Supporting Information

OligoA-tailed DNA for dense functionalization of gold nanoparticles and nanorods in minutes without thiol-modification: unlocking cross-disciplinary

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Table S1 The sequence information of DNA used for functionalization in the present study.

Name	Sequence (5' to 3')
A_2T_{12}	TTTTTTTTTTTTAA
A_2T_{12}-FAM	TTTTTTTTTTTTAA-FAM
A_{14}	AAAAAAAAAAAAAA
T_{14}	TTTTTTTTTTTTTT
C_{14}	CCCCCCCCCCCCCC
G_{14}	GGGGGGGGGGGGGG
N_{14}	CTGTCGCACCGCAG
A_5N_{12}	AAAAACTGTCGCACCGC
T_5N_{12}	TTTTTCTGTCGCACCGC
C_5N_{12}	CCCCCCTGTCGCACCGC
G_5N_{12}	GGGGGCTGTCGCACCGC
$N_{12}A_5$	CTGTCGCACCGCAAAAA
$N_{12}T_5$	CTGTCGCACCGCTTTTT
$N_{12}C_5$	CTGTCGCACCGCCCCCC
$N_{12}G_5$	CTGTCGCACCGCGGGGG
C_2T_{12}	TTTTTTTTTTTTCC
G_2T_{12}	TTTTTTTTTTTTGG
T_2A_{12}	AAAAAAAAAAAATT
C_2A_{12}	AAAAAAAAAAAACC
G_2A_{12}	AAAAAAAAAAAAGG
A_2C_{12}	CCCCCCCCCCCCAA
T_2C_{12}	CCCCCCCCCCCCTT
G_2C_{12}	CCCCCCCCCCCCGG
A_2G_{12}	GGGGGGGGGGGGAA
T_2G_{12}	GGGGGGGGGGGGTT
C_2G_{12}	GGGGGGGGGGGGCC
$A_2N_1T_{12}$	TTTTTTTTTTTTCAA
$A_2N_5T_{12}$	TTTTTTTTTTTTCGCACAA
$A_2N_5T_{12}$-FAM	TTTTTTTTTTTTCGCACAA-FAM
$A_2N_{10}T_{12}$	TTTTTTTTTTTTCTGTCGCACCAA
$A_2N_{15}T_{12}$	TTTTTTTTTTTTCTGTCGCACCCTGTCAA
$A_2N_{10}T_2$	TTCTGTCGCACCAA
$A_2N_{10}T_3$	TTTCTGTCGCACCAA
$A_2N_{10}T_4$	TTTTCTGTCGCACCAA
$A_2N_{10}T_5$	TTTTTCTGTCGCACCAA
$A_2N_{10}T_6$	TTTTTTCTGTCGCACCAA
$A_2N_{10}T_8$	TTTTTTTTCTGTCGCACCAA
$A_2N_{10}T_{10}$	TTTTTTTTTTCTGTCGCACCAA
$T_{12}A_2$	AATTTTTTTTTTTT
A_3T_{12}	TTTTTTTTTTTTAAA
A_4T_{12}	TTTTTTTTTTTTAAAA

Name	Sequence (5' to 3')
A_5T_{12}	TTTTTTTTTTTTAAAAA
A_2N_{16}	AATACGCCACCAGCTCTC
A_4N_{16}	AAAATACGCCACCAGCTCTC
A_6N_{16}	AAAAAATACGCCACCAGCTCTC
A_6N_{16}-FAM	FAM-AAAAAATACGCCACCAGCTCTC
Complementary DNA	GAGAGCTGGTGGCGTA
A_8N_{16}	AAAAAAAATACGCCACCAGCTCTC
$A_{10}N_{16}$	AAAAAAAAAATACGCCACCAGCTCTC
$A_{70}N_{10}$	GGTGCGACAGAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA AAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA AA

Table S2 The sequence information of the primer set and probe DNA used for the naked-eye LAMP detection.

Primer	Type	Sequence (5' to 3')
F3	Forward outer	AACCGGTGACCAAACATG
B3	Reverse outer	AGGAAGTAGAAGTCAGTGATG
FIP	Forward inner (F1c-F2)	CGTTAAAGTCGCCATCTTCCCA-TCTAGAGTCTATGATCGCTTC
BIP	Reverse inner (B1c-B2)	AGGTCTTATCGCATCTGATAAAGAC-TGGATATCTTTACGTTGGTAGT
LF	Forward loop	CCTTTAGCGCCTTGAACACCT
LB	Backward loop	GCAACTCTATGGGCTGGTAAAACT
A_6-probe$_{VP}$	probe	AAAAAATGCACAGTTTGCAGTTAAAGCTA

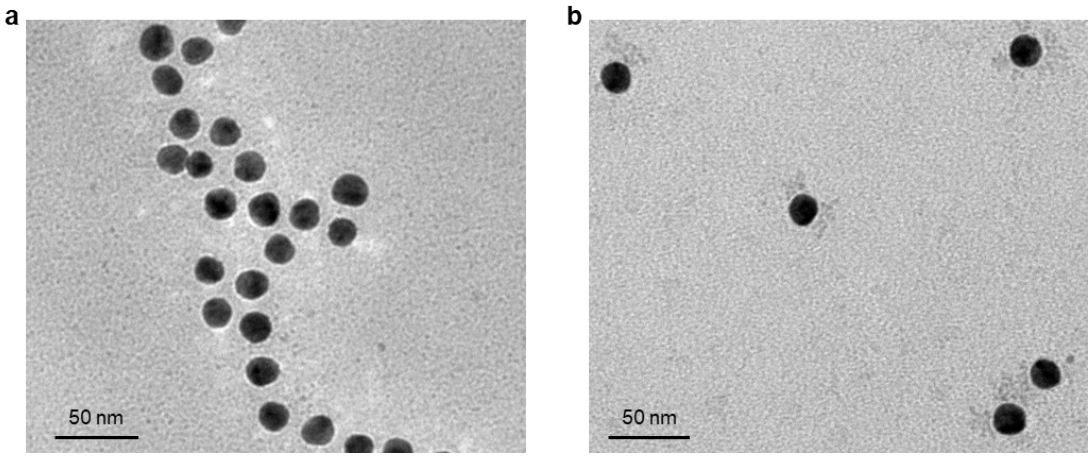

Figure S1. Representative TEM images of (a) the pristine AuNPs, and (b) the A_2T_{12}-AuNPs prepared by the dehydration method.

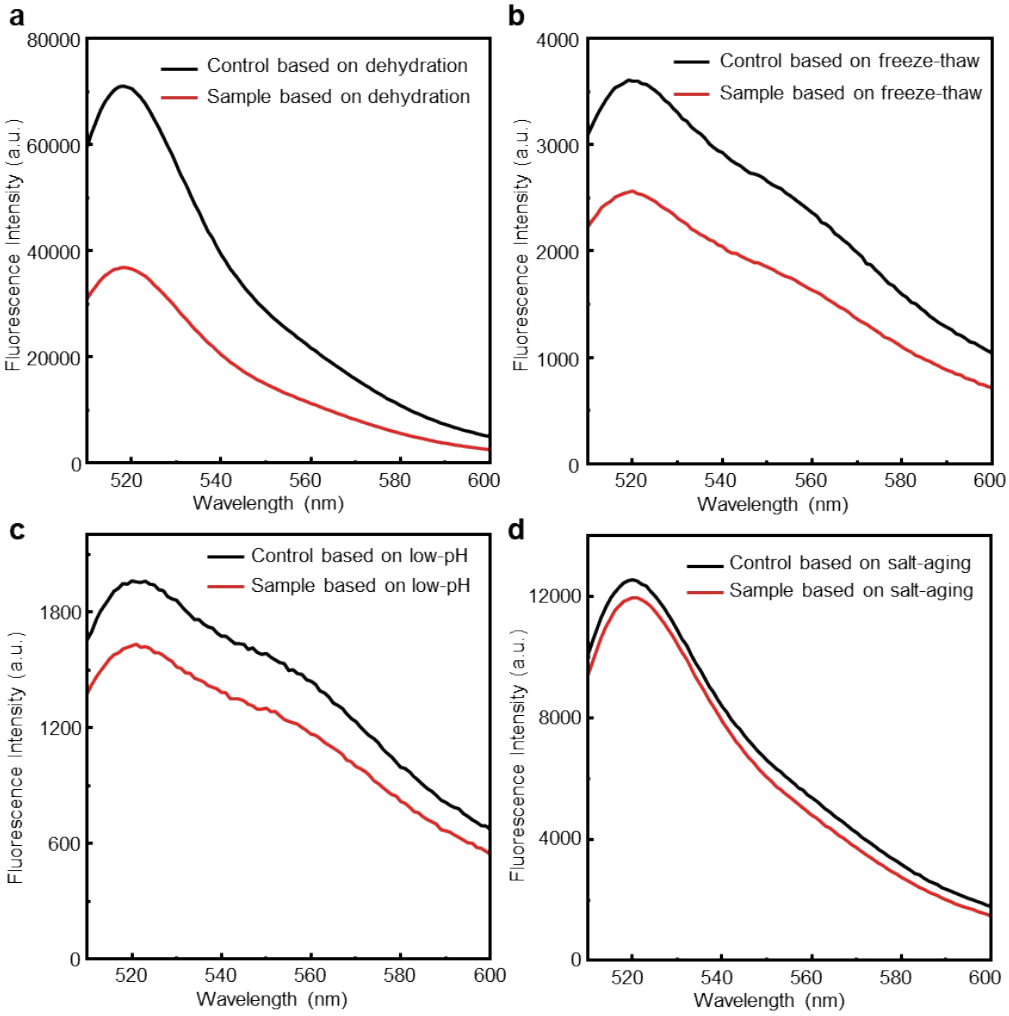

Figure S2. Fluorescence measurements of the number of A_2T_{12}-FAM on the functionalized AuNPs produced using different methods. (a-d) Fluorescence spectra of the supernatant of the mixture of A_2T_{12}-FAM and AuNPs after functionalization using (a) the dehydration, (b) the freeze-thaw, (c) the low-pH and (d) the salt-aging methods. The molar ratio of DNA to AuNP is 600:1. The solution of A_2T_{12}-FAM was used as the control.

Table S3 Calculation of the surface area occupied by each oligoA-DNA on the oligoA-DNA-AuNPs.

Method	DNA number per particle	Diameter of AuNP (nm)	Occupied area of each DNA (nm^2)
Salt-aging	36	20	35.0
Low-pH	94	20	13.4
Freeze-thaw	158	20	8.0
Dehydration	271	20	4.6

The calculation formula for oligoA-DNA density reads:

$$D = \frac{S_{AuNPs}}{N} = \frac{\pi \times d^2}{N}$$

where N is the number of DNA per AuNP, D is occupied area of each oligoA-DNA, S_{AuNP} is the surface area of a AuNP, and d is the diameter of a AuNP.

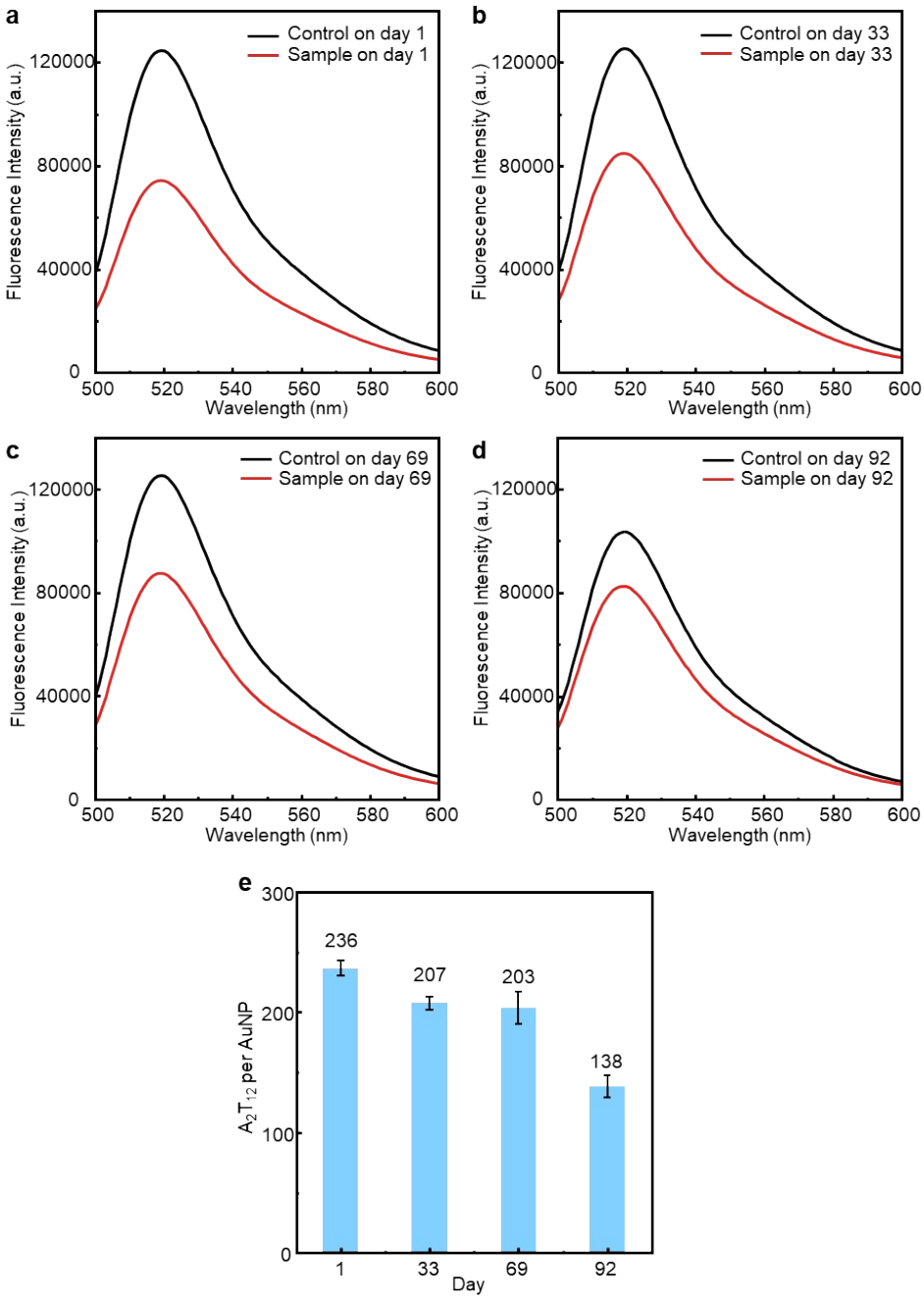

Figure S3. Attachment stability of A_2T_{12}-FAM on the AuNPs over time. (a-d) Fluorescence spectra of the supernatant of the mixture of A_2T_{12}-FAM and AuNPs after functionalization for (a) 1 day, (b) 33 days, (c) 69 days, and (d) 92 days, using the dehydration method. For each spectrum, the fluorescence spectrum of A_2T_{12}-FAM was used as the control. The molar ratio of DNA to AuNP in the mixture was 600:1. (e) Histogram depicting the measured numbers of DNA per AuNP after functionalization for different times.

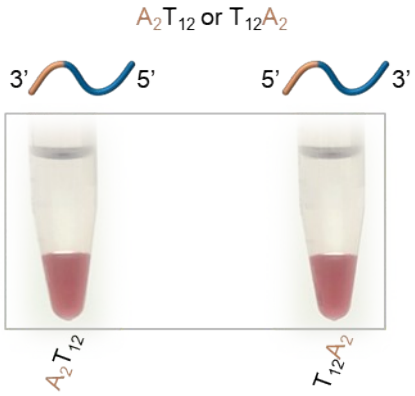

Figure S4. Photographs of the AuNPs functionalized with A_2T_{12} and $T_{12}A_2$. The dehydration was performed at room temperature using a molar ratio of 600:1 between DNA and AuNP, a final concentration of 20 mM $NaNO_3$, and a volume ratio of 7:1 between butanol and the colloidal AuNP system.

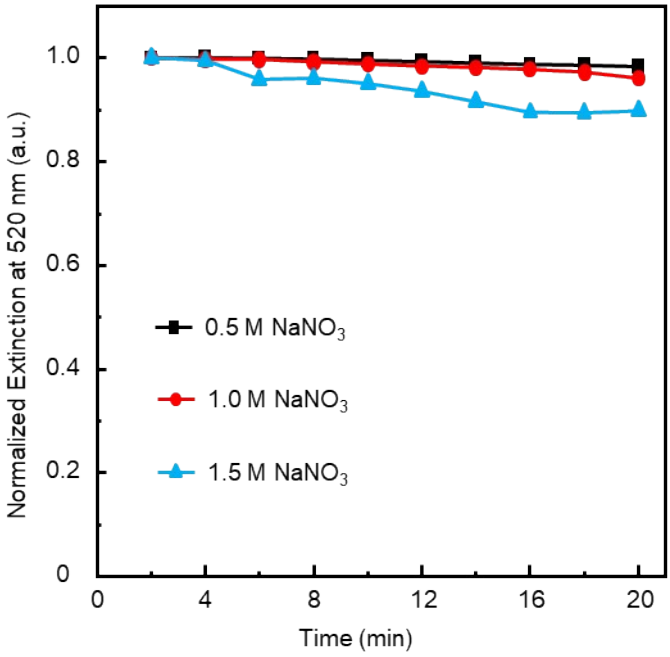

Figure S5. Test of the stability of the A_2T_{12}-AuNPs against high-concentration salt. Normalized UV-Vis absorption intensity of the A_2T_{12}-AuNPs at 520 nm at different concentrations of $NaNO_3$ over time.

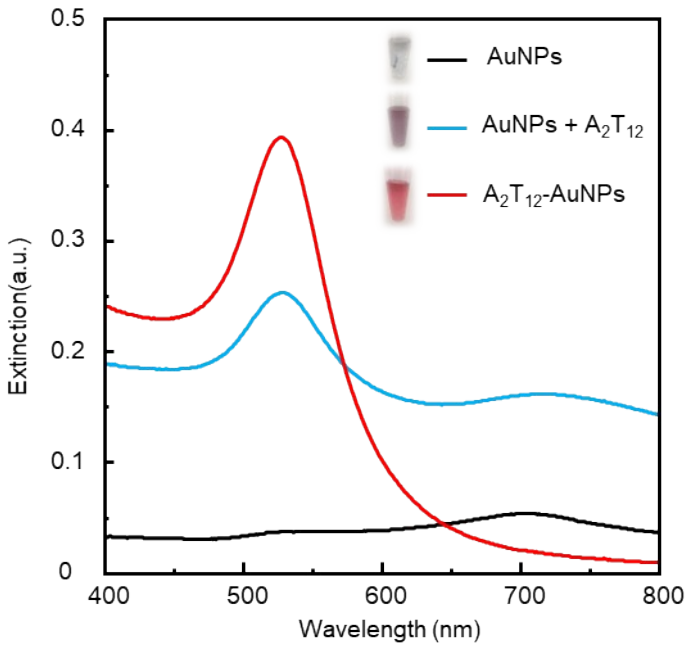

Figure S6. Test of the stability of the AuNPs before and after A_2T_{12} functionalization. The profiles show the extinction spectra of the AuNPs (2 nM), the A_2T_{12}-AuNPs (2 nM) and the mixture of AuNPs (2 nM) and A_2T_{12} (1.2 μM) in the presence of 167 mM of $NaNO_3$. The insets show the corresponding photographs of the AuNPs.

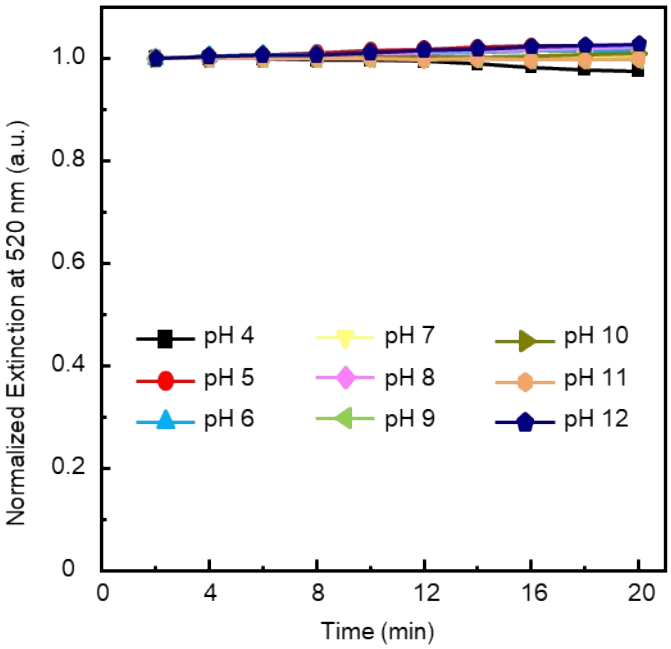

Figure S7. Test of the stability of the A_2T_{12}-AuNPs under different pH conditions. The profiles show the normalized UV-Vis absorption intensity of the A_2T_{12}-AuNPs at 520 nm under different pH conditions over time.

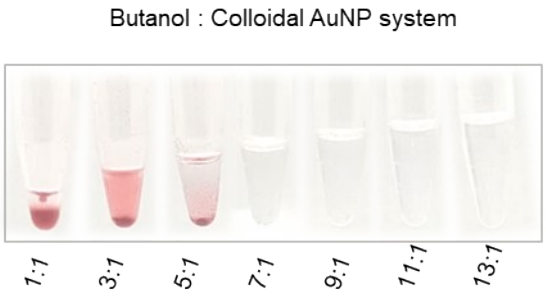

Figure S8. Optimization of the amount of butanol added for the dehydration method. The volume ratio of butanol to the colloidal AuNPs is optimized to be 7:1. The dehydration was performed at room temperature using a molar ratio of 600:1 between DNA and AuNP, and a final concentration of 20 mM of $NaNO_3$.

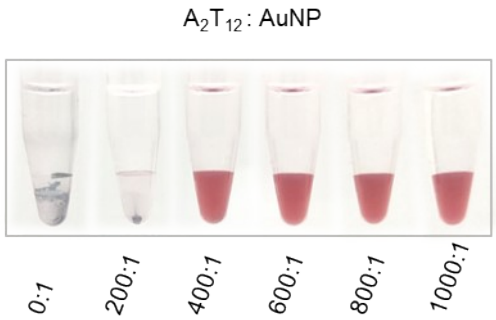

Figure S9. Optimization of the amount of A_2T_{12} added for functionalization of the AuNPs using the dehydration method. The ratio of A_2T_{12} to the AuNP is optimized to be 600:1. The dehydration was performed at room temperature using a final concentration of 20 mM of $NaNO_3$, and a volume ratio of 7:1 between butanol and the colloidal AuNP system.

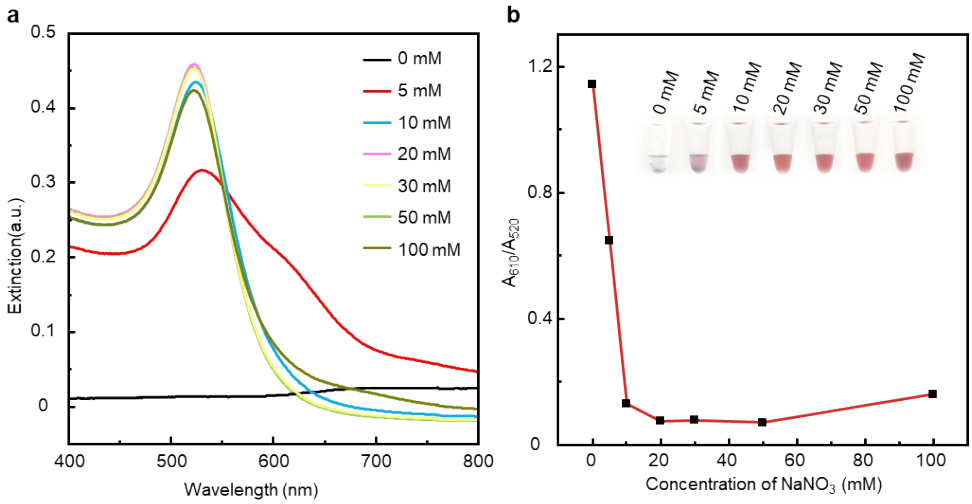

Figure S10. Optimization of the amount of $NaNO_3$ added for functionalization of the AuNPs using the dehydration method. The concentration of the $NaNO_3$ is optimized to be 20 mM. (a) UV-Vis spectra and (b) the UV-Vis absorption A_{610}/A_{520} ratio of the resultant AuNPs using A_2T_{12} for functionalization under different $NaNO_3$ concentrations. The dehydration was performed at room temperature using a molar ratio of 600:1 between DNA and AuNP, and a volume ratio of 7:1 between butanol and the colloidal AuNP system.

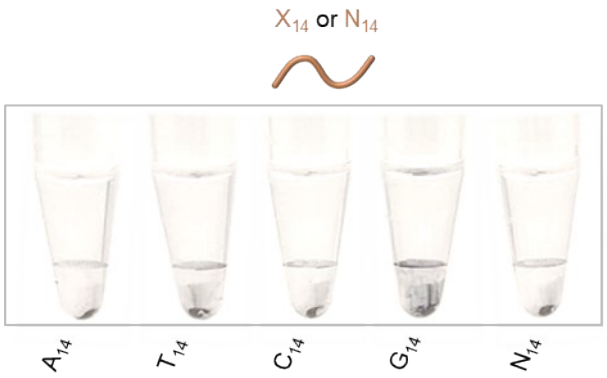

Figure S11. Photographs of the AuNPs functionalized with X_{14} or N_{14} using the dehydration method. The dehydration was performed at room temperature using a molar ratio of 600:1 between DNA and AuNP, a final concentration of 20 mM of $NaNO_3$, and a volume ratio of 7:1 between butanol and the colloidal AuNP system.

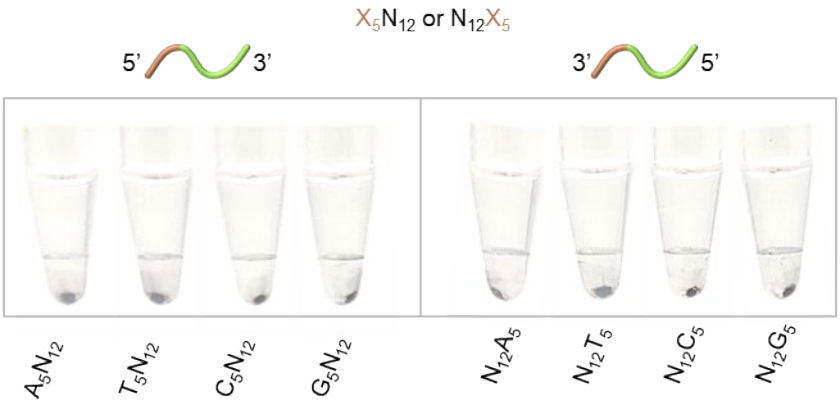

Figure S12. Photographs of the AuNPs functionalized with X_5N_{12} or $N_{12}X_5$ using the dehydration method. The dehydration was performed at room temperature using a molar ratio of 600:1 between DNA and AuNP, a final concentration of 20 mM $NaNO_3$, and a volume ratio of 7:1 between butanol and the colloidal AuNP system.

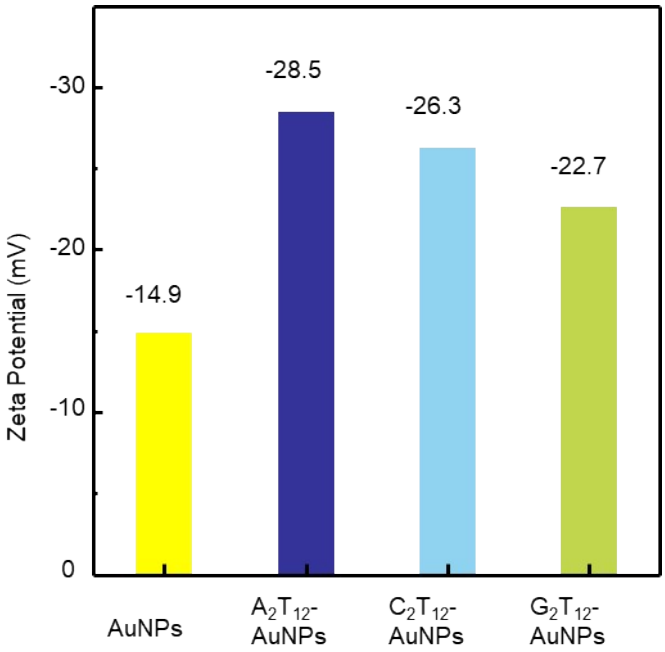

Figure S13. Zeta potential of the AuNPs, the A_2T_{12}-AuNPs, the C_2T_{12}-AuNPs and the G_2T_{12}-AuNPs produced using the dehydration method. The dehydration was performed at room temperature using a molar ratio of 600:1 between DNA and AuNP, a final concentration of 20 mM of $NaNO_3$, and a volume ratio of 7:1 between butanol and the colloidal AuNP system.

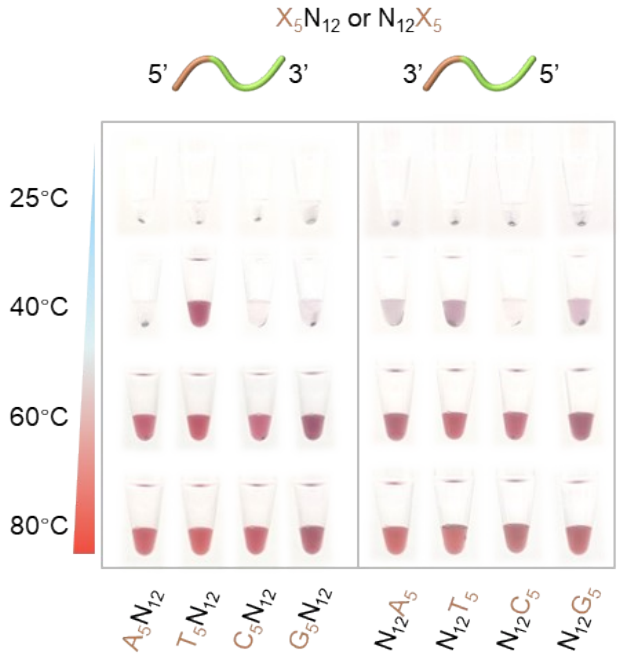

Figure S14. Photographs of the AuNPs functionalized with X_5N_{12} or $N_{12}X_5$ using the dehydration method. The dehydration was performed at different temperatures using a molar ratio of 600:1 between DNA and AuNP, a final concentration of 20 mM of $NaNO_3$, and a volume ratio of 7:1 between butanol and the colloidal AuNP system.

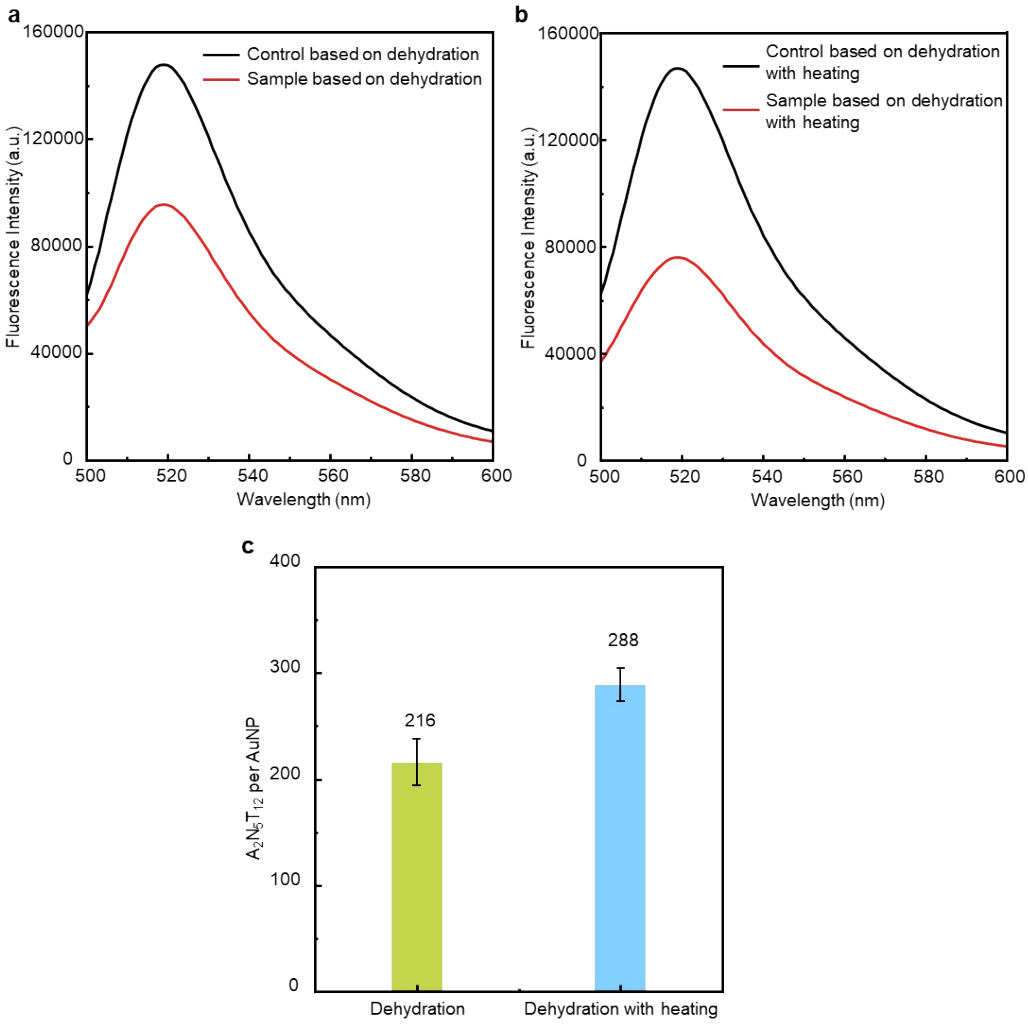

Figure S15. Measurements of the numbers of $A_2N_5T_{12}$-FAM on functionalized AuNPs produced using the dehydration method assisted with heating. (a, b) Fluorescence spectra of the supernatant of the mixture of $A_2N_5T_{12}$-FAM and AuNPs after functionalization using the dehydration method (a) without and (b) with the assistance of heating to 80°C. $A_2N_5T_{12}$-FAM was used as the control sample for the mixture in each case. (c) Histogram showing the measured numbers of $A_2N_5T_{12}$-FAM per AuNP produced using dehydration without (left) and with (right) the assistance of heating to 80°C. The molar ratio of DNA to AuNP is 600:1.

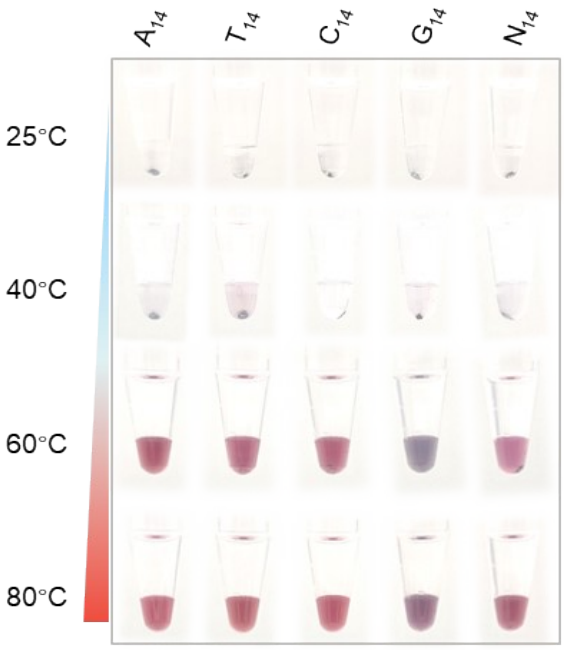

Figure S16. Photographs of the AuNPs functionalized with A_{14}, T_{14}, C_{14}, G_{14} or N_{14} using the dehydration method. The dehydration was performed at different temperatures using a molar ratio of 600:1 between DNA and AuNP, a final concentration of 20 mM of $NaNO_3$, and a volume ratio of 7:1 between butanol and the colloidal AuNP system.

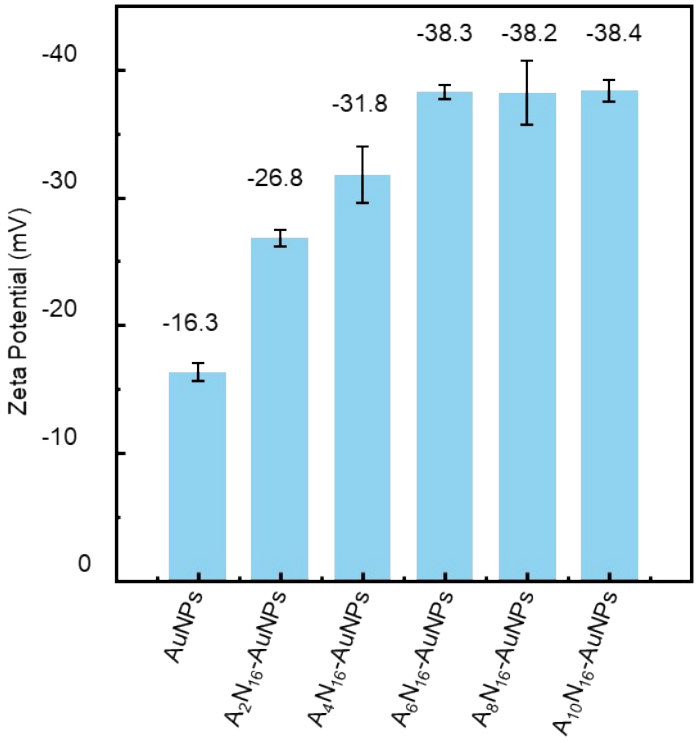

Figure S17. Zeta potential analysis for the AuNPs before and after A_nN_{16} functionalization. The dehydration was performed at 80°C using a molar ratio of 600:1 between DNA and AuNP, a final concentration of 20 mM of $NaNO_3$, and a volume ratio of 7:1 between butanol and the colloidal AuNP system.

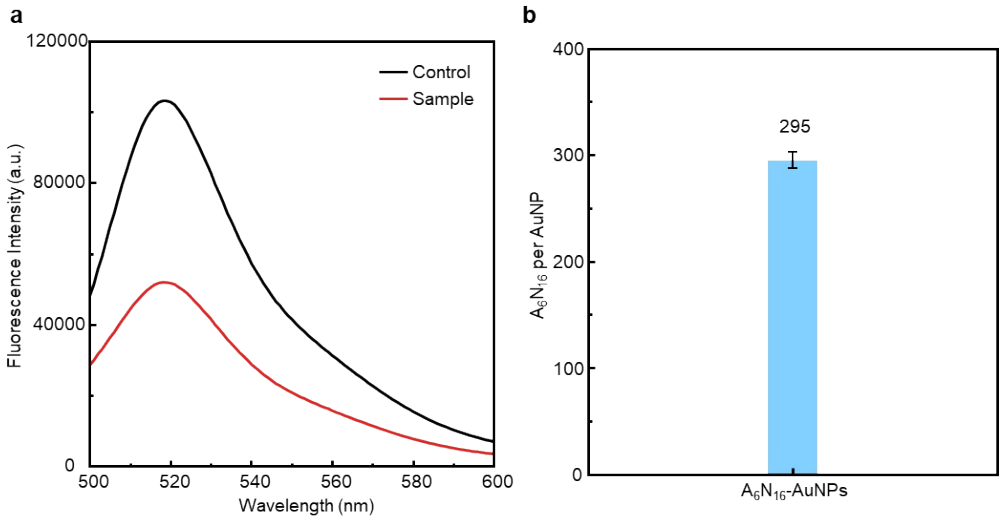

Figure S18. Measurement of the number of A_6N_{16}-FAM attached to the AuNPs produced using the dehydration method assisted with heating. (a) Fluorescence spectra of the supernatant of the mixture of A_6N_{16}-FAM and AuNPs after functionalization using the dehydration method assisted with heating to 80°C. A_6N_{16}-FAM was used as the control sample. (b) Histogram showing the measured number of A_6N_{16}-FAM attached to each AuNP based on the dehydration method assisted with heating to 80°C. The molar ratio of DNA to AuNP was 600:1.

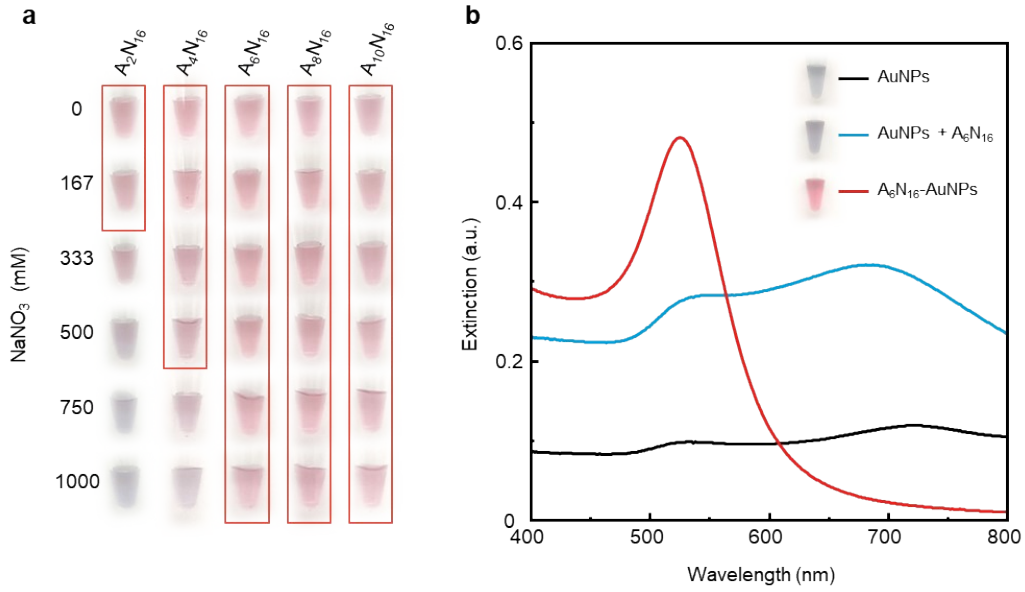

Figure S19. Test of the stability of the AuNPs before and after A_nN_{16} functionalization using the dehydration method assisted with heating. (a) Photograph of the A_nN_{16}-AuNPs (n: 2-10) (1 nM) in the presence of $NaNO_3$ at different concentrations. (b) Extinction spectra of the AuNPs (2 nM), the A_6N_{16}-AuNPs (2 nM) and the mixture of the AuNPs (2 nM) with A_6N_{16} (1.2 μM) in the presence of 167 mM of $NaNO_3$. The insets show the corresponding photographs of the AuNPs.

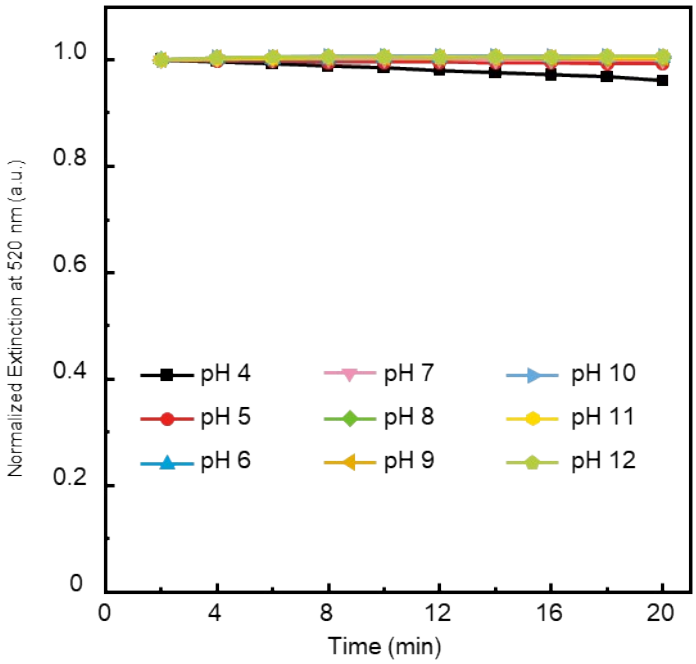

Figure S20. Stability test for the A_6N_{16}-AuNPs under different pH conditions. The curves show the normalized UV-Vis absorption intensity of the A_6N_{16}-AuNPs at 520 nm under different pH conditions over time.

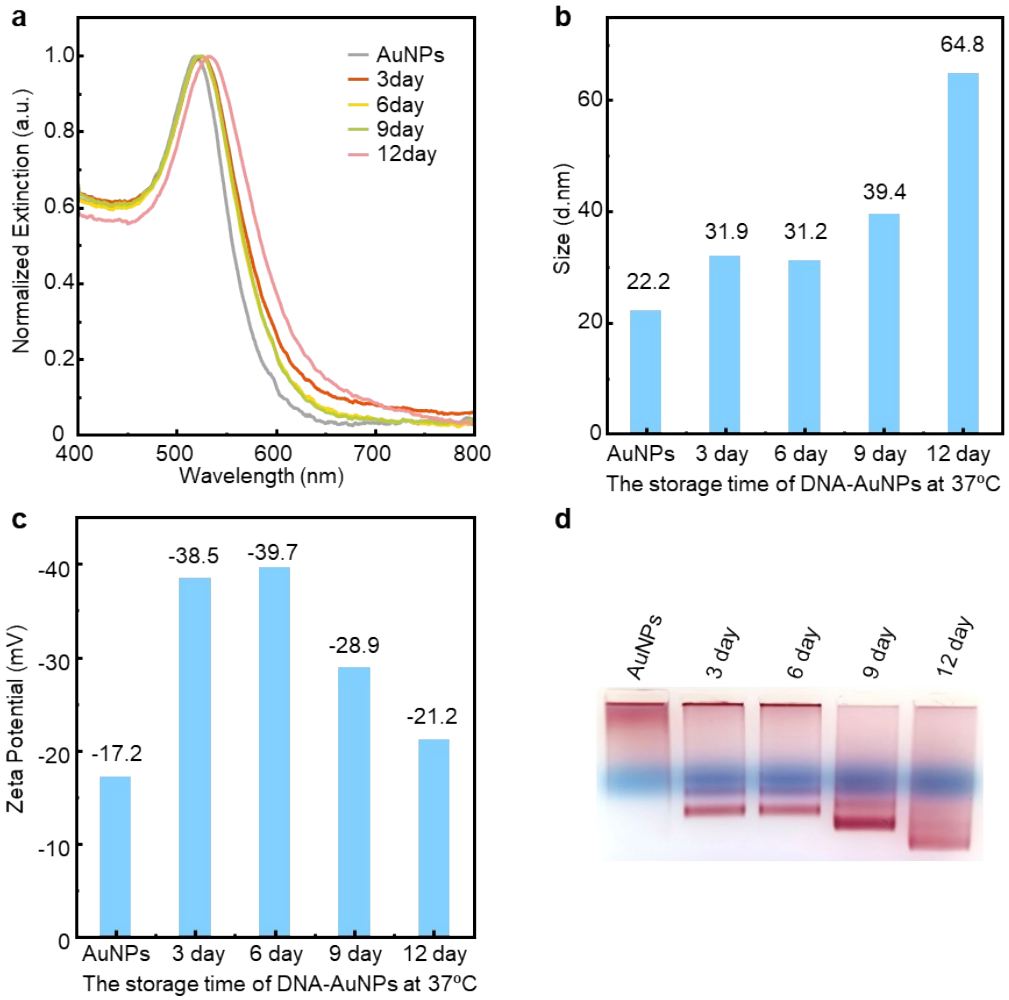

Figure S21. Stability of A_6N_{16}-AuNPs after storage. (a) Normalized extinction spectra, (b) DLS measurement results, (c) zeta potential measurement results, and (d) agarose gel electropherograms of the A_6N_{16}-AuNPs after storage for 3 days, 6 days, 9 days, and 12 days at 37°C.

Estimation of the shelf life of the DNA-AuNPs

Q_{10} is defined as the temperature sensitivity of a reaction by which the reaction rate increases for per 10°C increase in temperature, and the Q_{10} rule is often used for the accelerated shelf-life tests (*Food Control* **1993**, 4, 125-133; *Ecol. Modell.* **2020**, 431, 109127).

The formulas for calculating shelf life can be given as:

$$Q_{10} = \left[\frac{k(T_2)}{k(T_1)}\right]^{\left(\frac{10}{T_2 - T_1}\right)} \quad (1)$$

where k is reaction rate and T is temperature. Since shelf life is inversely proportional to reaction rate (*J. Chem. Educ.* **1984**, 6, 348; https://www.hemdahl.com/content/resources/Hemdahl_WP_103_Shelf_Life_Prediction.pdf, visited on 28th, Feb., 2025), we have:

$$S_T = S_{ref} \cdot Q^{\left(\frac{T_{ref} - T}{10}\right)}_{10} \quad (2)$$

where S_T is the shelf life at T (4°C), S_{ref} is the shelf life at reference temperature (T_{ref}, 37°C). It is generally accepted that the reaction rate doubles with every 10°C increase. Therefore, Q_{10} is approximately equal to 2 when (T_2−T_1) is 10°C (*Proc. Natl. Acad. Sci. U. S. A.* **2016**, 113, 3832-3837). According to the calculation, the shelf life of the DNA-AuNPs stored at 4°C is about 88 days.

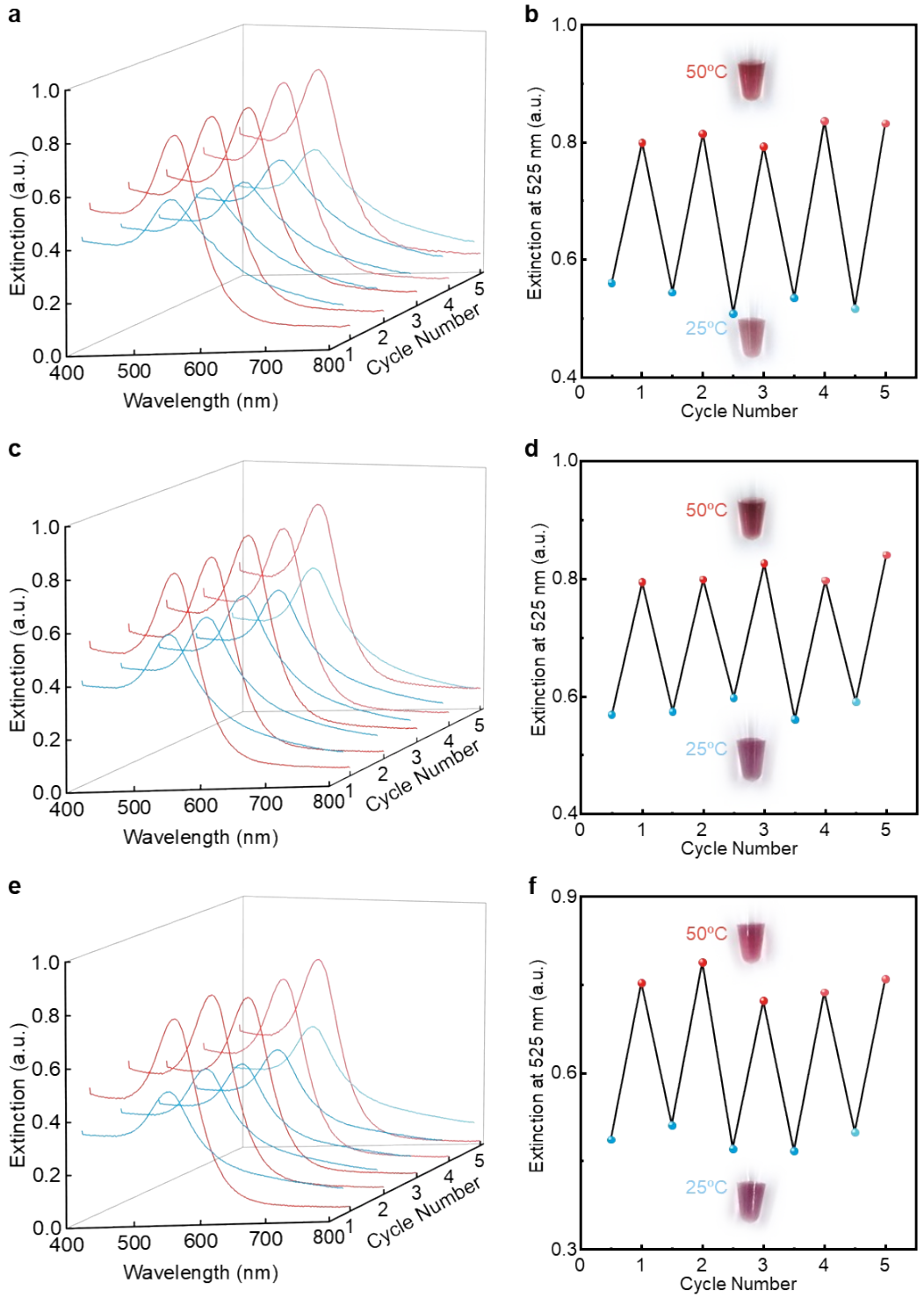

Figure S22. Thermo-responsive assembly of A_6N_{16}-AuNPs after storage for (a, b) 3 days, (c, d) 12 days, and (e, f) 21 days. The insets denote the photographs of the colloidal A_6N_{16}-AuNPs at the corresponding temperatures. The temperature was changed in the range of 25-50°C for A_6N_{16}-AuNPs. Note that the colloid contains 2.5 nM dsDNA-AuNPs, 500 mM salt, and 10 mM HEPES (pH 7.4).

Table S4 Sequence information of the target gene sequence (GenBank: JF747207.1) of *V. parahaemolyticus*.

Type	Sequence 5'-3'
Nucleotide sequence (1209 bp)	ATGAAAAAAGTAAGTGGTATTGCAGCGGCTGTTGCTGCAACT TTAGCTGCTGGTTCTGCTTTCGCAGTGGACTTTAATGGTTACA TGCGCGCCGGTACTGGTATTAGTGCTGAAAGCGGTGGTGACG TATCATTTATGAAAAATGGTATTGGCCGTCTAGGTAACGAAG ATGACAACTACTCTGAGTTTGGTTTTGCTGAGGATCTAAAAA CCGGTGACCAAACATGGCGTCTAGAGTCTATGATCGCTTCAG GTGTTCAAGGCGCTAAAGGTTGGGAAGATGGCGACTTTAACG TTGCACAGTTTGCAGTTAAAGCTAAAGGTCTTATCGCATCTG ATAAAGACGCAACTCTATGGGCTGGTAAAACTTACTACCAAC GTAAAGATATCCACATCACTGACTTCTACTTCCTAAACACTTC TGGTACTGGTGGTGGTATCGAAAACCTTTCTGTAGGTAACCA AAAGCTATCTCTAGCAATCATTCAAGACGGTGAAGATGAAAA CGGTGCGGGTTACATTGCAGACGCTCGTTTAGCGAACATTGG TCTTTGGGAAGATGCATCTCTTGAAGTTGCGCTAGCGTACAA CTTCTCAACAGAAAGCAAAAATGGCAAATACGATGGCGATG ATGGCCTGCTTGCAAGTGGTATCATTCACCAAAACATGAGCA ATGGTTTCAACCAGACTGTAGTTCAAGTTGGTACAGCTGGCT ACGGTATTCAGATGGCAAACTTCTGGGGTGCTGGTGCATATT ACGACCGCTCTGGAGACCAAAATGATGGCTTCGGTTACCGTG TTATTAACTGGGGTGTTATGAACTTGGGCGAAAACTGGGAAA TGGGTCACCAGTTGGCTTACCTAGCAGGTTCAGATTTAGGTA CAACTAAGTACGACTCTAGCCAGTACTCAATTGTCGCTCGTC CAATGTACAAATGGAACGATACTATGCGTACTATTTTTGAAG CTGGCTACAATGCTGGTGAAGTGGATGATGTTGACTTCGGTG GTGCTAAATTTACCGTAGCTCAAGCATGGGCAATGGGTGATA GCTTCTGGGCTCGTCCTGAAATTCGTGTATACGGTAGCTACCT AATGGATCTAGAAAACGATAGTTTTGGTGAAGTTAAAAATGA CGCTGGTGTAGTTACAGCAGCTGGCACAGACAACGACTTTGT TGTTGGCATCCAAGTCGAAGCTTGGTGGTAA

Table S5 The information of the binding regions of the primers and probe$_{VP}$ in the target gene sequence.

Type	Type	Sequence 5'-3'
Primers and Probe$_{VP}$		F3 **191** 5'-GTTTTGCTGAGGATCTAAA*AACCGGTGACCAAACATG*GCG-3' 3'-CAAAACGACTCCTAGATTTTTGGCCACTGGTTTGTACCGC-5'
		F2 **231** 5'-*TCTAGAGTCTATGATCGCTTC*AGGTGTTCAAGGCGCTAAA-3' 3'-AGATCTCAGATACTAGCGAAG*TCCACAAGTTCCGCGATTT*-5' LF
		Probe$_{VP}$ **271** 5'-GGTTGGGAAGATGGCGACTTTAACGT*TGCACAGTTTGCAG*-3' 3'-*CC*A*ACCCTTCTACCGCTGAAATTGC*AACGTGTCAAACGTC-5'
		F1c B1c **311** 5'-*TTAAAGCTA*A*AGGTCTTATCGCATCTGATAAAGACGCAAC*-3' 3'-AATTTCGATTTCCAGAATAGCGTAGACTATTTCTGCGTTG-5'
		LB **351** 5'-*TCTATGGGCTGGTAAAACT*TACTACCAACGTAAAGATATC-3' 3'-AGATACCCGACCATTTTGAA*TGATGGTTGCATTTCTATAG*-5' B2
		391 5'-CACATCACTGACTTCTACTTCCTAAACACTTCTGGTACTG-3' 3'-*GTGTAGTGACTGAAGATGAAGGA*TTTGTGAAGACCATGAC-5' B3

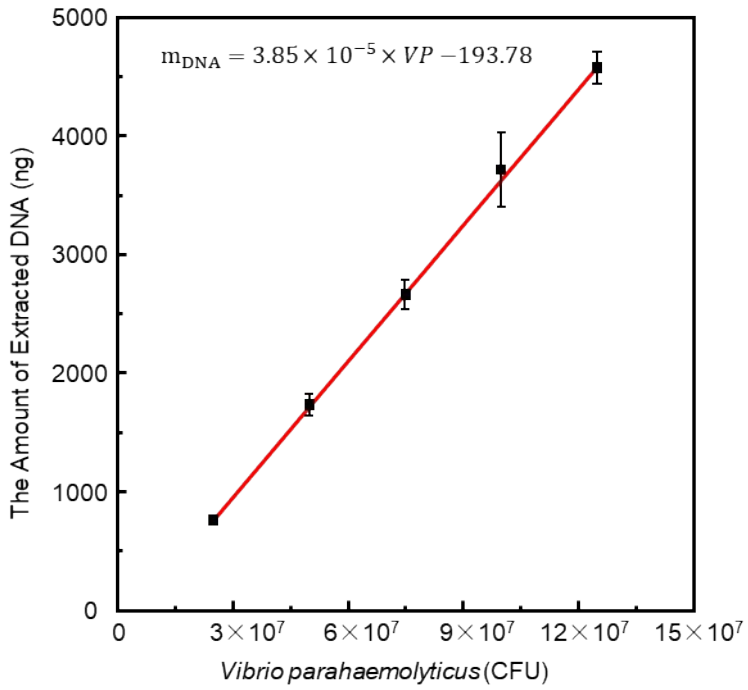

Figure S23. Calibration curve showing the linear relationship between the CFU of *V. parahaemolyticus* (*VP*) and the mass of the extracted DNA (m_{DNA}).

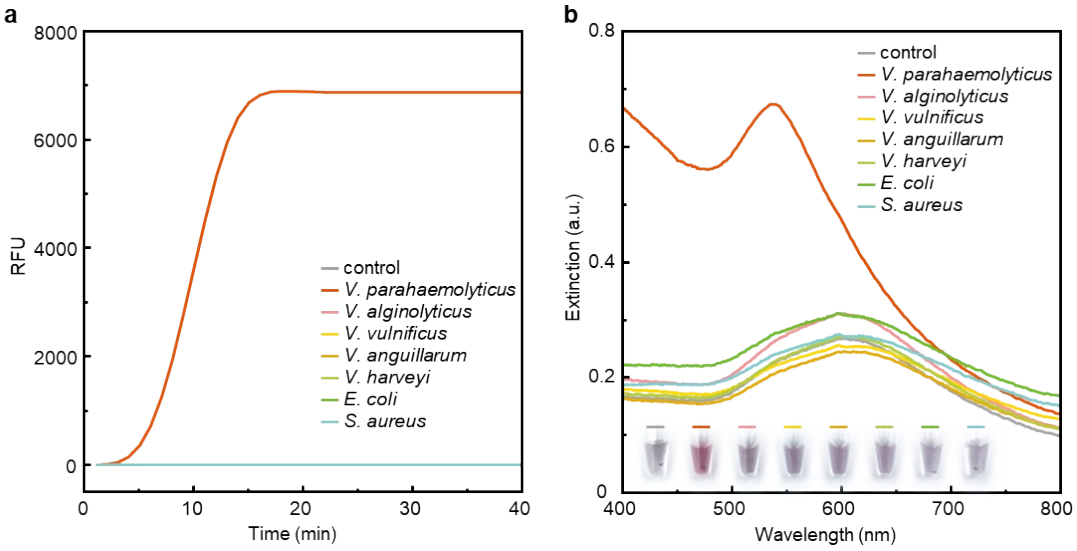

Figure S24. Sensitivity test of naked-eye LAMP assay. (a) Real-time fluorescence quantification curve for the specificity of the LAMP assay. (b) Extinction spectra of A_6-Probe$_{VP}$-AuNPs for the specificity of the LAMP assay; the inset was actual photographs. The concentration of different bacteria was 1 ng/μL. Note that the LAMP reactions were performed in a reaction mixture containing FIP (1.4 μM), BIP (1.4 μM), F3 (0.2 μM), B3 (0.2 μM), LF (0.4 μM), and LB (0.4 μM) primers, dNTPs mix (1.2 mM each), 1× thermopol-supplied reaction buffer, $MgSO_4$ (4 mM), 0.24 U of Bst DNA polymerase.

Table S6 Comparison of the present method with the previous LAMP methods based on DNA-AuNPs.

Testing method	Type of DNA probe	Preparation method of the DNA-AuNPs	Preparation time of the DNA-AuNPs	Characteristics	Reference
SERS	Thioated DNA	Salt-aging	~72 h	Need for specified instrument	*Theranostics* **2016**, *6*, 522-532
Colorimetric	Thioated DNA	Salt-aging	~72 h	High cost and long nanoprobe preparation	*J. Nanobiotechnol.* **2013**, *11*, 38
Colorimetric	Thioated DNA	Salt-aging	~72 h	High cost and long nanoprobe preparation	*J. Nanopart. Res.* ***2016****, 18, 351*
Colorimetric	Thioated DNA	Salt-aging	~72 h	High cost and long nanoprobe preparation	*LWT* **2023**, *185*, 115190
Colorimetric	Thioated DNA	Salt-aging	~72 h	High cost and long nanoprobe preparation	*ACS Infect. Dis.* **2024**, *10*, 2668-2678
Colorimetric	OligoA-DNA	Salt-aging	~72 h	long nanoprobe preparation	*ACS Comb. Sci.* **2018**, *20*, 472-481
Colorimetric	OligoA-DNA	Dehydration	A few seconds	No need for specified instrument, low cost, and short nanoprobe preparation	This work

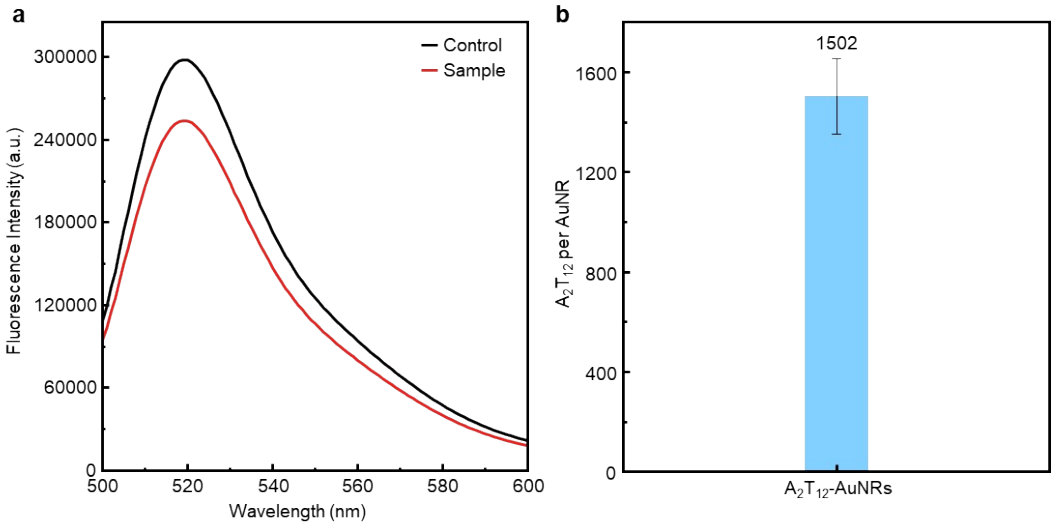

Figure S25. Fluorescence measurement of the number of A_2T_{12}-FAM on the functionalized AuNRs produced using the dehydration method assisted with heating. (a) Fluorescence spectra of the supernatant of the mixture of A_2T_{12}-FAM and the AuNRs after functionalization using the dehydration method assisted with heating to 80°C. A_2T_{12}-FAM was used as the control sample. (b) Histogram showing the measured number of A_6N_{16}-FAM per functionalized AuNR. The molar ratio of DNA to AuNR was 15000:1.

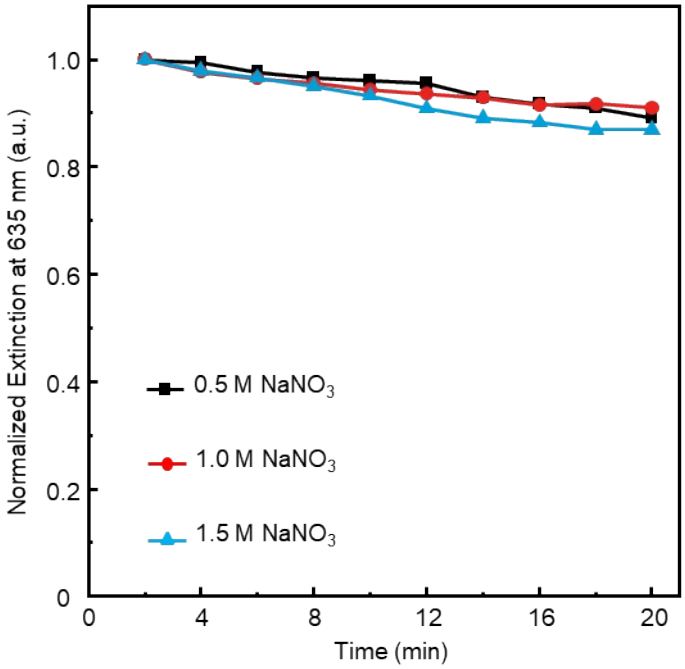

Figure S26. Stability test for the A_2T_{12}-AuNRs at high salt concentrations. The curves show the normalized UV-Vis absorption intensity of the A_2T_{12}-AuNRs at 635 nm at different concentrations of $NaNO_3$ over time.

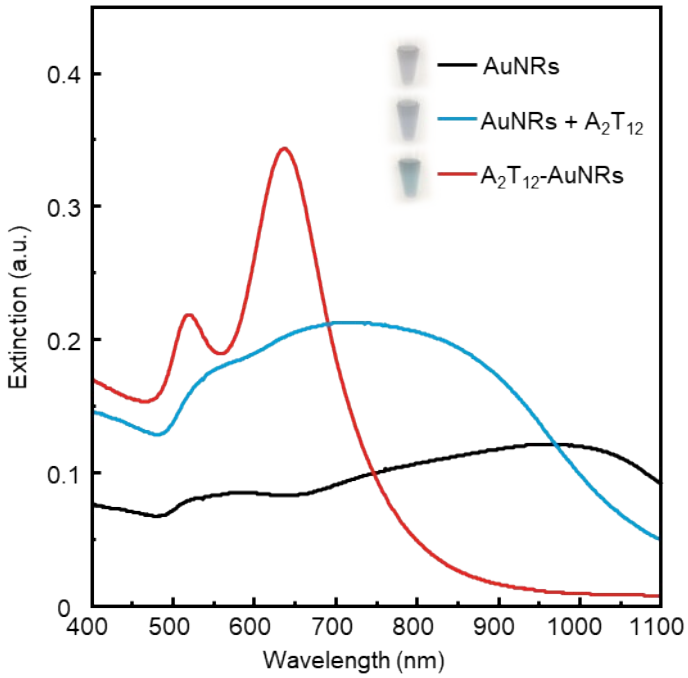

Figure S27. Stability test for the AuNRs before and after A_2T_{12} functionalization using the dehydration method assisted with heating. The profiles show the extinction spectra of the AuNRs (0.5 nM), the A_2T_{12}-AuNRs (0.5 nM) and the mixture of the AuNRs (0.5 nM) with A_2T_{12} (3.75 μM) in the presence of 167 mM of $NaNO_3$. The insets show the photographs of the corresponding AuNPs.

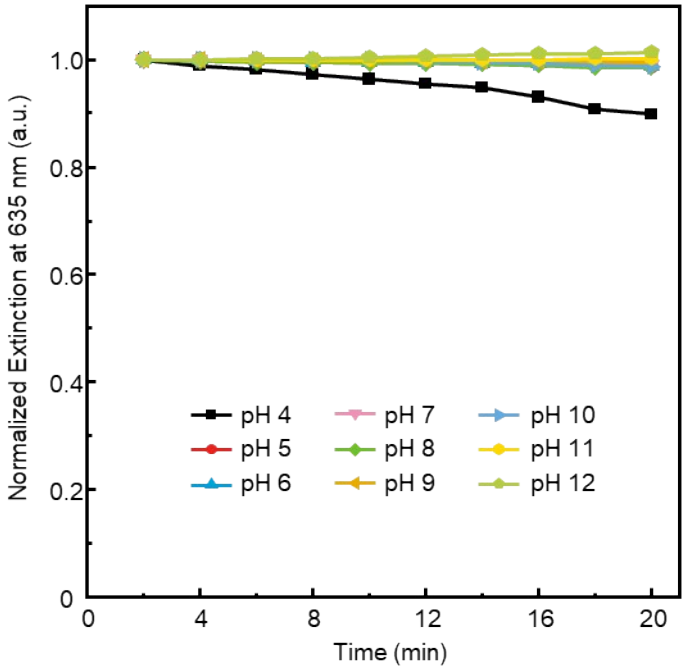

Figure S28. Stability test for the A_2T_{12}-AuNRs under different pH conditions. The curves show the normalized UV-Vis absorption intensity of the A_2T_{12}-AuNRs at 635 nm under different pH conditions over time.

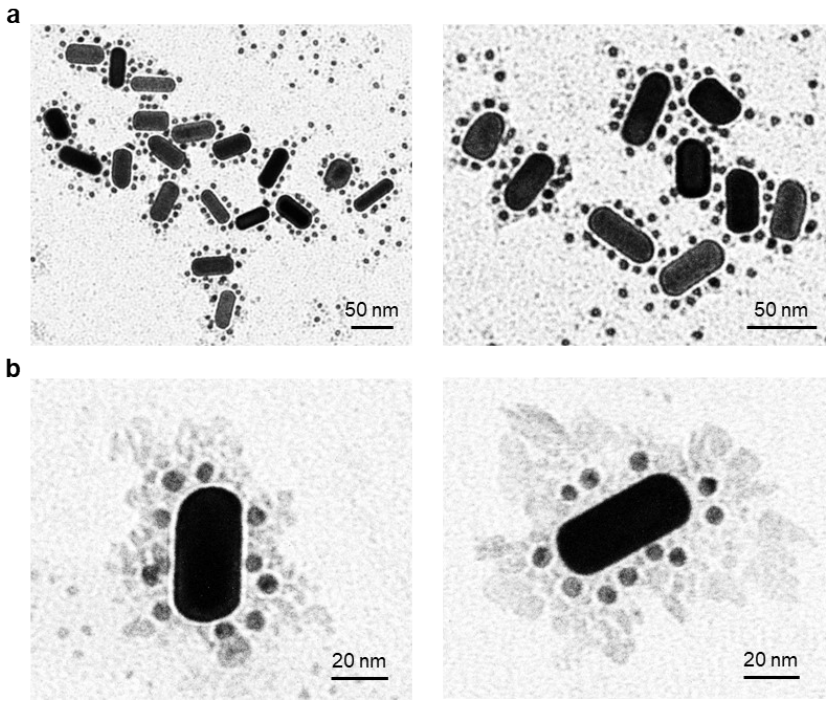

Figure S29. Representative TEM images at (a) small and (b) large magnifications of the core-satellite nano-assemblies produced by DNA hybridization of the $A_2N_{10}T_{10}$-AuNRs (cores) and the $A_{70}N_{10}$-AuNPs (5 nm, satellites).