Electronic Supplementary Information (ESI) for Chemical Communications

Effect of Single Xeno Nucleic Acid Replacement on the Fluorescence of DNA-encapsulated Silver Nanoclusters

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Materials and Methods

Oligonucleotides and Chemicals: All oligos were obtained from mBiotech, (IDT company, Korea) or Bioneer Co., Ltd., Korea. Oligos were dissolved in DEPC water to obtain a stock concentration of 100 μM. AgNCs were synthesized using silver nitrate ($AgNO_3$, >99.99%) and sodium borohydride ($NaBH_4$, 99.99%) from Sigma-Aldrich. 40% Acrylamide: Bis-acrylamide (19:1) was obtained from Sigma Aldrich (Yongin City, Korea), TEMED (tetramethylethylenediamine) from Bio-Rad (Hong Kong, China).

Synthesis of AgNCs: For the synthesis of fluorescent AgNCs, a reaction mixture of 25 μL containing 15 μM of the DNA oligo, 25 mM $NaNO_3$ and 20 mM Tris–acetate buffer (pH 6.5) were denatured at 95 °C for 10 mins annealed at 25 °C for 20 mins. Then $AgNO_3$ and $NaBH_4$ were added and vortexed each to a final concentration of 250 μM in a reaction mixture of 50 μL. These samples were prepared systematically to obtain optimal results. The concentration of nucleic acid here is given for a total reaction volume of 50 μL. The DNA/AgNCs were incubated for 1 h at 25 °C and eventually used for further analysis. The excitation and emission spectra were measured after dilution to 200 μL using DEPC water. All fluorescent measurements were carried in a 96-well disposable plate using CLARIOstar from BMG Labtech.

Time-dependent stability studies: For time-dependent on stability studies, samples were prepared as mentioned above. The fluorescence of the XNA/AgNCs samples was measured at 1, 2, 3 and 6 h. The emission intensities were recorded at an Ex/Em: 560/640 nm and 480/590 nm.

In-gel Fluorescence Assay: The as-synthesized DNA/AgNCs were analysed using native polyacrylamide gel electrophoresis (PAGE) (12%) in a MiniPROTEAN Tetra Cell system (Bio-Rad) with TBE buffer (Tris base (44.5 mM), Boric acid (44.5 mM), EDTA (1 mM)). DNA/AgNCs

were prepared as depicted above. To half of the 50 μL reaction mixture, with 2.25 μL of 50% glycerol and 0.5 μL of 200X SYBR Gold dye (SYBR Gold Nucleic Acid Gel Stain, Thermo Fisher Scientific S11494) were added. NATIVE PAGE was performed at 90 V/1 h followed by visualization under the UV transilluminator. A Canon EOS 750D DSLR camera with an EF-S 18-55mm lens was used to capture the gel images under UV light.

Time-Correlated Single-Photon Counting (TCSPC) Measurement The fluorescence lifetimes of the nanoclusters were measured using a TCSPC. A solution containing DNA/AgNCs in water was placed onto a coverslip and measured using a confocal microscope (HC PL APO CS2 40 × /1.10 WATER CORR CS2, Leica TCS SP8). The DNA/AgNCs were excited by laser beams (20 MHz 488 nm) and detected by HyD SMD (between 580 nm) connected to a TCSPC board (HydraHarp 400, Picoquant). The decay curves were fitted by a double-exponential function using SymPhoTime software (Picoquant).

ESI-MS spectroscopy DNA-encapsulated silver nanoclusters were analyzed using Electrospray Ionization Mass Spectrometry (ESI-MS) in negative ion mode on a SolariX XR Bruker mass spectrometer, with a mass range spanning 150 to 4000 m/z. The nanoclusters were prepared according in the described protocols and used without further purification. Immediately prior to analysis, aliquots of 5–25 μL were diluted in 300–600 μL of 10 mM ammonium formate (pH 7) and directly injected into the mass spectrometer at a flow rate of 420 μL h^{-1}. The instrument was operated under optimized conditions. Data acquisition was performed over 16 to 64 scans within a targeted mass window of 400–800 m/z, capturing signals of interest with high resolution. All data were collected and processed using Compass FTMS Control and Compass DataAnalysis software.

UV-Vis and CD spectroscopy

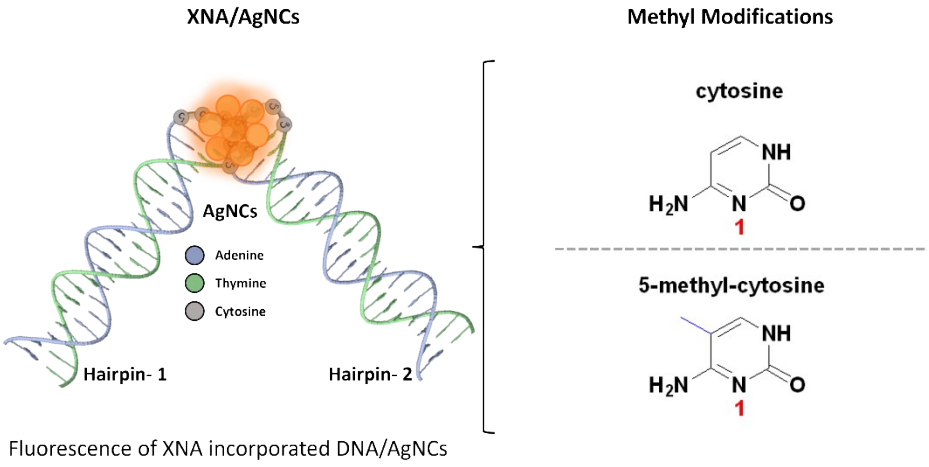
UV-Vis absorbance data was recorded in the visible to near infrared range on a Jasco V-780 at room temperature using a 1 cm quartz cuvette for AgNCs formed from DNA sequences with RNA, LNA, and ANA- sugar backbone modifications at the 2^{nd} and 7^{th} position of the 7C loop. Similarly, far-UV Circular dichroism (CD) spectroscopy using a Jasco J-1500 CD instrument at room temperature in a 1 mm quartz cell in the range of 360 nm to 180 nm in continuous mode with a bandwidth of 1 nm, in the data pitch of 1 nm and a scanning speed of 50 nm/min. The D.I.T was set to 4 s and each sample was averaged based on 10 scans. Jasco Spectra Analysis software was used for CD and UV-Vis data analysis. A corresponding reference spectrum of a buffer solution was subtracted from each sample spectrum. Data sets were truncated in the presented figures to exclude invalid data due to high sample absorbance.

Table S1. 5′ to 3′ sequences of DNA oligomers used.

Sugar modification	Sequence
DNA	AAAAAAAAACCCCCCCTTTTTTTTTTTTTTTTTTTTTTTTTTTT
1-LNA	AAAAAAAAAC_LCCCCCCTTTTTTTTTTTTTTTTTTTTTTTTTTTT
2-LNA	AAAAAAAAACC_LCCCCCTTTTTTTTTTTTTTTTTTTTTTTTTTTT
3-LNA	AAAAAAAAACCC_LCCCCTTTTTTTTTTTTTTTTTTTTTTTTTTTT
4-LNA	AAAAAAAAACCCC_LCCCTTTTTTTTTTTTTTTTTTTTTTTTTTTT
5-LNA	AAAAAAAAACCCCC_LCCTTTTTTTTTTTTTTTTTTTTTTTTTTTT
6-LNA	AAAAAAAAACCCCCC_LCTTTTTTTTTTTTTTTTTTTTTTTTTTTT
7-LNA	AAAAAAAAACCCCCCCC_LTTTTTTTTTTTTTTTTTTTTTTTTTTTT
1-ANA	AAAAAAAAAC_ACCCCCCTTTTTTTTTTTTTTTTTTTTTTTTTTTT
2-ANA	AAAAAAAAACC_ACCCCCTTTTTTTTTTTTTTTTTTTTTTTTTTTT
3-ANA	AAAAAAAAACCC_ACCCCTTTTTTTTTTTTTTTTTTTTTTTTTTTT
4-ANA	AAAAAAAAACCCC_ACCCTTTTTTTTTTTTTTTTTTTTTTTTTTTT
5-ANA	AAAAAAAAACCCCC_ACCTTTTTTTTTTTTTTTTTTTTTTTTTTTT
6-ANA	AAAAAAAAACCCCCC_ACTTTTTTTTTTTTTTTTTTTTTTTTTTTT
7-ANA	AAAAAAAAACCCCCCCC_ATTTTTTTTTTTTTTTTTTTTTTTTTTTT
1-RNA	AAAAAAAAAC_RCCCCCCTTTTTTTTTTTTTTTTTTTTTTTTTTTT
2-RNA	AAAAAAAAACC_RCCCCCTTTTTTTTTTTTTTTTTTTTTTTTTTTT
3-RNA	AAAAAAAAACCC_RCCCCTTTTTTTTTTTTTTTTTTTTTTTTTTTT
4-RNA	AAAAAAAAACCCC_RCCCTTTTTTTTTTTTTTTTTTTTTTTTTTTT
5-RNA	AAAAAAAAACCCCC_RCCTTTTTTTTTTTTTTTTTTTTTTTTTTTT
6-RNA	AAAAAAAAACCCCCC_RCTTTTTTTTTTTTTTTTTTTTTTTTTTTT
7-RNA	AAAAAAAAACCCCCCCC_RTTTTTTTTTTTTTTTTTTTTTTTTTTTT
2-(5MeC) DNA	AAAAAAAAAC$C_{(O\text{-}Me)}$CCCCCTTTTTTTTTTTTTTTTTTTTTTTTTTTT
4-(5MeC) DNA	AAAAAAAAACCCC$C_{(O\text{-}Me)}$CCCTTTTTTTTTTTTTTTTTTTTTTTTTTTT
7-(5MeC) DNA	AAAAAAAAACCCCCCC$C_{(O\text{-}Me)}$TTTTTTTTTTTTTTTTTTTTTTTTTTTT
7C-30T-miR-21-22bp	TAGCTTATCAGACTGATGTTGACCCCCCCTTTTTTTTTTTTTTTTTTTTTTTTTTTT
7C-9A-miR-21-22bp	AAA AAA AAACCCCCCCTCAACATCAGTCTGATAAGCTA
6C-miR-159-8bp	GAGCTCTACCCCCCTAGAGCTCCCTTCAATCCAAA
10A-10T-7C-10T	AAAAAAAAAATTTATTTTTTTTTTCCCCCCCTTTTTTTTTT
9A-6C-30T	AAAAAAAAACCCCCCTTTTTTTTTTTTTTTTTTTTTTTTTTTT

Notes: The table indicates the 5′ to 3′ oligo sequences wherein the red Cytosines indicate the silver binding site, while purple, green, blue and gold colors denote the site of sugar backbone modifications LNA (L), ANA (A), RNA (R), and methylated DNA

respectively.



Scheme 1 Schematic representation of XNA/AgNCs. Schematic representation of the XNA/AgNCs encapsulated in the respective template DNA (left). The chemical structures of Cytosine and 5-methyl-Cytosine (5MeC) nitrogenous bases (right).

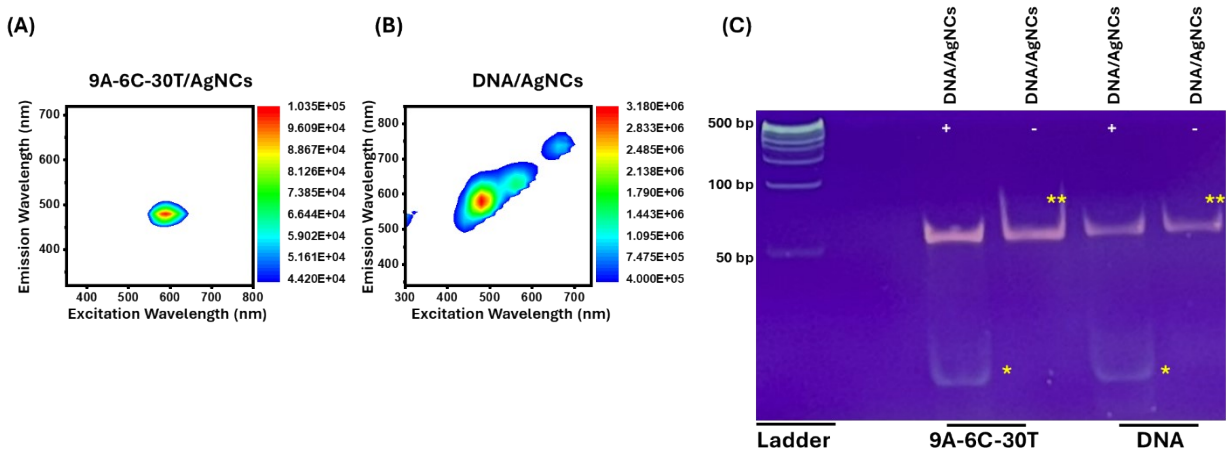


Fig. S1 In-gel Fluorescence assay of DNA/AgNCs. (A, B) Fluorescence emission profile of DNA/AgNCs encapsulated in their respective 9A-6C-30T (A) and DNA (9A-7C-30T) (B) templates. Samples were excited for every 10 nm from 320 nm to 720 nm. (C) Comparative structural analysis of DNA/AgNCs corresponding to the 9A-6C-30T and DNA templates. Samples were visualized either with SYBR Gold DNA staining (+) or AgNCs (-). Ladder indicates from bottom 50 bp. Single and double asterisks indicate the monomeric and dimeric bands, respectively.

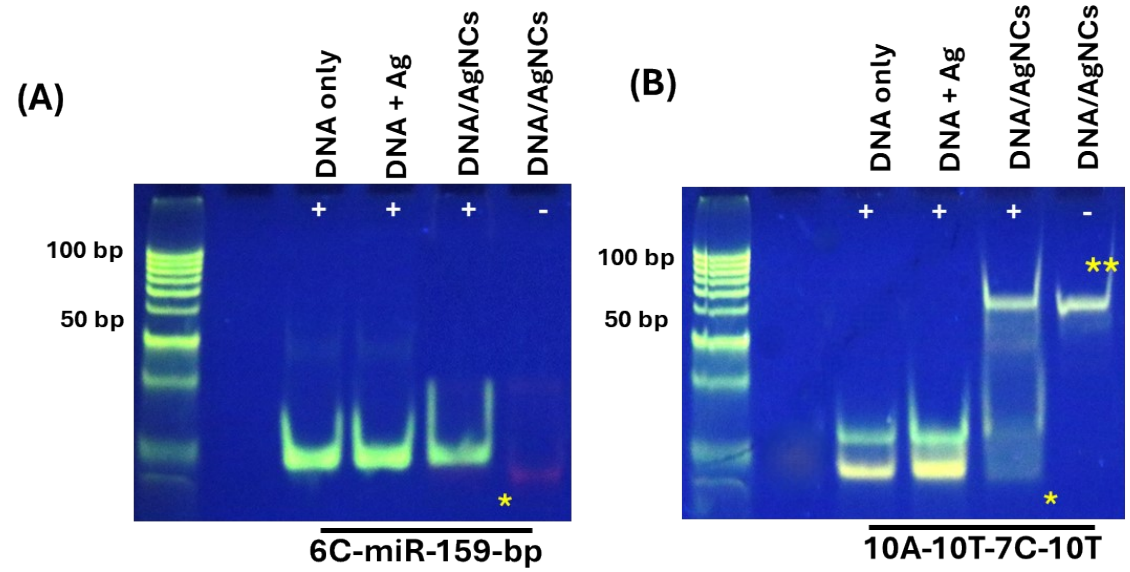


Fig. S2 In-gel Fluorescence assay of DNA/AgNCs. (A) Comparative structural analysis of hairpin DNA/AgNCs (A), with respect to the 10A-10T-7C-10T-dimer DNA/AgNCs (B). Samples were visualized either with SYBR (+) or without (-) SYBR Gold DNA staining. Single and double asterisks indicate the monomeric and dimeric bands.

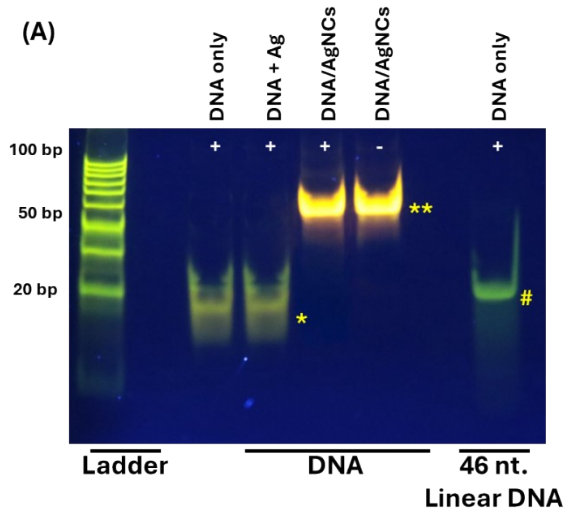


Fig. S3 Structural analysis using In-gel fluorescence assay. (A) In gel fluorescence assay of corresponding DNA, DNA+Ag and DNA/AgNCs corresponding to the DNA (9A-7C-30T) template along with a 46-nucleotide linear DNA. Samples were visualized either with SYBR Gold DNA staining (+) or AgNCs (-). Single and double asterisks indicate the monomeric and dimeric bands, while the hashtag indicates the linear DNA respectively.

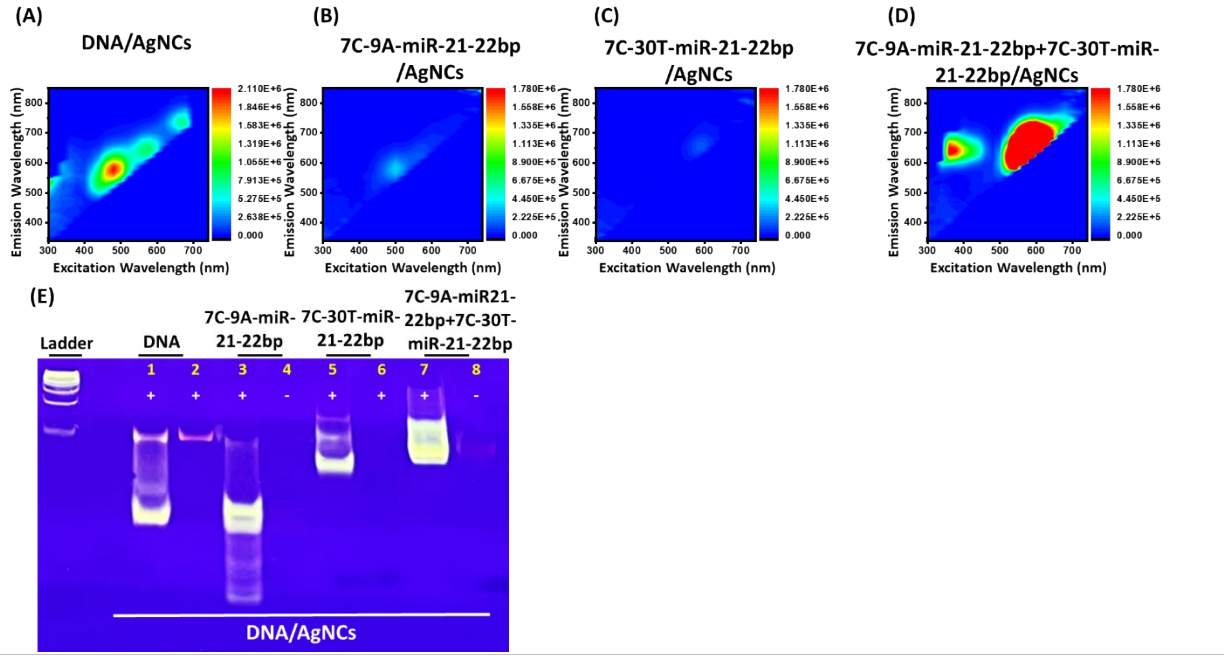


Fig. S4 Non-canonical head-to-head heterodimer mediated by AgNCs. (A-D) Excitation Vs. Emission plot depicting the change in fluorescence of homo and heterodimeric DNA/AgNCs respectively. (D) Secondary structure analysis mediated by AgNCs visualized by In-gel

fluorescence assay. All DNA sequences have 7-Cytosine loop incorporated. Samples were either treated with 50% glycerol or SYBR Gold DNA staining solution as indicated. All reactions were treated in the presence of 25 mM Na^{2+} and 20 mM Tris Acetate buffer (pH 6.5) or otherwise specified. All images were treated similarly to avoid bias. The lane numbers are indicated in yellow along with the + and – indicate the samples stained with and without SYBR Gold DNA staining solution, respectively.

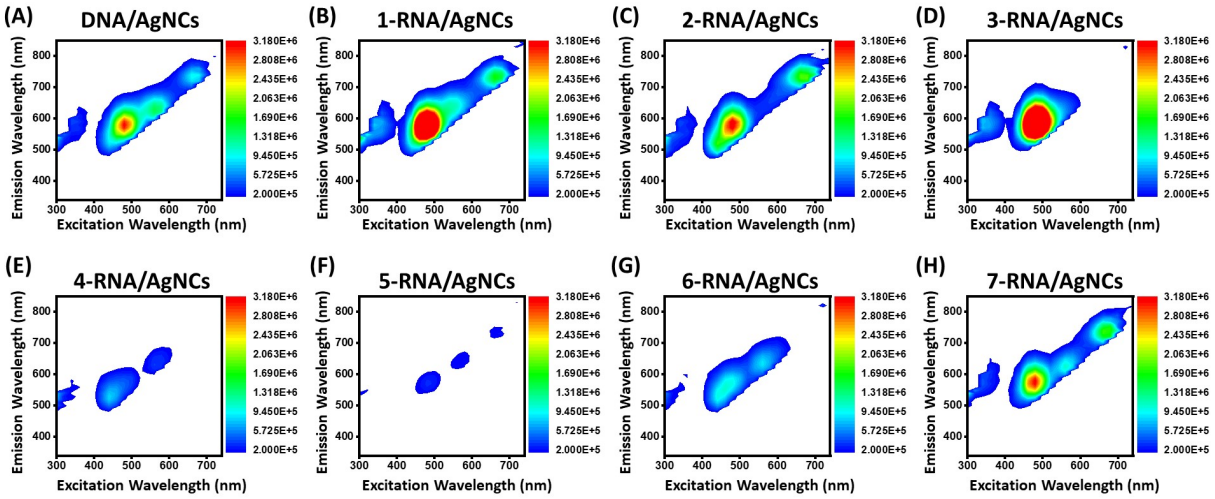


Fig. S5 Effect of RNA-sugar backbone on the emission tuning of XNA/AgNCs. (A-F) Excitation Vs. Emission plot depicting the change in fluorescence of XNA/AgNCs encapsulated by the corresponding nucleic acid templates modified with a DNA (A) sugar backbone and RNA sugar backbone on the 1st (B), 2nd (C), 3rd (D), 4th (E), 5th (F) and the 7th (H) positions, respectively.

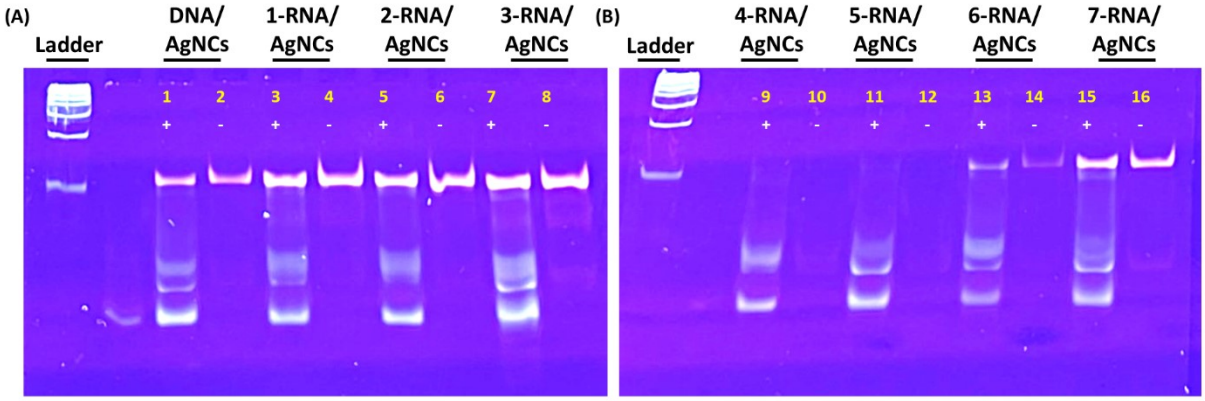


Fig. S6 In-gel Fluorescence assay of RNA/AgNCs. (A) Secondary structure analysis of DNA/AgNCs and the effect of RNA sugar backbone on the 1st to 7th positions of the XNA/AgNCs using In-gel fluorescence assay. Samples were either treated with 50% glycerol or SyBr Gold DNA staining solution as indicated. All reactions were treated in the presence of 25 mM Na^+ and 20 mM Tris Acetate buffer (pH 6.5). All images were treated similarly to avoid bias. The lane numbers are indicated in yellow along with the + and – indicate the samples stained with and without SyBr Gold DNA staining solution, respectively.

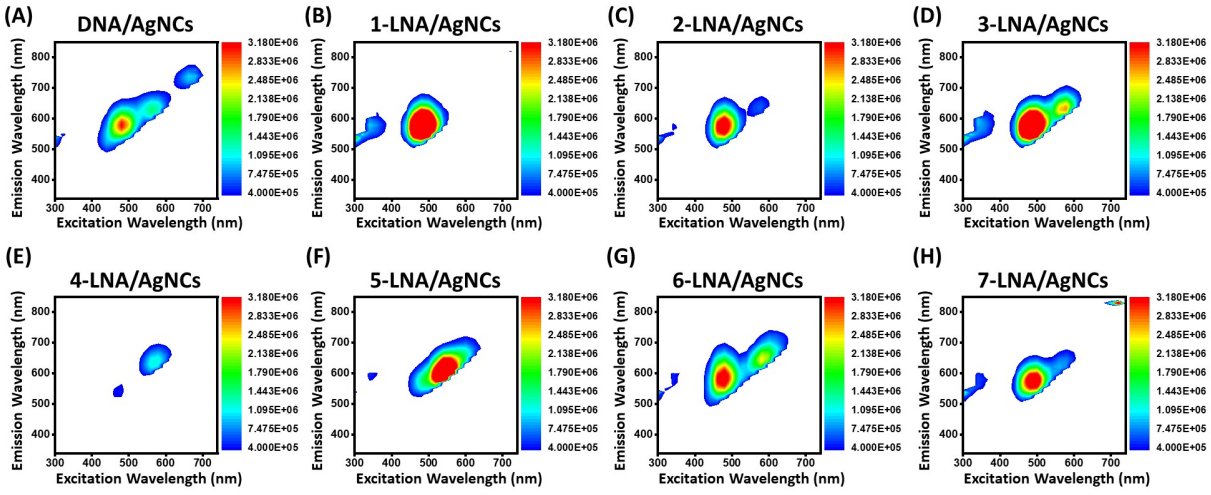


Fig. S7 Effect of LNA-sugar backbone on the emission tuning of XNA/AgNCs. (A-F) Excitation Vs. Emission plot depicting the change in fluorescence of XNA/AgNCs encapsulated by the corresponding nucleic acid templates modified with a DNA (A) sugar backbone and LNA sugar

backbone on the 1st (B), 2nd (C), 3rd (D), 4th (E), 5th (F) and the 7th (H) positions respectively

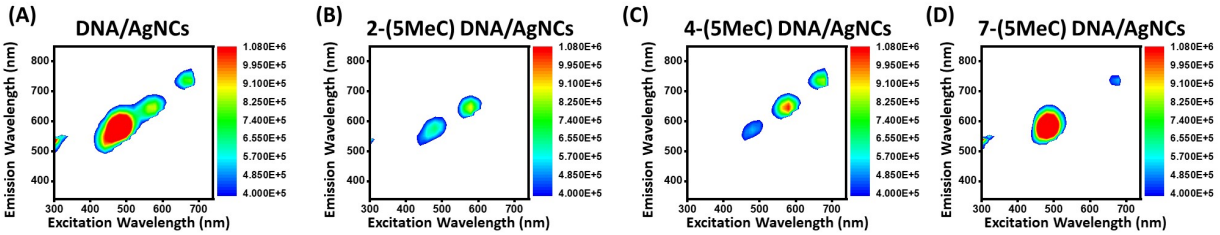


Fig. S8 Effect of 5-methyl-cytosine on the emission tuning of XNA/AgNCs in different positions of the 7C loop in DNA sequence. (A-F) Excitation Vs. Emission plot depicting the change in fluorescence of XNA/AgNCs encapsulated by the corresponding nucleic acid templates modified with a DNA (A) cytosine and 5-methyl-cytosine in the 2nd (B), 4th (C) and the 7th (D) positions, respectively

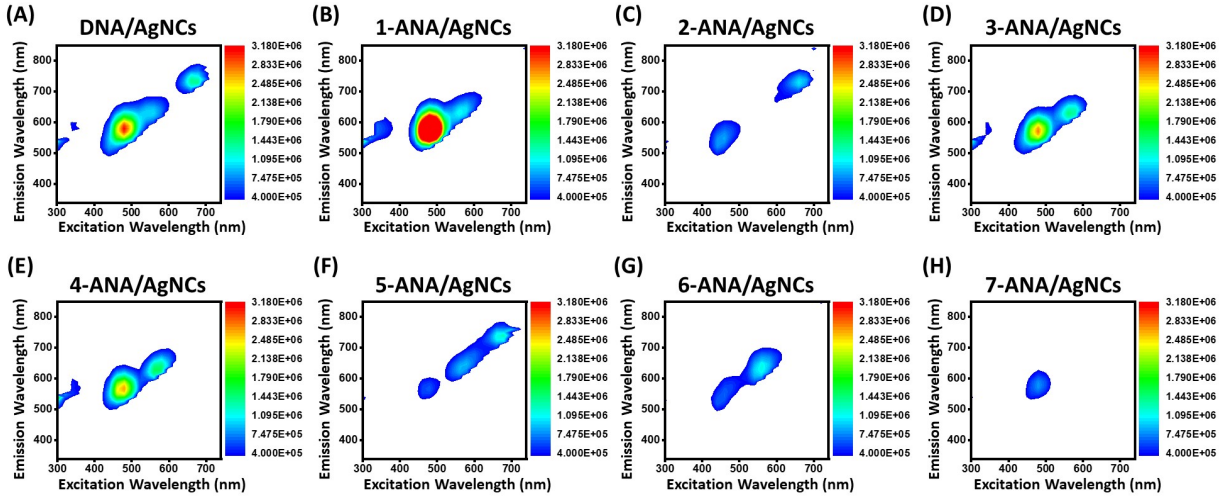


Fig. S9 Effect of ANA-sugar backbone on the emission tuning of XNA/AgNCs. (A-F) Excitation Vs. Emission plot depicting the change in fluorescence of XNA/AgNCs encapsulated by the corresponding nucleic acid templates modified with a DNA (A) sugar backbone and ANA sugar backbone on the 1st (B), 2nd (C), 3rd (D), 4th (E), 5th (F) and the 7th (H) positions respectively.

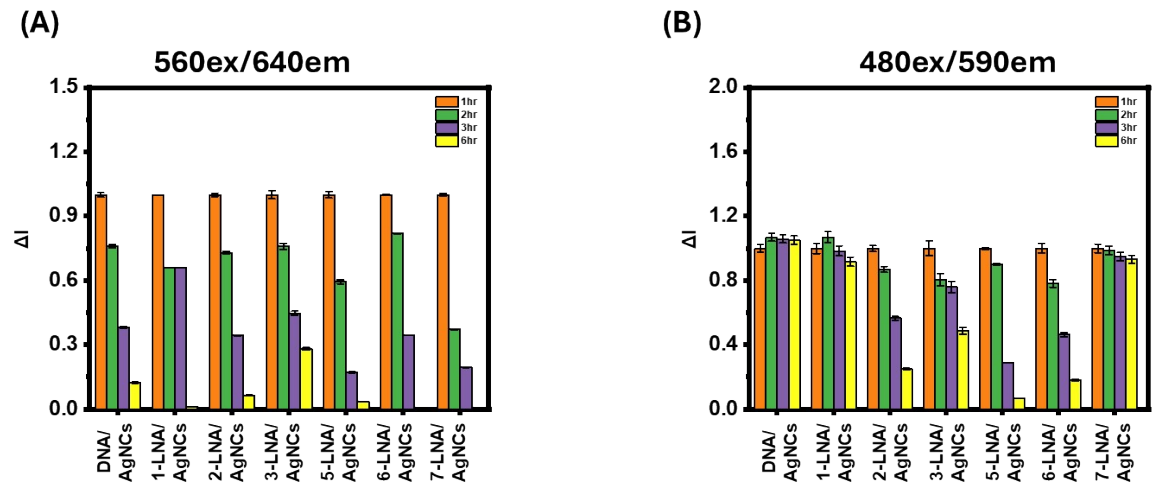


Fig. S10 Effect of position of LNA on fluorescence emission profiles. (A, B) Time dependent stability studies determining the change in red (A) and orange (B) relative fluorescence intensity profiles of LNA/AgNCs encapsulated by the corresponding nucleic acid templates. Samples were excited for every 10 nm. ΔI represents the relative emission intensity of XNA/AgNCs normalized to 1 h emission of respective XNA/AgNCs.

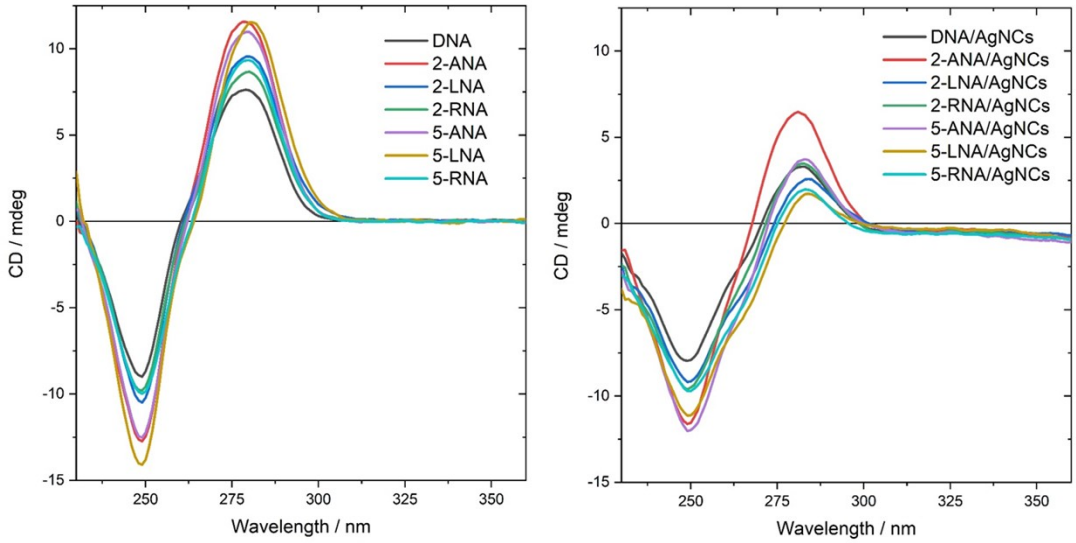


Fig. S11. Analysis of Secondary structure of XNA/AgNCs. CD spectroscopy data for sequences with DNA and the RNA-, LNA-, and ANA- modified sugar backbones at the 2nd and 5th positions at positions 2- and 5 - of the 7C loop after formation of AgNCs

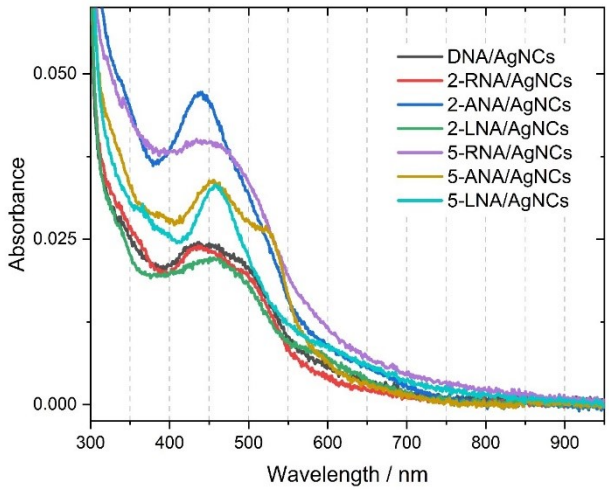


Fig. S12. UV-Vis Spectroscopic studies. UV-Vis absorbance data for sequences with DNA, RNA-, LNA-, and ANA-variants at positions 2- and 5- of the 7C loop after formation of AgNCs

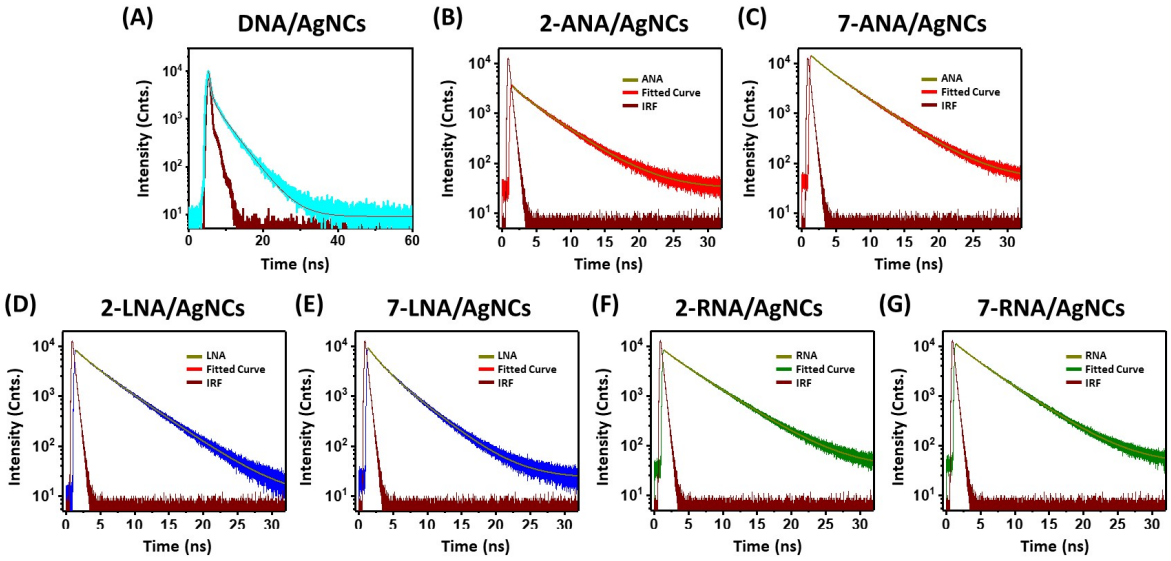


Fig. S13 Fluorescence lifetime for XNA/AgNCs. Fluorescence lifetime for XNA/AgNCs showing the change in lifetime of XNA/AgNCs encapsulated by the corresponding DNA (A), ANA (B-C), LNA (D-E) and RNA (F-G) modified sugar backbones at the 2nd and 7th positions, respectively, corresponding to IRF (Maroon curve). The samples were excited at 480 nm, and the fluorescence decay was monitored at 590 nm. (D) Table with the values from a biexponential fit of the

fluorescence decays. Fluorescence decay times (τ) in nanoseconds and (A) amplitudes.

Sample	α_1	τ_1	Species$_1$	α_2	τ_2	Species$_2$	Average τ	A_Sum[kCnts]
DNA/AgNCs	0.12	0.2	93.02%	0.01	4.01	6.98%	2.49	1.73
2-LNA/AgNCs	6.48	4.621	78.1%	1.81	1.93	21.9%	4.034	8.29
2-ANA/AgNCs	2.877	4.582	81.3%	0.6607	1.5	18.7%	4.002	3.538
2-RNA/AgNCs	7.17	4.928	87.3%	1.03	1.81	12.7%	4.536	8.207
7-LNA/AgNCs	5.38	3.97	58.2%	3.86	1.553	41.8%	2.961	9.23
7-ANA/AgNCs	11.6	4.672	81.9%	2.57	1.6	18.1%	4.116	14.16
7-RNA/AgNCs	9.31	4.705	84.4%	1.71	1.92	15.2%	4.274	11.02

Table S2. Table for fluorescence lifetime for XNA/AgNCs.

Notes: The table indicates the change in fluorescence lifetime decays based on the biexponential fit when the XNA/AgNCs are modified with their respective sugar backbones at different positions. Fluorescence decay times (tau) in nanoseconds and (alpha) amplitudes.

No.	XNA/AgNCs	Ex/Em Wavelengths (nm)	Structure AgNCs	Ag number
1	**DNA/AgNCs**	Ex/Em: **480/590** nm; 560/630 **nm**; 680/730 nm	Dimer	6,9,13,14
2	**2-LNA/AgNCs**	Ex/Em: 420/530 nm; **480/590 nm**	Dimer	6,9,14
3	**2-ANA/AgNCs**	Ex/Em: 440/520 nm; **660/730 nm**; 580/660 nm	Mono-,Dimer	6,9,10,13,14
4	**2-RNA/AgNCs**	Ex/Em: 420/530 nm; **480/590 nm**; 680/730 nm	Dimer	6,8,9,10,11,12,13
5	**5-ANA/AgNCs**	Ex/Em: 480/570 nm; **660/740 nm**;	N.D	6,9,13
6	**5-RNA/AgNCs**	Ex/Em: 420/530 nm; **560/640 nm**; 680/730 nm	N.D	13
7	**5-LNA/AgNCs**	Ex/Em: 480/590 nm; **540/620 nm**	N.D	13

Table S3. Summary of XNA/AgNCs properties.

Notes: The table indicates the highest Ex/Em range followed by the secondary structure and the Ag number (Mass spectrometry) of the XNA/AgNCs encapsulated by the corresponding nucleic acid templates modified with DNA, LNA, ANA and RNA respectively. The highest Ex/Em values are highlighted in bold. N.D indicates no detectable DNA structure encapsulating AgNCs based on gel electrophoresis.

Table S4. Comparison of ESI-mass spectrometry (negative mode) analysis of the XNA/AgNCs present in synthesis products of DNA, RNA-, LNA-, and ANA-variants at positions 2- and 5- of the 7C loop.

XNA/ AgNCs	Observed Clusters							
	Ag_6	Ag_8	Ag_9	Ag_{10}	Ag_{11}	Ag_{12}	Ag_{13}	Ag_{14}
DNA	Ag^+_4/Ag^0_2		Ag^+_5/Ag^0_4				Ag^+_5/Ag^0_8	Ag^+_7/Ag^0_7
2-LNA	Ag^+_3/Ag^0_3		Ag^+_5/Ag^0_4					Ag^+_8/Ag^0_6
5-LNA							Ag^+_6/Ag^0_7	
7-LNA	Ag^+_3/Ag^0_3		Ag^+_5/Ag^0_4				Ag^+_7/Ag^0_6	
2-ANA	Ag^+_4/Ag^0_2		Ag^+_5/Ag^0_4	Ag^+_6/Ag^0_4			Ag^+_7/Ag^0_6	Ag^+_8/Ag^0_6
3-ANA	Ag^+_3/Ag^0_3		Ag^+_5/Ag^0_4				Ag^+_6/Ag^0_7	
5-ANA	Ag^+_3/Ag^0_3		Ag^+_5/Ag^0_4				Ag^+_6/Ag^0_7	
2-RNA	Ag^+_4/Ag^0_2	Ag^+_4/Ag^0_4	Ag^+_5/Ag^0_4	Ag^+_5/Ag^0_5	Ag^+_5/Ag^0_6	Ag^+_6/Ag^0_6	Ag^+_6/Ag^0_7	
5-RNA							Ag^+_5/Ag^0_8	
2-(MeC) DNA	Ag^+_5/Ag^0_1		Ag^+_7/Ag^0_2				Ag^+_7/Ag^0_6	
7-(MeC) DNA	Ag^+_3/Ag^0_3		Ag^+_5/Ag^0_4				Ag^+_5/Ag^0_8	Ag^+_7/Ag^0_7

Notes: The observed ESI-MS peaks exhibited typical charge states of -5, -6, and -7. A peak was considered detected if its intensity was at least three times the background level and displayed a reasonable isotope pattern. Uncertainties in charge distribution between Ag^+ and Ag^0 is +/- 1 or the peak could be a mixture of such species. 2-RNA/AgNC also contained small amounts of 2-RNA/Ag_{17} NC and $_2$-RNA/Ag_{19}NC. The observed peaks for the native sequences of $A_9C_7T_{30}$ incorporating ANA or RNA corresponded to the expected masses. In contrast the observed peak for 2-LNA and 5-LNA had a higher mass than the DNA sequence equivalent to the addition of the extra atoms $OCCH_2$, showing that, LNA in addition to the bridge, includes an extra CH_3 group.