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Electronic Supplementary Information

DNA-functionalized silver nanoclusters as a chemopalette: tunable fluorescence for turn-on detection of cysteine†

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Experimental Section

Materials and Measurements:

Silver nitrate ($AgNO_3$), 99.9995%, and sodium borohydride ($NaBH_4$), 98%, were purchased from Alfa Aesar and used without further purification. The DNA oligonucleotides were purchased from Sangon (Shanghai, China). All other reagents were all of analytical grade. All solutions were prepared with MilliQ water (18.2 MΩ. cm) from a Millipore system. Unless otherwise noted, experiments were carried out in 10 mM Tris/HCl buffer solution (pH 7.0).

Fluorescence measurements were carried out by using an FLS-920 picosecond fluorescence lifetime spectrometer (Edinburgh Instruments, UK). UV-vis spectra were recorded by an Agilent 8453 UV-vis spectrophotometer (Agilent Technologies Co. Ltd., USA). XPS measurements were performed on a Thermo ESCALAB 250 instrument configured with a monochromated $Mg_{K\alpha}$ (1486.8 eV) 150 W X-ray source, 0.5 mm circular spot size, a flood gun to counter charging effects, and the analysis chamber base pressure $< 1 \times 10^{-9}$ mbar; data were collected with FAT = 20 eV. The takeoff angle, defined as the angle between the substrate normal and the detector, was fixed at 0º. The samples were dropped on the surface of the silicon substrate by natural evaporation. All binding energies were calibrated using either the $Au_{4f7/2}$ peak (84.0 eV) or the C_{1s} carbon peak (284.6 eV). HR-TEM images were taken with a JEM-2011 high-resolution transmission electron microscopy operating at 200 kV. The as-prepared Ag nanoclusters were dried on carbon-coated copper grids by slow natural evaporation. The fluorescence lifetimes were measured with Fluorescence lifetime spectrometer C11367 instrument.

Preparation of silver nanoclusters (Ag NCs):

For the preparation of the DNA-Ag NCs, certain volume of $AgNO_3$ solution was introduced into aliquot volume of DNA solution in 10 mM Tris/HCl buffer solution (pH 7.0). The mixture was kept at 0 °C for 15 minutes. Then, the mixture was reduced by quickly adding $NaBH_4$ (must be freshly prepared before use) under vigorously shaking for 2 min.[1] Final concentrations were 15 μM in the DNA template, 90 μM in $AgNO_3$ and 90 μM in $NaBH_4$. The reaction mixture was kept in the dark at 4 °C for another 3 hours before use.

DNA-Ag NCs based sensor for fluorescence turn-on (or turn-off) detection of cysteine:

Cysteine was activated by tris-(2-carboxyethyl)-phosphine (TCEP) solution (40 mM, freshly prepared) before used. Certain volume of DNA-Ag NCs was added into test tube. Then, a series of dilutions of cysteine were pipetted into the test tubes by using microsyringes before fluorescence measurement. Fluorescence spectra were made based on the data collected on the first minute after the addition of cysteine.[2]

References

1 J. T. Petty, J. Zheng, N. V. Hud and R. M. Dickson, *J. Am. Chem. Soc.,* 2004, **126**, 5207-5212.

2 Z. G. Chen, G. L. Liu, M. H. Chen, Y. R. Peng and M. Y. Wu, *Anal. Biochem.,* 2009, **384**, 337-342.

Table S1. DNA sequences of the preparation of DNA-Ag NCs. The sequences of all oligonulceotides are listed in the 5' to 3' direction.

Name	Sequences (5'-3')	DNA size (bp)
C-rich ssDNA	$(CCCTAA)_3CCCTA$	23
m1 ssDNA	$(CGCTAA)_3CGCTA$	23
m2 ssDNA	$(GCCTAA)_3GCCTA$	23
m3 ssDNA	$(CCGTAA)_3CCGTA$	23
m4 ssDNA	$(CGGTAA)_3CGGTA$	23
m5 ssDNA	$(GGCTAA)_3GGCTA$	23
m6 ssDNA	$(GCGTAA)_3GCGTA$	23
m7 ssDNA	$(GGGTAA)_3GGGTA$	23

Table S2. Optical properties of ssDNA templated silver nanoclusters.

Nanocluster	Excitation peak (nm)	Emission peak (nm)	Color
C-rich ssDNA-Ag NCs	468	560	yellow
m1 ssDNA-Ag NCs	466	554	yellow green
m3 ssDNA-Ag NCs	439	495	cyan green
m5 ssDNA-Ag NCs	453	515	green
m6 ssDNA-Ag NCs	548	623	orange
m7 ssDNA-Ag NCs	560	626	red

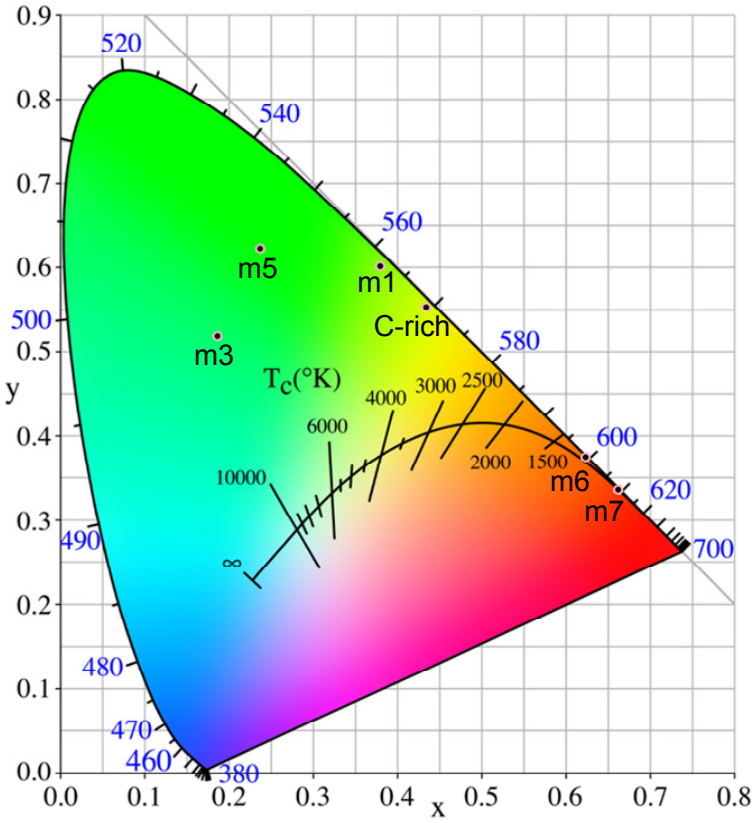


Figure S1. The image of CIE1931 Chromaticity Coordinate Calculation of ssDNA templated Ag NCs (Ag NCs). (a), C-rich ssDNA-Ag NCs; (b), m1 ssDNA-Ag NCs; (c), m3 ssDNA-Ag NCs; (d), m5 ssDNA-Ag NCs; (e), m6 ssDNA-Ag NCs; (f), m7 ssDNA-Ag NCs.

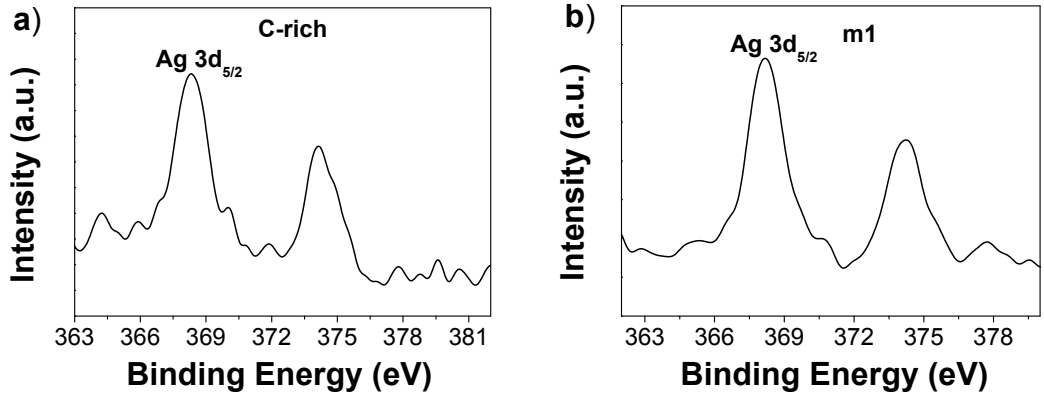


Figure S2. The Ag3d XPS spectra of synthesized (a) C-rich ssDNA-Ag NCs, (b) m1 ssDNA-Ag NCs.

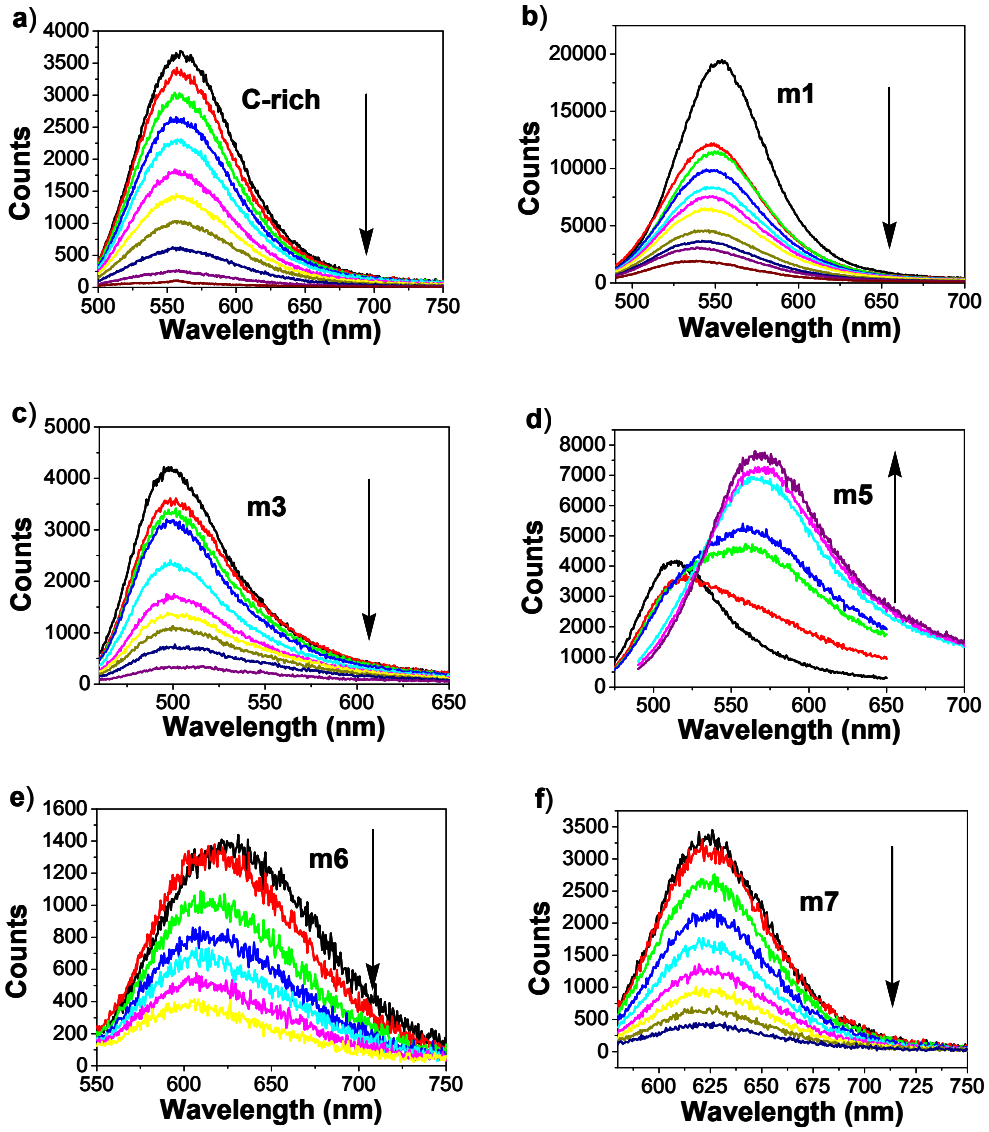


Figure S3. Fluorescence spectra of ssDNA-Ag NCs in the presence of increasing cysteine concentrations. (a) 1-9 (top-bottom): 1, rich-C ssDNA-Ag NCs; 2-9, 1 + cysteine (μM): 0.5, 1, 1.5, 2, 3, 8, 13, 18. (b) 1-11 (top-bottom): 1, m1 ssDNA-Ag NCs; 2-9, 1 + cysteine (μM): 0.0333, 0.0666, 0.567, 1.067, 1.567, 4.067, 9.067, 14.067, 19.067, 24.067. (c) 1-10 (top-bottom): 1, m3 ssDNA-Ag NCs; 2-10, 1 + cysteine (μM): 0.5, 1, 3.5, 8.5, 13.5, 18.5, 23.5, 28.5, 33.5. (d) 1-7 (bottom-top): 1 (black), m5 ssDNA-Ag NCs; 2-10, 1 + cysteine (μM): 0.5 (2, red), 1.5 (3, green), 2.5 (4, blue), 5 (5, cyan), 7.5 (6, magenta), 12.5 (7, purple). (e) 1-7 (top-bottom): 1, m6 ssDNA-Ag NCs; 2-10, 1 + cysteine (μM): 0.5, 1.5, 2.5, 3.5, 6, 8.5. (f) 1-9 (top-bottom): 1, m7 ssDNA-Ag NCs; 2-10, 1 + cysteine (μM): 0.5, 1.5, 2.5, 3.5, 4.5, 5.5, 6.5, and 7.5.

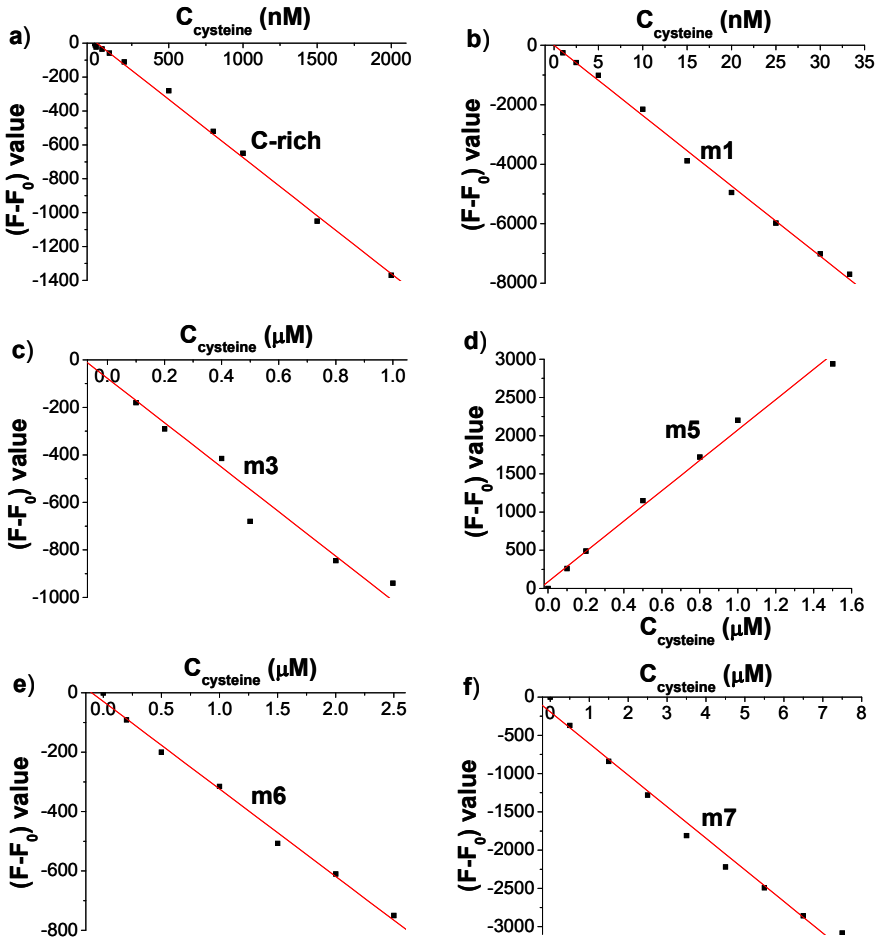
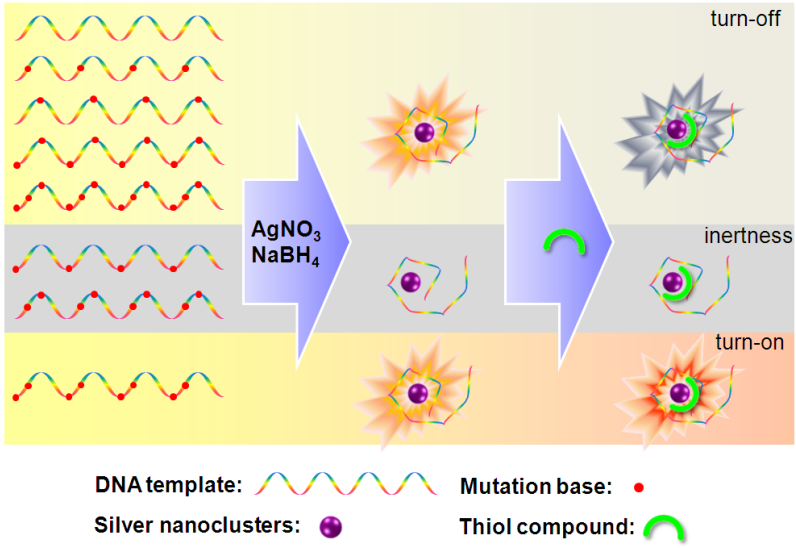


Figure S4. Linear plots of the enhancement value or reduction values of fluorescence intensities versus the concentration of cysteine. (a), C-rich ssDNA-Ag NCs; (b), m1 ssDNA-Ag NCs; (c), m3 ssDNA-Ag NCs; (d), m5 ssDNA-Ag NCs; (e), m6 ssDNA-Ag NCs; (f), m7 ssDNA-Ag NCs. The solid line represents a linear fit to the data. All experiments were performed in three times.

Table S3. Relationship between the concentration of cysteine (c) and the change of fluorescence intensity (ΔI_{FL}) by using different templated silver nanoclusters sensors.

DNA-Ag NCs	Linear ranges	Regression equation	R value	Detection Limit
C-rich ssDNA	50-2000 (nM)	ΔI_{FL} = -13.62 + 0.69c (nM)	0.9985	20 nM
m1-ssDNA	1-35 (nM)	ΔI_{FL} = -2.77 + 236.51c (nM)	0.9982	0.5 nM
m3-ssDNA	100-1000 (nM)	ΔI_{FL} = 77.37 + 936.14c (μM)	0.9784	50 nM
m5-ssDNA	100-1500 (nM)	ΔI_{FL} = 87.53 + 1987.14c (μM)	0.9967	45 nM
m6-ssDNA	200-2500 (nM)	ΔI_{FL} = 28.99 + 294.81c (μM)	0.9967	90 nM
m7-ssDNA	400-7500 (nM)	ΔI_{FL} = 193.75 + 412.73c (μM)	0.9926	150 nM



Scheme S1. Schematic illustration of the fluorescence response patterns of ssDNA-templated Ag NCs to thiol compounds.

Table S4. Comparison of the linear range and detect limit for the detection of cysteine using different fluorescent probes.

Method	Linear range	Detect limit	Reference
Au NPs-based near-infrared fluorescent	4-250 μM	10 nM	14a
PMAA-Ag NCs	25-6000 nM	20 nM	14b
CdTe/CdSe quantum dots	0.2-100 μM	131 nM	15
DNA-Ag NCs	8-100 nM	4 nM	13a
DNA-Ag NCs	25-200 nM	2.1 nM	13b
DNA-Ag NCs (turn-on pattern)	100-1500 nM	45 nM	This work
DNA-Ag NCs (turn-off pattern)	1-7500 nM	0.5 nM	This work

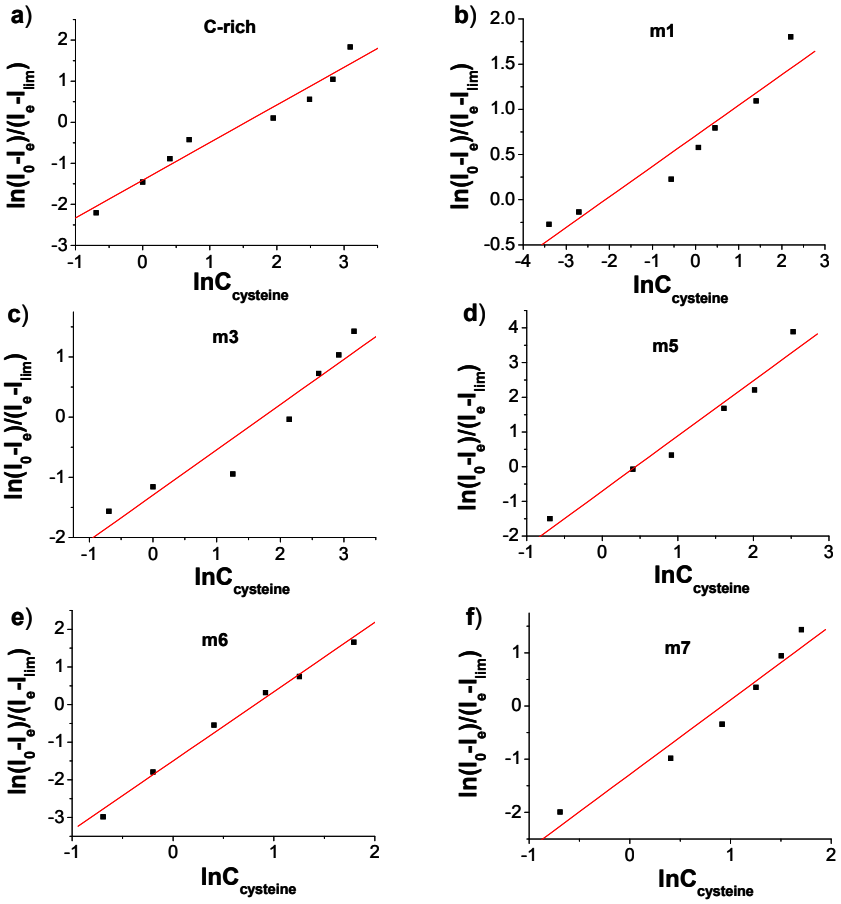


Figure S5. The linear plots of calculating the stability constant. (a), C-rich ssDNA-Ag NCs; (b), m1 ssDNA-Ag NCs; (c), m3 ssDNA-Ag NCs; (d), m5 ssDNA-Ag NCs; (e), m6 ssDNA-Ag NCs; (f), m7 ssDNA-Ag NCs. The solid line represents a linear fit to the data.

According to the change of intensity located at the maximum emission wavelength versus the concentration of cysteine, the stability constant of nanoclusters with cysteine can be calculated by using the equation as follows:[3]

$$\ln\frac{I_0 - I_e}{I_e - I_{\lim}} = \ln K_s + n\ln C$$

Where, I_0 and I_e represent the fluorescent intensity of silver nanoclusters in the absence or presence of cysteine while I_{lim} represents the limit value of fluorescent intensity of silver nanoclusters in the presence of cysteine. C is the concentration of added cysteine. n is the number of bound cysteine molecules per nanoclusters (stoichiometry). K_s is the stability constant of nanoclusters with cysteine.

References

3 A. P. De Silver, D. B. Fox, T. S. Moody and S. M. Weir, *Trends in Biotechnology,* 2001, **19**, 29-33.

Table S5. The parameters of the interaction between DNA-Ag NCs and cysteine.

DNA-Ag NCs	Stability Constant (K_s, L/mol)	Stoichiometry (n)
C-rich ssDNA	0.244×10^6	0.9174
m1-ssDNA	2.03×10^6	0.3380
m3-ssDNA	0.274×10^6	0.7525
m5-ssDNA	0.495×10^6	1.5906
m6-ssDNA	0.223×10^6	1.8415
m7-ssDNA	0.276×10^6	1.4025

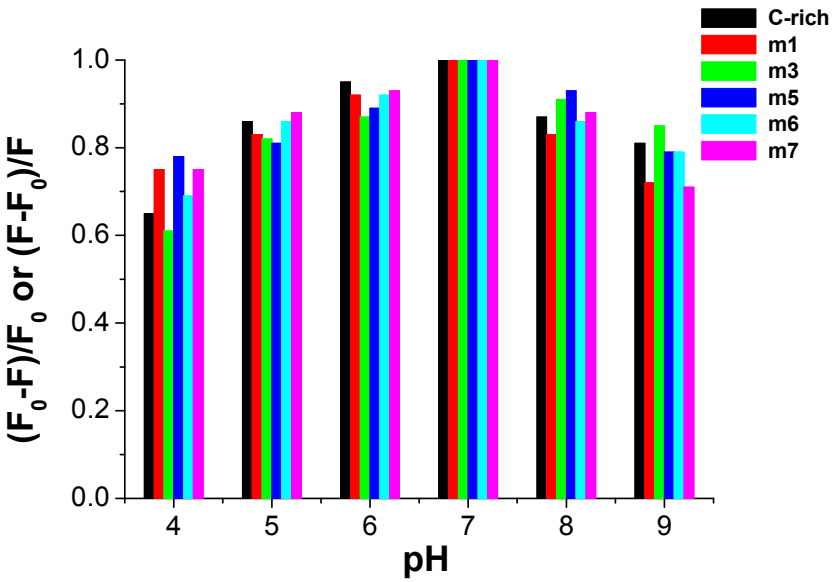


Figure S6. Effect of pH on the ssDNA-Ag NCs sensor based assay for detecting cysteine in Tris/HCl buffer. (A), black, C-rich ssDNA-Ag NCs; (B), red, m1 ssDNA-Ag NCs; (C), green, m3 ssDNA-Ag NCs; (D), blue, m5 ssDNA-Ag NCs; (E), cyan, m6 ssDNA-Ag NCs; (F), magenta, m7 ssDNA-Ag NCs.

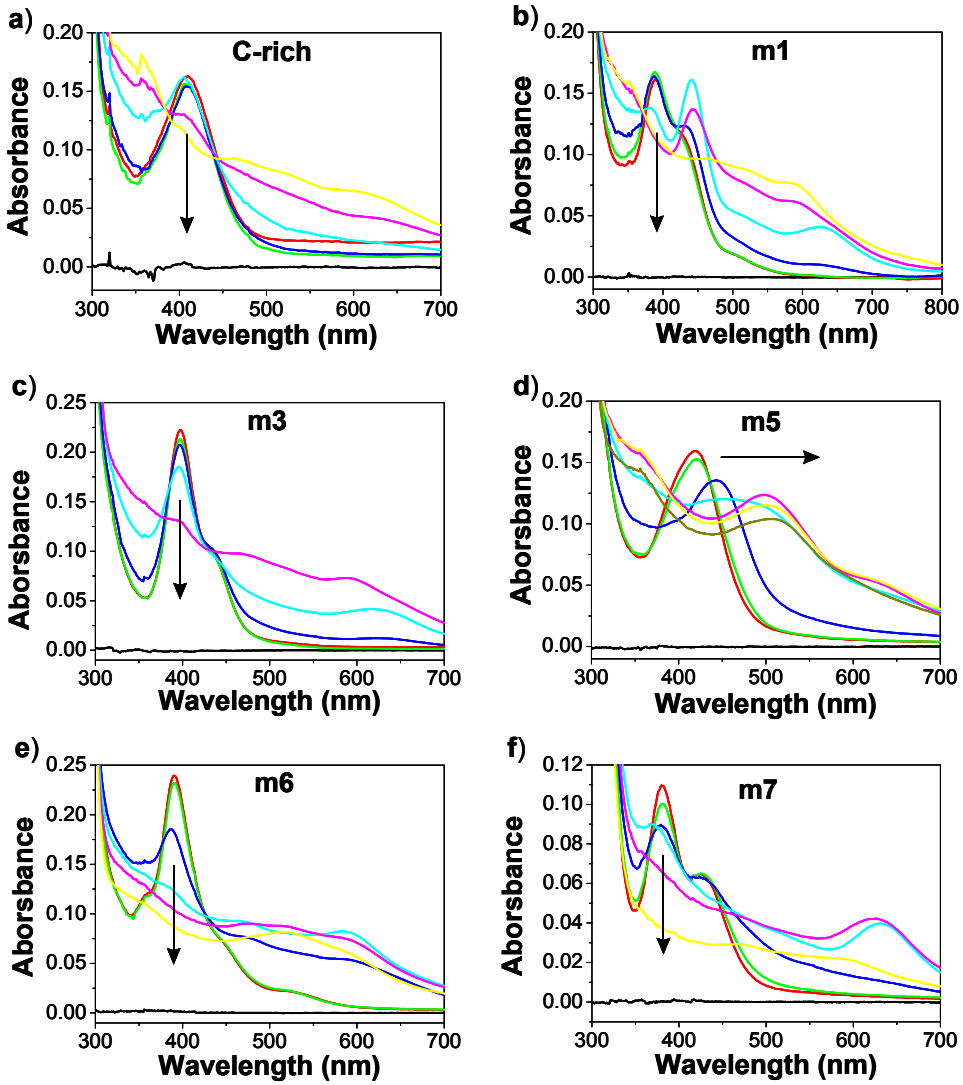


Figure S7. UV-vis spectra of the ssDNA-Ag NCs in the absence and presence of cysteine solution at room temperature.

(a) 1 (black), buffer solution; 2 (red), rich-C ssDNA-Ag NCs; 3-7: 2 + cysteine (μM): 1 (green), 3 (blue), 13 (cyan), 33 (magenta), 83 (yellow).

(b) 1 (black), buffer solution; 2 (red), m1 ssDNA-Ag NCs; 3-7: 2 + cysteine (μM): 1 (green), 11 (blue), 31 (cyan), 81 (magenta), 131 (yellow).

(c) 1 (black), buffer solution; 2 (red), m3 ssDNA-Ag NCs; 3-6: 2 + cysteine (μM): 1 (green), 11 (blue), 31 (cyan), 51 (magenta).

(d) 1 (black), buffer solution; 2 (red), m5 ssDNA-Ag NCs; 3-8: 2 + cysteine (μM): 1 (green), 11 (blue), 31 (cyan), 51 (magenta), 71 (yellow), 121 (dark yellow).

(e) 1 (black), buffer solution; 2 (red), m6 ssDNA-Ag NCs; 3-7: 2 + cysteine (μM): 1 (green), 51 (blue), 101 (cyan), 151 (magenta), 251 (yellow).

(f) 1 (black), buffer solution; 2 (red), m7 ssDNA-Ag NCs; 3-7: 2 + cysteine (μM): 2 (green), 12 (blue), 32 (cyan), 82 (magenta), 182 (yellow).