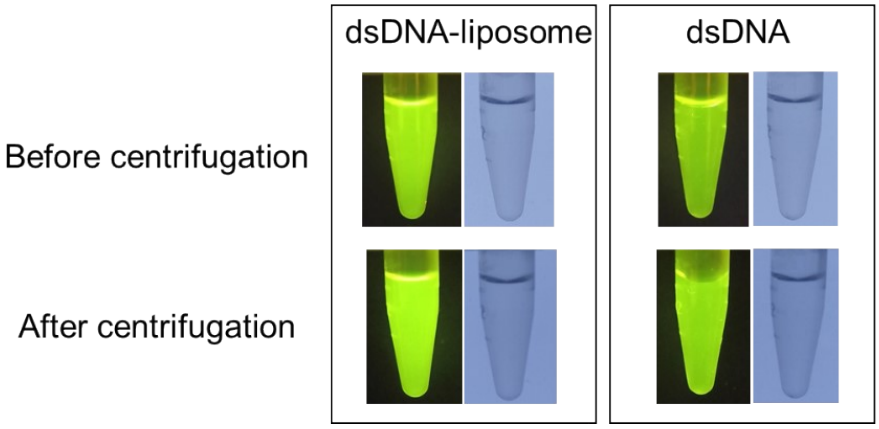

Figure S5. Images showing the effect of high-speed centrifugation (14,000 rpm, 10 min, 20 ˚C) on separating a mixture of 100 nM dsDNA (Chol-9T24 and FAM-cDNA24) and dsDNA /liposome conjugates. Centrifugation in pure buffer failed to separate dsDNA-liposome conjugates from the mixture.

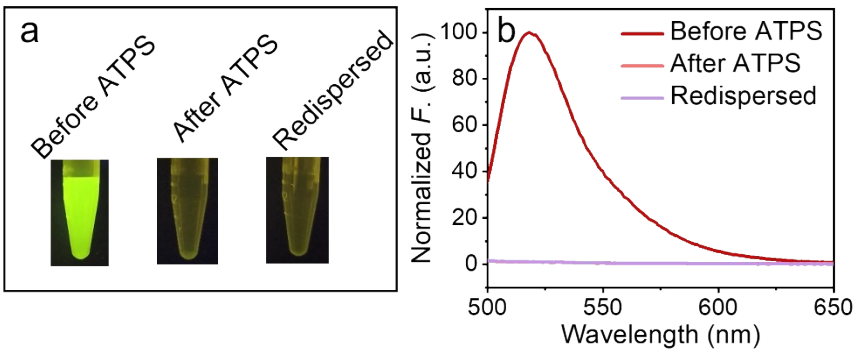

Figure S6. (a) Fluorescent images and (b) plots showing 100 nM dsDNA (Chol-9T24 and FAM-cDNA24) alone before ATPS, after ATPS, and redispersed in buffer. No dsDNA remained after five times of centrifugation and it thus is unlikely to be isolated as side products.

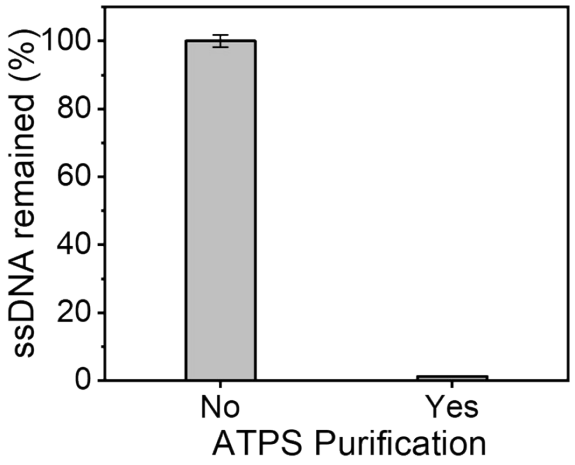

Figure S7. Quantification of ssDNA in dsDNA-TDN mixtures in the top PEG-rich phase. Only 1.2% probe DNA was left, suggesting a high purification efficiency. Error bars are standard deviations based on three independent experiments.

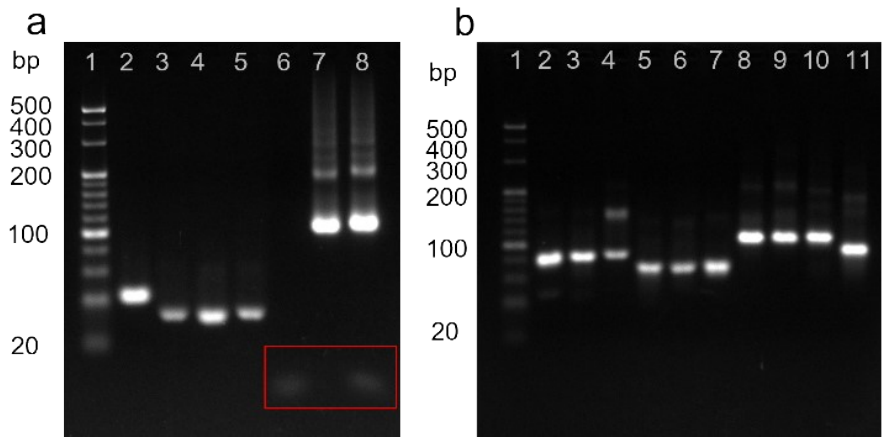

Figure S8. Agarose gel electrophoresis (3%) analysis of the gradual formation of TDN under the 590 nm channel. (a) Lane 1: 20 bp ladder maker; Line 2: TDN1-9T12; Line 3: TDN2; Line 4: TDN3; Line 5: TDN4; Line 6: FAM-cDNA12; Line 7: TDN; Line 8: dsDNA-TDN. (b) Line 1: 20 bp ladder marker; Line 2: TDN1-9T12+TDN2; Line 3: TDN1-9T12+TDN3; Line 4: TDN1-9T12+TDN4; Line 5: TDN2+TDN3; Line 6: TDN2+TDN4; Line 7: TDN3+TDN4; Line 8: TDN1-9T12+TDN2+TDN3; Line 9: TDN1-9T12+TDN2+TDN4; Line 10: TDN1-9T12+TDN3+TDN4; Line 11: TDN2+TDN3+TDN4.

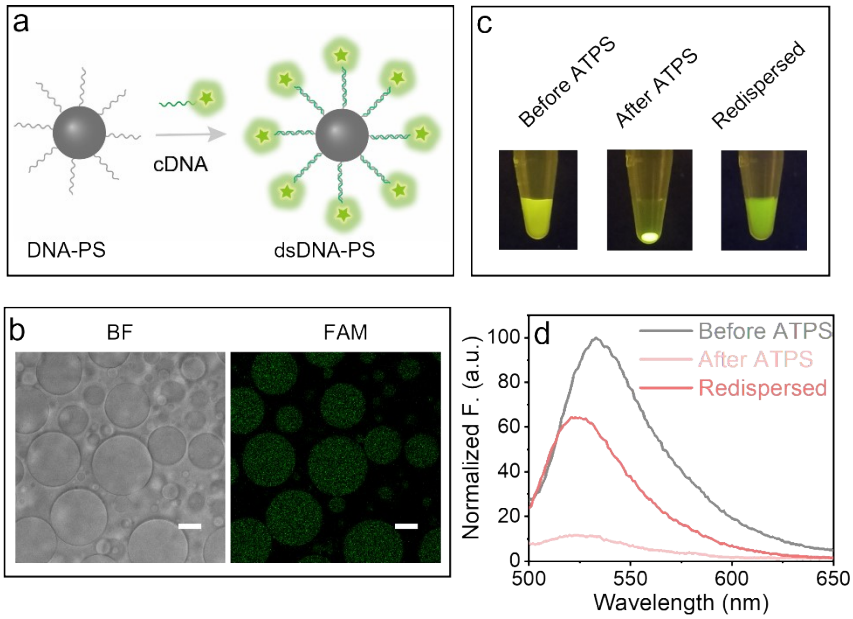

Figure S9. ATPS purification of DNA-PS conjugates. (a) A schematic showing the conjugation of FAM-labelled DNA with PS. (b) Bright-field and CLSM images of dextran-rich droplets containing DNA-PS conjugates after purification. (c) Fluorescent images and (d) spectra of DNA-PS conjugates before ATPS, after ATPS, and re-dispersed in buffer. The ATPS used for purification contained PEG 20K (10%) and dextran 40K (1%) in 10 mM HEPES buffer (pH 7.6). Scale bars, 20 μm.

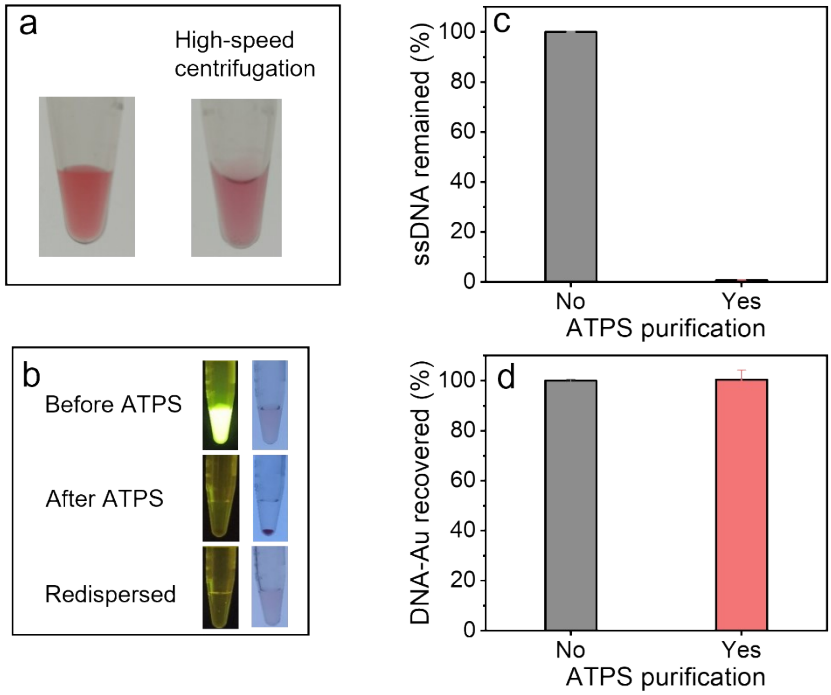

Figure S10. ATPS purification of non-thiolated DNA-AuNPs conjugates (13 nm). (a) Images of DNA-AuNPs conjugates before and after high-speed centrifugation (14,800 rpm, 10 min). (b) Images of non-thiolated DNA-AuNPs conjugates before ATPS, after ATPS, and re-dispersed in buffer. (c) ssDNA remained in the top PEG-rich phase. (d) The percentages of DNA-AuNPs recovered in the dextran-rich phase.

Additional References

[S1] J. Liu, Y. Lu, *Nature Protocols* **2006**, *1*, 246-252.
[S2] N. J. Lang, B. Liu, J. Liu, *Journal of Colloid and Interface Science* **2014**, *428*, 78-83.
[S3] B. Liu, J. Liu, *Journal of the American Chemical Society* **2017**, *139*, 9471-9474.