Carbon-Dot-Triggered Aggregation/Dispersion of Gold Nanoparticles for Colorimetric Detection of Nucleic Acid and Its Application in Visualization of Loop-Mediated Isothermal Amplification

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Supporting Information

Preparation of the CDs

Briefly, 5 mL *p*PDA (10 mM) was added to 15 mL deionized water and incubated for 2 h. The resulting solution turned red, indicating the formation of a radical cation or semiquinone radical. The solution was then hydrothermally treated in a 50-mL Teflon-lined autoclave at 160 °C for 4 h. After the reaction, the autoclave was allowed to cool naturally, and the resulting pink solution was centrifuged at 10,000 rpm to remove larger residues. Following centrifugation, the solution was filtered through a Millipore membrane (0.45 μm) and dialyzed with a dialysis bag to eliminate unreacted precursor molecules. The obtained solution was freeze-dried to yield the CDs (~ 15 mg).

Synthesis of the AuNPs

AuNPs were prepared by reducing $HAuCl_4$ with trisodium citrate. Briefly, 0.1% trisodium citrate was rapidly added to a boiling aqueous solution containing 0.01% $HAuCl_4$. Then, the solution was heated for 20 min, and the appearance of a wine-red color indicated the formation of AuNPs (AuNPs concentration: 15 nM).[1] The products were cooled at room temperature and then stored in a refrigerator at 4 °C for further use.

Material Characterization

UV-Vis absorption and emission spectra were recorded using a Tecan Infinite 200 Pro microplate reader equipped for absorption and fluorescence measurements. The emission and excitation profiles of the CDs were obtained at their respective excitation wavelengths. UV-Vis absorption titration experiments of the AuNPs/CDs probe in the absence and presence of DNA were performed at room temperature (pH = 7). The FT-IR spectra of the CDs were acquired by pelleting it with KBr (JASCO, Japan). The NMR spectra of the CDs were obtained using a Bruker Avance 400-MHz instrument. The HR-TEM images of the AuNPs and CDs were obtained using an HR-TEM instrument (JEOL JEM 2100) operating at 200 kV. The average particle sizes of the AuNPs and CDs were determined using ImageJ software. The LAMP reaction was performed using a PCR machine (Thermo Fisher Scientific). The surface charge analysis was conducted using a Malvern ZetaSizer. To calculate the limit of detection (LOD) of the Au NP/CD probe, the following relationship was utilized: $LOD = 3S/m$, where S represents the standard deviation of blank measurements (n = 10) and m is the slope obtained from the calibration plot. The fluorescence quantum yield (QY) of the CDs relative to Rhodamine 6G was determined using Equation 1;

$$Q_S = Q_R\ (I_S/I_R)\ (OD_R/OD_S)\ n_S^2/n_R^2 \qquad (1)$$

where “Q” is the fluorescence quantum yield, “I” is the integrated fluorescence intensity, “n” is the refractive index of the solvent, and “OD” is the optical density obtained from absorbance measurements. The subscript “S” denotes sample, while “R” denotes the reference standard, which in this case is Rhodamine 6G, with a known quantum yield of 0.95.

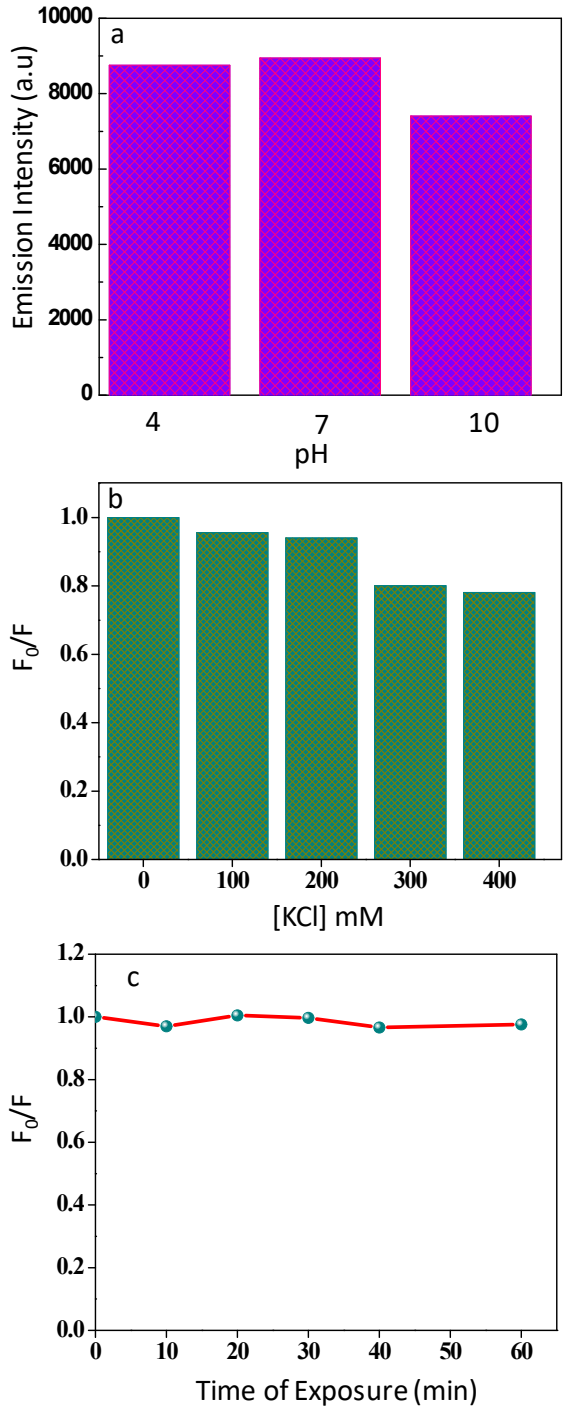

Figure S1. The Influence of (a) different pH conditions, (b) ionic strength and (c) UV irradiation time on the fluorescence intensity of CDs.

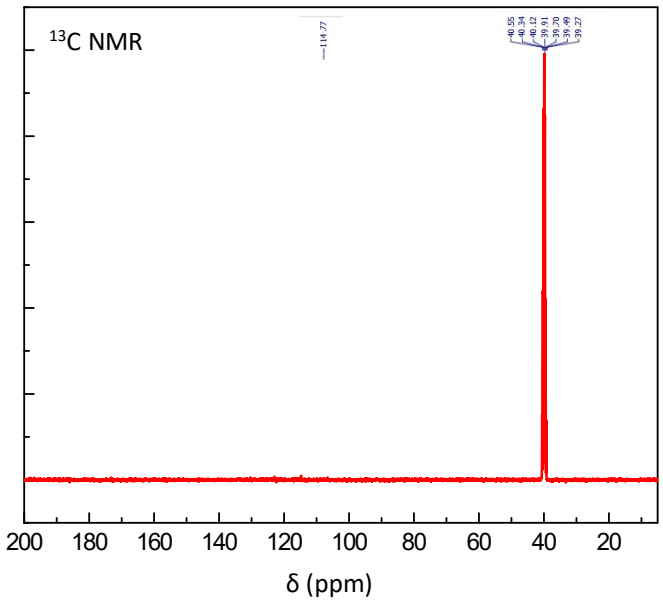

Figure S2. ^{13}C NMR spectrum of CDs.

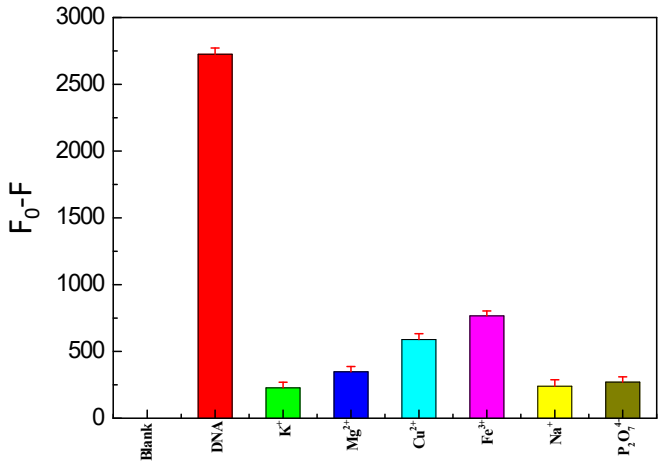

Figure S3. The fluorescence response of CDs toward DNA (14 nM) and other metal/buffering ions (10 μM).

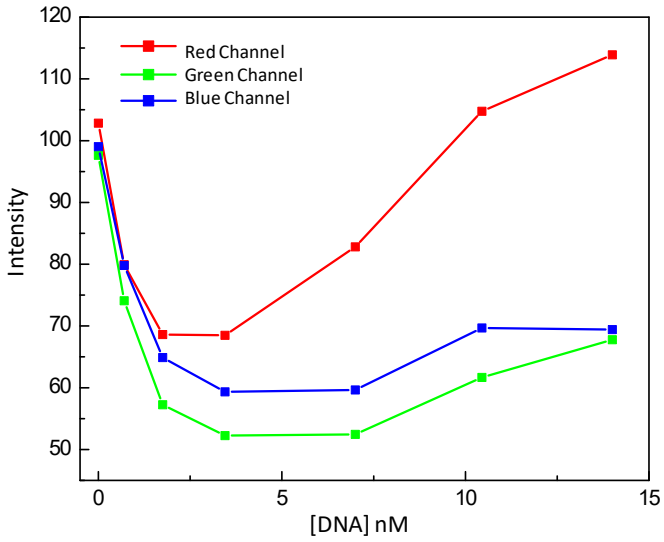

Figure S4. Plot of inensity of the individual red, green and blue components (RGB) of AuNPs/CDs *vs* different concentration of DNA.

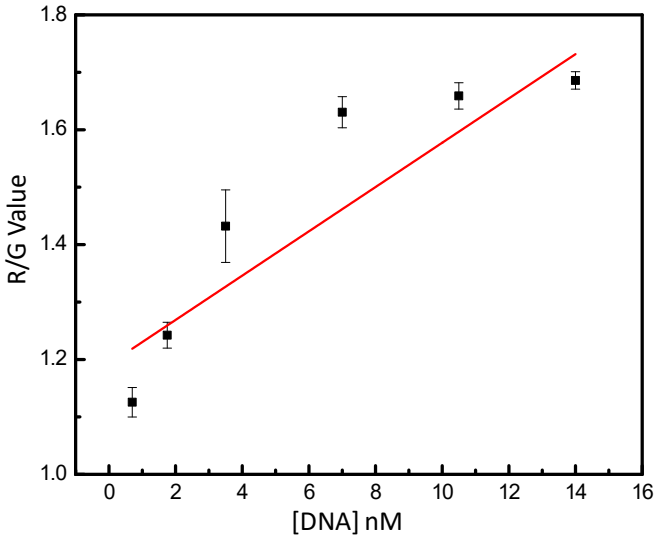

Figure S5. Plot of R/G inensity ratio of AuNPs/CDs *vs* different concentration of DNA.

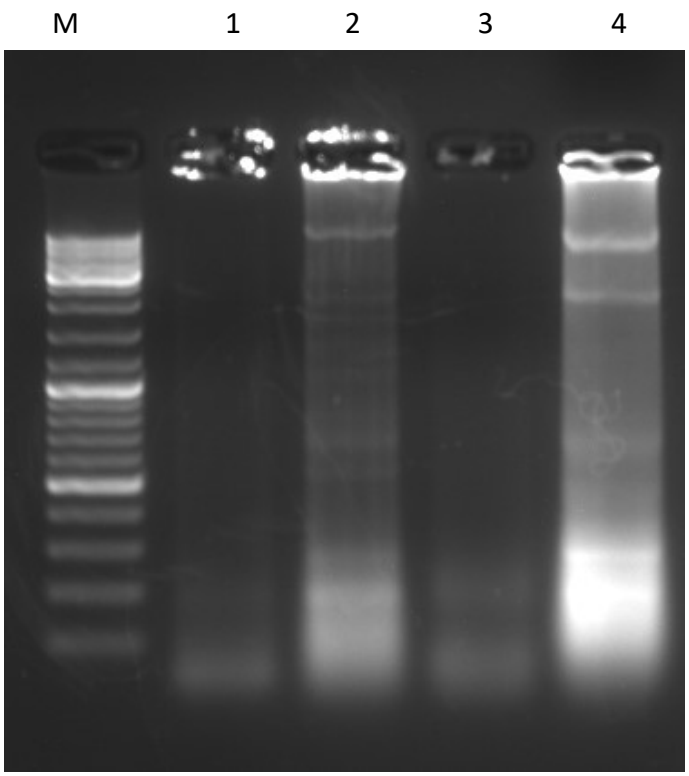

Figure S6. Agarose gel electrophoresis: Lanes 1 and 3 represent NTC samples, respectively, and lanes 2 and 4 represent the amplified samples.

Table S1. LAMP primer sequences used for the detection of *E. coli* targeting the malB region.[2]

Primer name	Primer sequence (5' - 3')
F3	GCCATCTCCTGATGACGC
B3	ATTTACCGCAGCCAGACG
FIP (F1c + F2)	CTGGGGCGAGGTCGTGGTATTCCGACAAACACCACGAATT
BIP (B1 + B2c)	CATTTTGCAGCTGTACGCTCGCAGCCCATCATGAATGTTGCT
LF	CTTTGTAACAACCTGTCATCGACA
LB	ATCAATCTCGATATCCATGAAGGTG

Table S2. Comparison of different detection strategies for detection of nucleic acids.

System	Detecting strategy	Analyte	LOD	Ref
AuNPs-SYBR Green I	Colorimetric	DNA	8 pM	[1]
Fluorescent carbon dots	Fluorescence	RNA	0.062 μg/mL	[3]
Carboxylic carbon quantum dots	Fluorescence	DNA	17.4 nM	[4]
Silver ion reduction	Colorimetric	DNA	1 nM	[5]
Dextrin-capped AuNPs	Colorimetric	DNA	2.9 fM	[6]
AuNPs/CDs	Colorimetric	DNA	1.70 nM	This work

References

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