Supporting Information

Analytical Method for the Determination of the Absorption Coefficient of DNA-Stabilized Silver Nanoclusters

Giacomo Romolini[a], Cecilia Cerretani[a], Vanessa Rück[a], Mikkel Baldtzer Liisberg[a], Christian Brinch Mollerup[b], Tom Vosch[a]**

[a] Department of Chemistry, University of Copenhagen, Universitetsparken 5, DK-2100 Copenhagen, Denmark

[b] Department of Forensic Medicine, University of Copenhagen, Frederik V's Vej 11, DK-2100 Copenhagen, Denmark

E-mail: tom@chem.ku.dk; cece@chem.ku.dk

1. HPLC chromatograms:

DNA600-AgNC

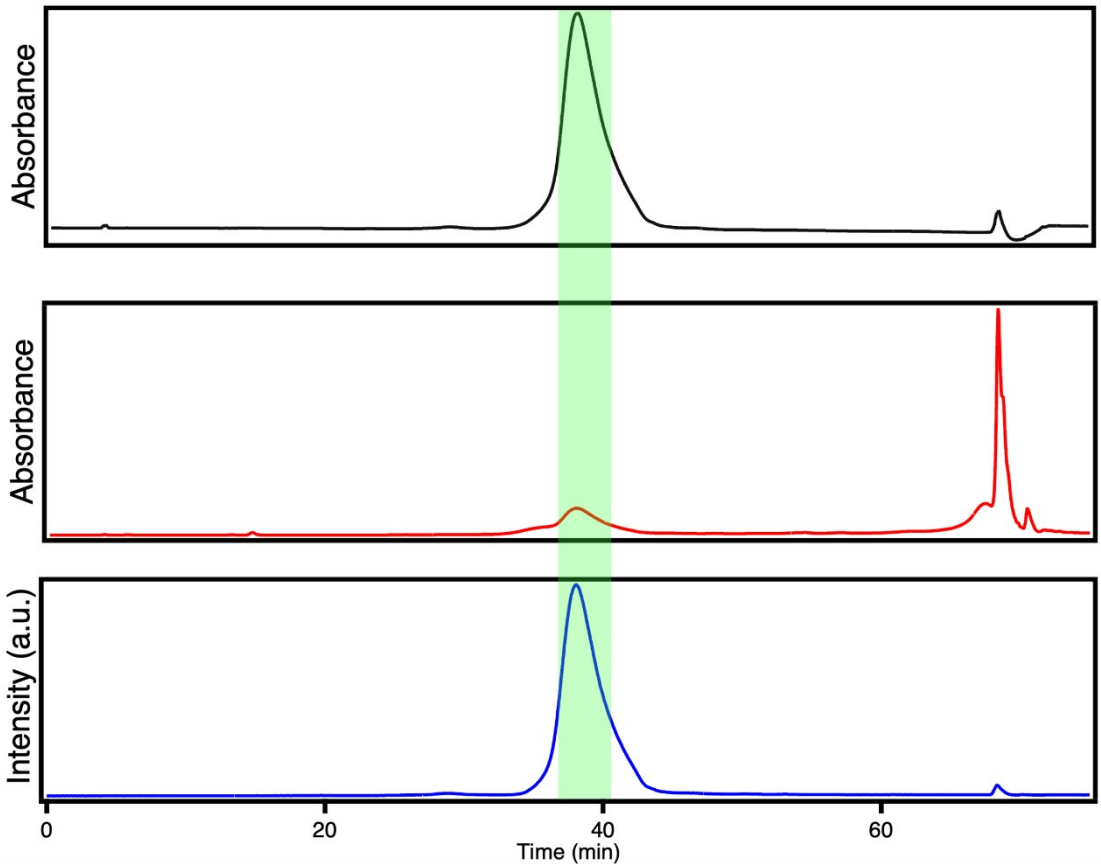

Figure S1. HPLC chromatograms of DNA600-AgNCs monitoring the absorption at 600 nm (top panel) and 260 nm (middle panel), and the fluorescence signal at 670 nm, exciting at 600 nm (bottom panel). The green highlighted area is the fraction collected between 36 and 41 min and used in the study.

DNA750-AgNC

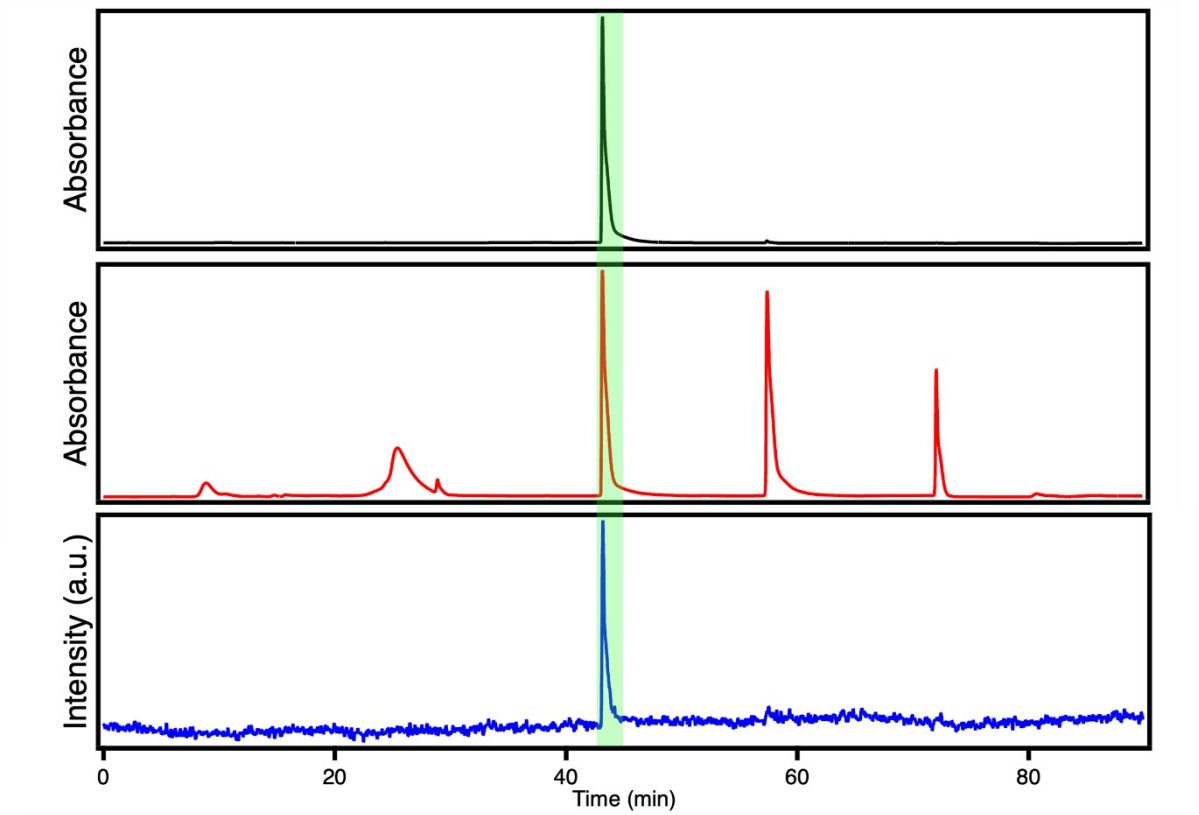

Figure S2. HPLC chromatograms of DNA750-AgNCs monitoring the absorption at 730 nm (top panel) and 260 nm (middle panel), and the fluorescence signal at 850 nm, exciting at 730 nm (bottom panel). The green highlighted area is the fraction collected between 43 and 44.5 min and used in our study.

2. Mass spectrometry data:

Mass Spectrometry data analysis

Determining the exact number of silver atoms of the HPLC-purified DNA-AgNCs is not a trivial task and often involves combining prior knowledge and experience in identifying the relevant peaks. While we are confident in the peaks we picked, we would like to point out that there is no general procedure to do so. The general strategy is to identify most intense peak at the highest m/z that is in line with the expected number of reduced silver atoms (N_0) in the AgNC. Due to the fact that Ag^+ cations like to bind to nucleobases, peaks above the molecular ion peak are sometimes present with one Ag^+ m/z unit difference. For DNA600-AgNC we took 15, for DNA750-AgNC we took 21 and for DNA575-AgNC we took 14. Structural information is available for DNA470-AgNC (partial) and DNA525-AgNC, and in those cases the atomic composition reflects well the mass-spectrometry data. Previously reported mass-spectrometry data for DNA575-AgNC, DNA600-AgNC and DNA750-AgNC are in line with our findings. Mass spectrometry data reported by Schultz *et al.* shows that DNA575-AgNC is formed by one DNA strand encapsulating 14 silvers, of which 8 are cationic.[1] For DNA600-AgNC, one DNA strand was found to stabilize 15 silvers,[1] consistent with our value of 6 metallic atoms and 9 Ag+ cations. Inductively coupled plasma-atomic emission spectroscopy by Petty *et al.*[2] revealed that DNA750-AgNC is formed by 9.6 ± 0.8 Ag atoms per DNA strand, and the mass spectrum reported by Schultz *et al.*[3] mention 20 silvers in the construct with 2 DNA strands. Note that we found 21, but this is only a difference of 5% and future structural characterization might provide a more final answer on this. Hence, we are confident that the data we selected from the mass-spectrometry is representative for each of the DNA-AgNCs at this point in time.

When calculating the molecular mass of the DNA-AgNCs, it is also worth noticing that the synthetic DNA strands purchased from IDT lack the phosphate group at the 3'-terminus. Table S1 provides information on the mass spectra reported in Figures S3, S4 and S5.

Table S1. Center of Gaussian fits, x_0, for the experimentally measured mass spectra (x_0^{exp}) shown in Figures S3, S4 and S5, and the corresponding theoretical mass distributions (x_0^{th}). The last column corresponds to the absolute error calculated as $x_0^{exp} - x_0^{th}$.

Sample	Molecular Formula	Charge state (z)	x_0^{exp}	x_0^{th}	error
DNA575-AgNC	$DNA[Ag_{14}]^{8+}$	5-	1605.0803	1605.0379	0.0424
		4-	2006.5874	2006.5496	0.0378
DNA600-AgNC	$DNA[Ag_{14}]^{8+}$	5-	2016.6611	2016.7022	-0.0411
	$DNA[Ag_{15}]^{9+}$	5-	2038.084	2038.0737	0.0103
	$DNA[Ag_{16}]^{10+}$	5-	2059.4216	2059.4467	-0.0251
DNA750-AgNC	$DNA_2[Ag_{20}]^{10+}$	5-	2289.2498	2289.2543	-0.0045
	$DNA_2[Ag_{21}]^{11+}$	5-	2310.6275	2310.6272	0.0003
	$DNA_2[Ag_{22}]^{12+}$	5-	2331.9865	2331.9994	-0.0129

DNA575-AgNC

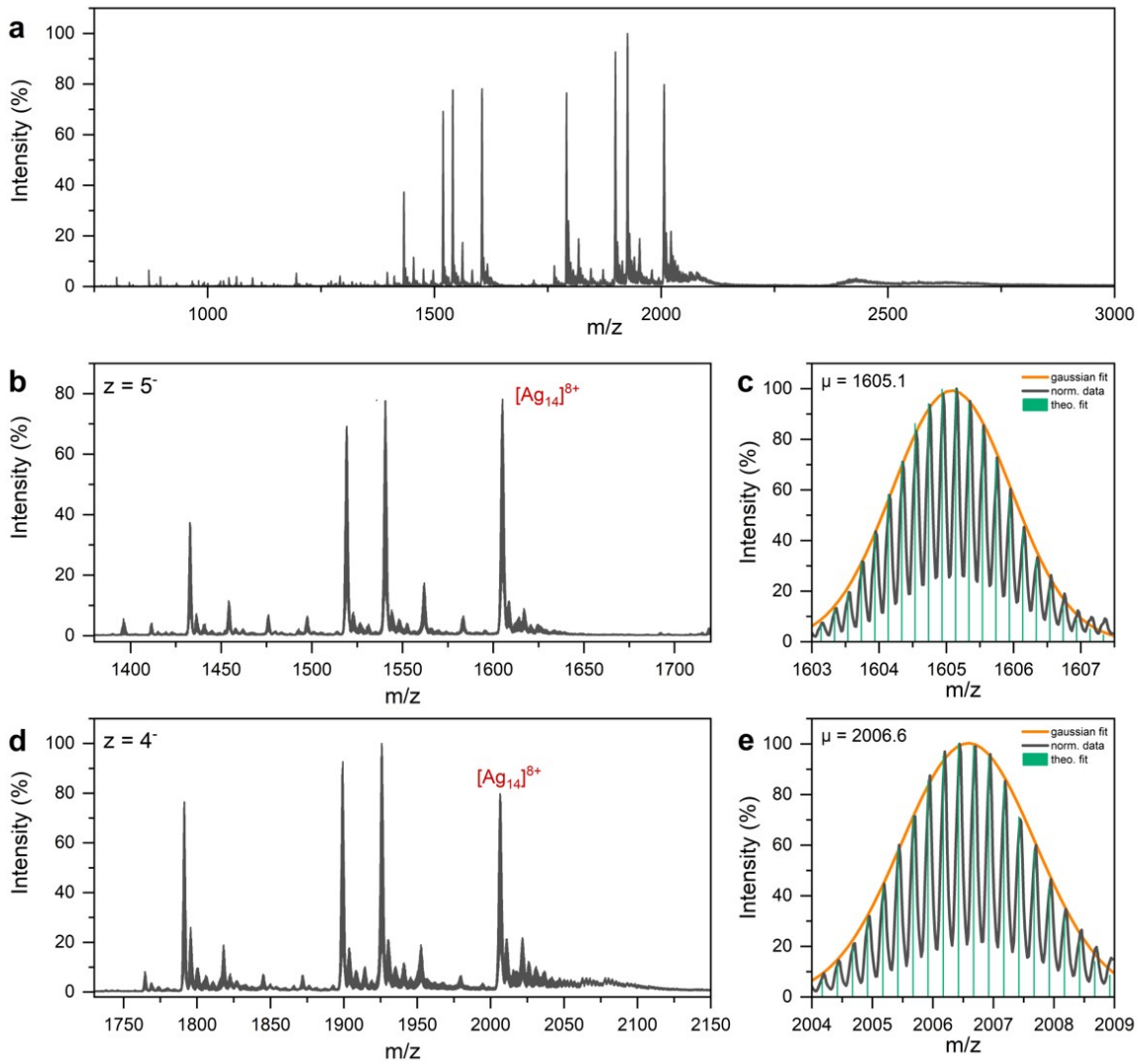

Figure S3. (a) ESI mass spectrum of DNA575-AgNCs measured in negative ion mode. Zoomed-in views of the mass spectrum showing charge state 5^- (b) and 4^- (d). Most peaks are related to fragments/dissociation products of DNA$[Ag_{14}]^{8+}$. Normalized peaks for charge state 5^- (c) and 4^- (e) with corresponding theoretical isotopic distributions (green) and Gaussian fits (orange) of the experimental isotopic distributions of DNA$[Ag_{14}]^{8+}$ ($H_{261}C_{207}N_{72}O_{132}P_{21}[Ag_{14}]^{8+}$). The molecular mass is 8,030.2942 g/mol.

DNA600-AgNC

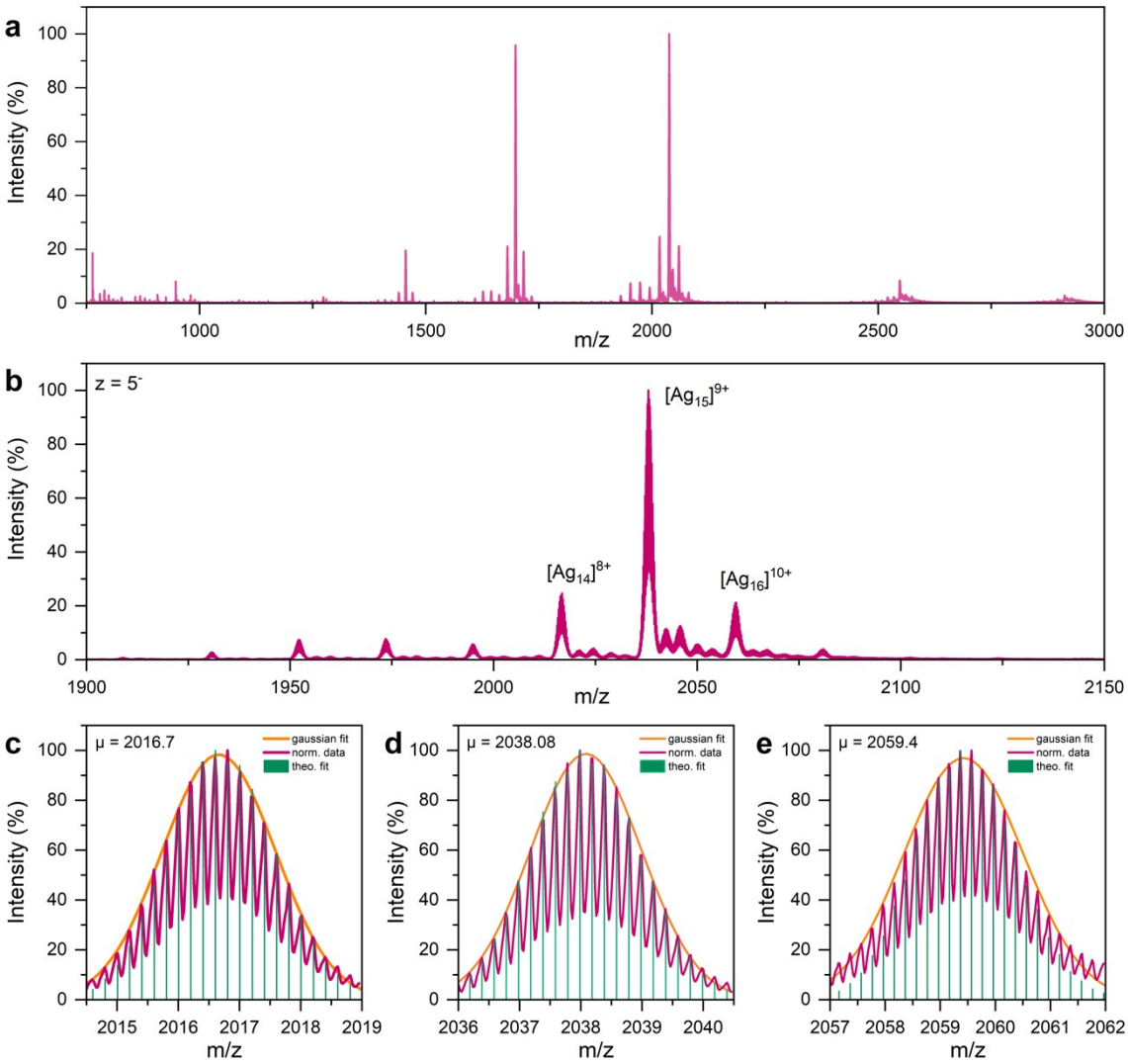

Figure S4. (a) ESI mass spectrum of DNA600-AgNCs measured in negative ion mode. (b) Zoomed-in view of the mass spectrum for z= 5^- peaks. (c-e) Normalized peaks with theoretical isotopic distributions (green) and corresponding Gaussian fits (orange) of the experimental isotopic distributions for the highlighted peaks in b. (c) $DNA_2[Ag_{14}]^{8+}$ ($H_{338}C_{273}N_{99}O_{171}P_{27}[Ag_{14}]^{8+}$) with a molecular mass of 10,088.6119 g/mol, (d) $DNA_2[Ag_{15}]^{9+}$ ($H_{337}C_{273}N_{99}O_{171}P_{27}[Ag_{15}]^{9+}$) with a molecular mass of 10,195.4721 g/mol, and (e) $DNA_2[Ag_{16}]^{10+}$ ($H_{336}C_{273}N_{99}O_{171}P_{27}[Ag_{16}]^{10+}$) with a molecular mass of 10,302.3324 g/mol.

DNA750-AgNC

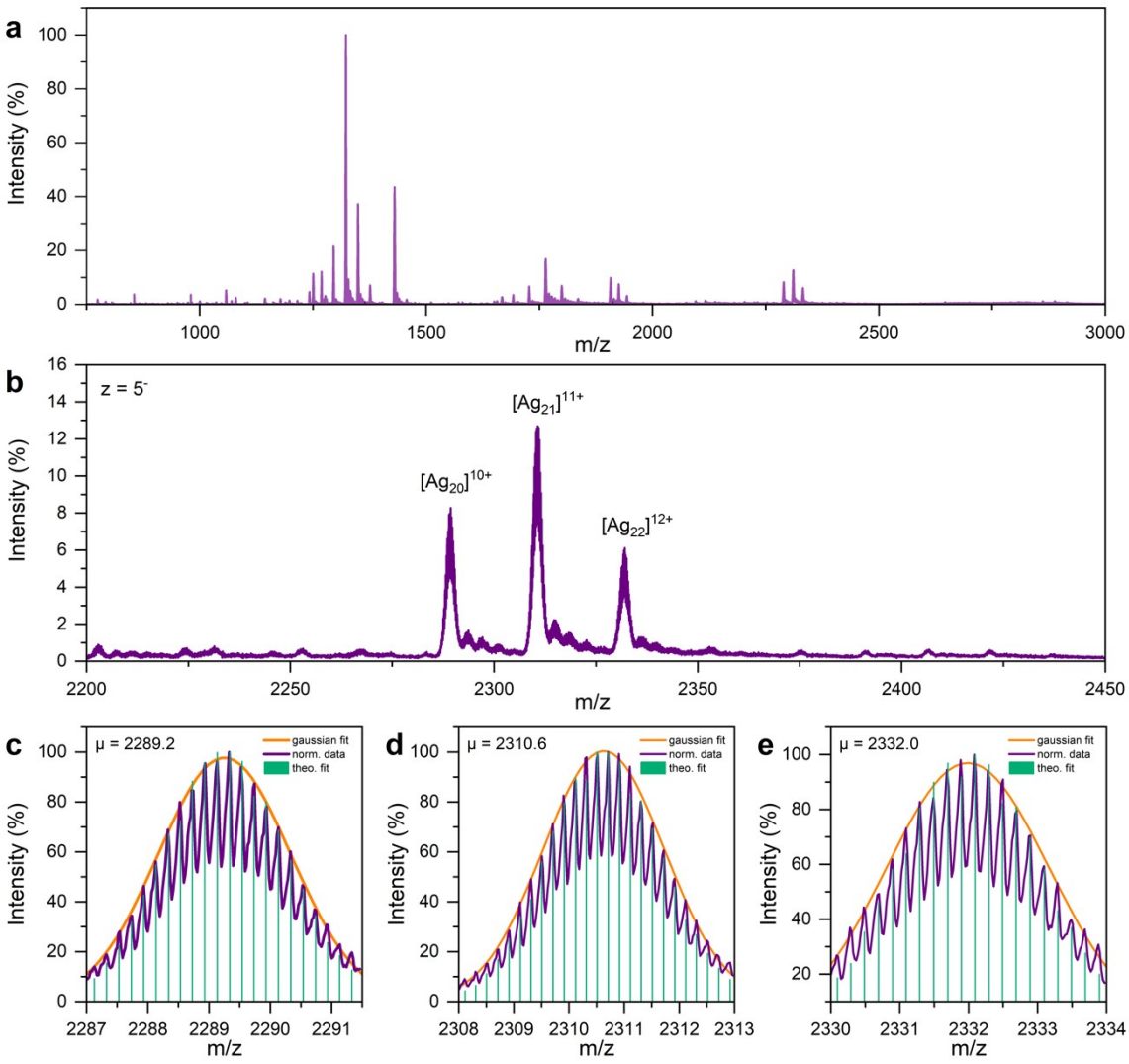

Figure S5. (a) ESI mass spectrum of DNA750-AgNCs measured in negative ion mode. (b) Zoomed-in view of the mass spectrum for z = 5^- peaks. (c-e) Normalized peaks with theoretical isotopic distributions (green) and corresponding Gaussian fits (orange) of the experimental isotopic distributions for the highlighted peaks in b. (c) $DNA_2[Ag_{20}]^{10+}$ ($H_{378}C_{296}N_{106}O_{184}P_{30}[Ag_{20}]^{10+}$) with a molecular mass of 11,451.3452 g/mol, (d) $DNA_2[Ag_{21}]^{11+}$ ($H_{377}C_{296}N_{106}O_{184}P_{30}[Ag_{21}]^{11+}$) with a molecular mass of 11,558.2054 g/mol, and (e) $DNA_2[Ag_{22}]^{12+}$ ($H_{376}C_{296}N_{106}O_{184}P_{30}[Ag_{22}]^{12+}$) with a molecular mass of 11,665.0657g/mol. Note that DNA750-AgNC highly fragments/dissociates under applied ESI-MS conditions.

3. Absorption versus excitation spectra:

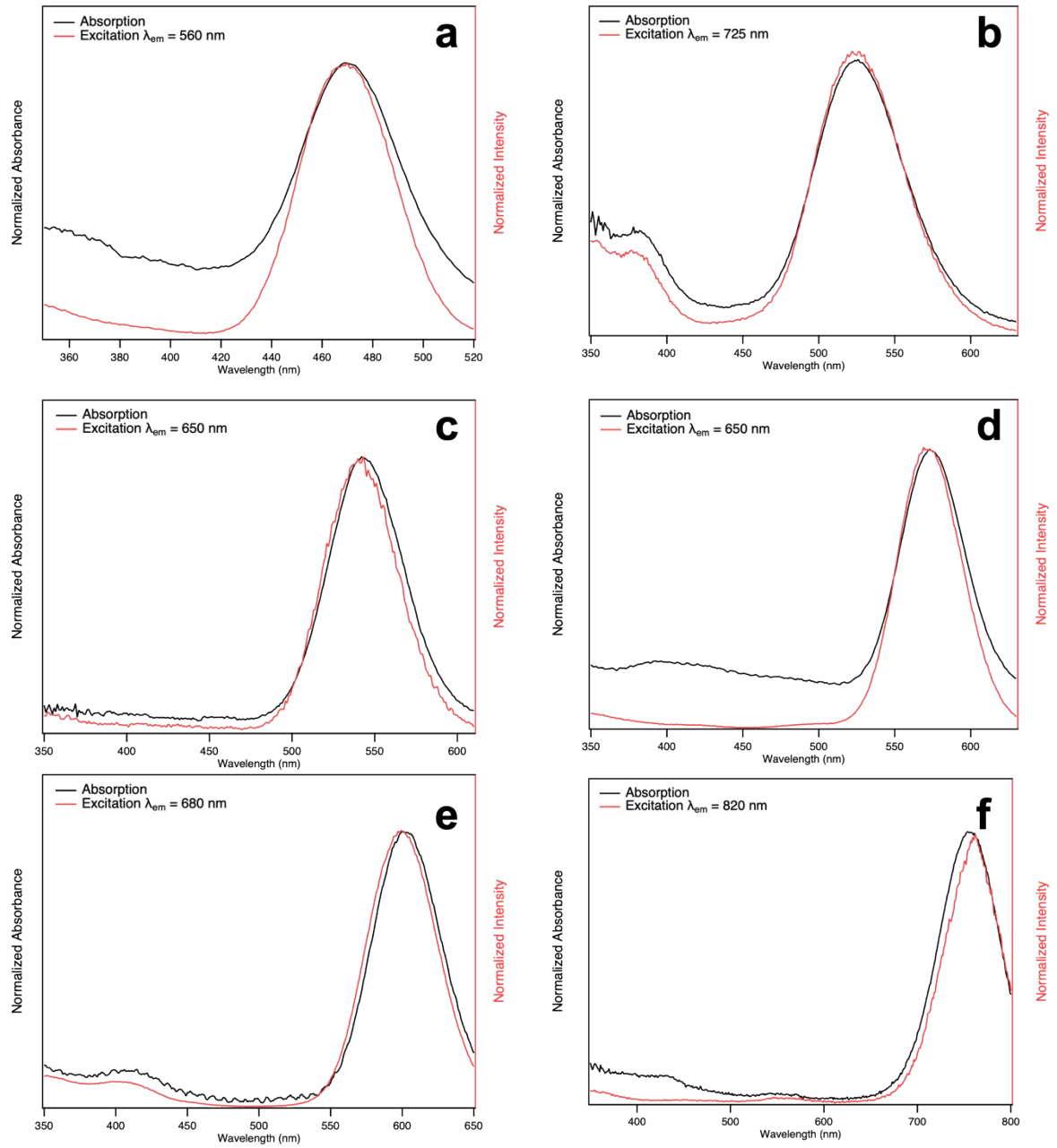

Figure S6. Comparison of normalized absorption (black) and excitation (red, measured at the maximum emission wavelength) spectra of: a) DNA470-AgNCs, b) DNA525-AgNCs, c) DNA540-AgNCs, d) DNA575-AgNCs, e) DNA600-AgNCs, and f) DNA750-AgNCs.

4. Fluorescence quantum yield and decay time of DNA750-AgNC:

The absorption measurements were performed on a Cary 300 UV-Vis spectrophotometer from Agilent Technologies using a deuterium lamp for ultraviolet radiation and a halogen lamp for visible and near-infrared radiation. The measurements were carried out in a single-beam configuration with a 0/100% transmittance baseline correction. Every spectrum was subtracted by the corresponding blank absorption spectrum.

The emission spectra were recorded using a FluoTime300 system (PicoQuant), exciting at 726 nm with a picosecond-pulsed laser (PicoQuant, LDH-P-C-730). Reference-based quantum yield measurements require an accurate determination of the emission spectra of the reference compound and the DNA-AgNC of interest.

Therefore, to circumvent the low detector efficiency of the FluoTime300 above 800 nm, we used our home-built confocal microscope.[4] A continuum white-light laser (NKT Photonics, SuperK EXTREME EXB-6) was used as an excitation source delivering a wavelength of 720 nm by sending the continuum output through an acousto-optic tunable filter (NKT Photonics, SuperK SELECT). The output of the laser was expanded (GBE05-B, Thorlabs) before it was reflected by a 30:70 beam splitter (Omega Optical, XF122) and sent through an air objective (Olympus, CPlanFLN 10x, NA= 0.3). Standard 1 cm quartz cuvettes (Hellma) were filled with either H_2O (used for subtracting residual laser scatter), an aqueous solution of the DNA750-AgNC, or an aqueous solution of Alexa-750 (reference dye). The cuvettes were placed on top of the microscope's sample stage, and the laser was focused around 1 mm into the solutions ensuring that the spectra of the three cuvettes were recorded under identical conditions. The same objective collected the fluorescence. The emission passed a 100 µm pinhole and it was sent through a spectrograph (SP 2356 spectrometer, 300 grooves/mm, Acton Research) onto a nitrogen cooled CCD camera (SPEC-10:100B/LN-eXcelon, Princeton Instruments) for the recording of spectra. The emission spectra of the DNA750-AgNC and reference samples were corrected for residual laser scatter by subtracting the spectrum recorded with the H_2O filled cuvette. Finally, the emission spectra were intensity corrected as reported previously.[3]

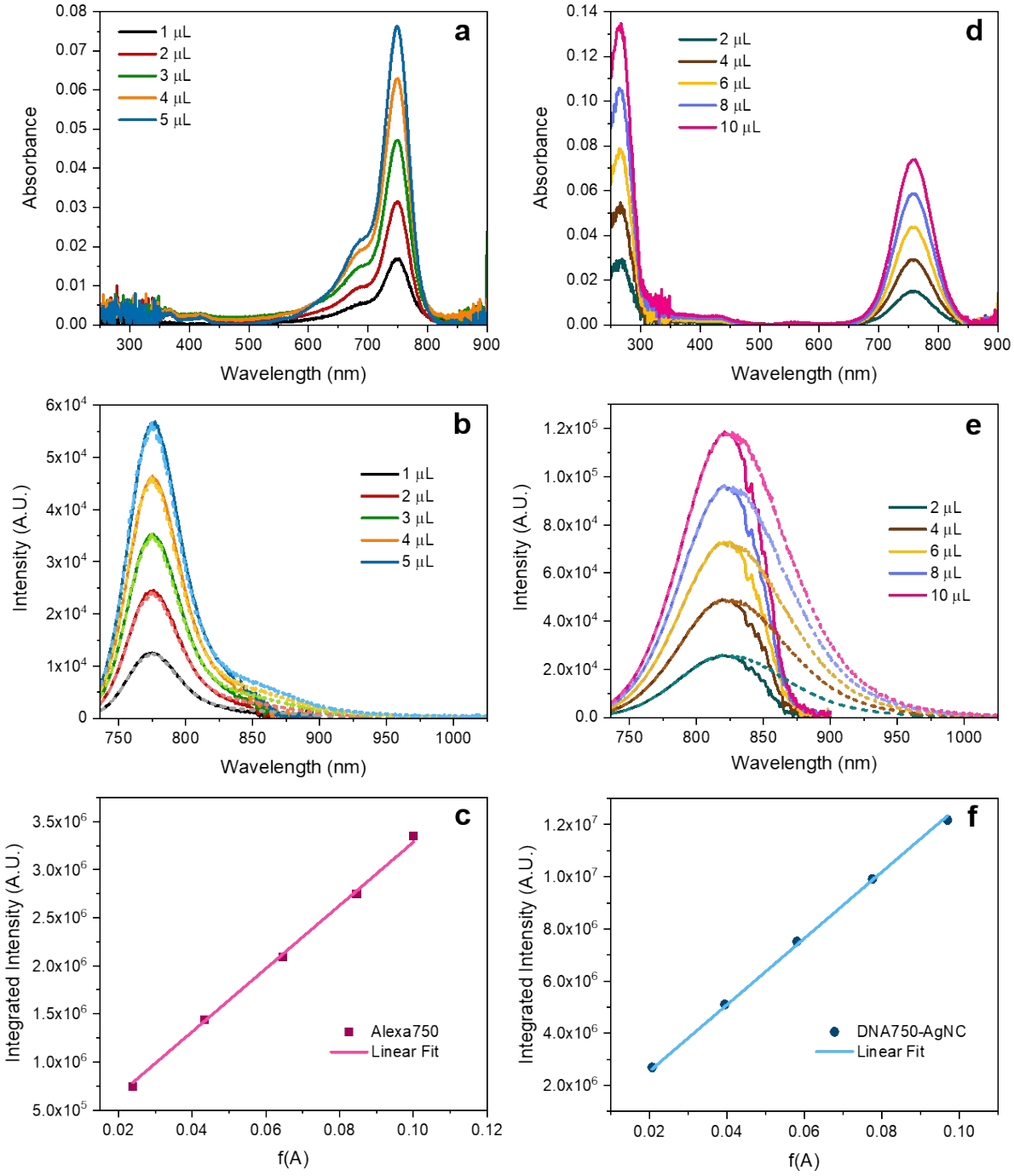

Figure S7. Quantum yield data of DNA750-AgNC at 25 ˚C. (a) Absorption and (b) emission spectra (λ_{exc}= 720 nm) of Alexa750 in PBS (pH=7.2). (d) Absorption and (e) emission spectra (λ_{exc}= 720 nm) of DNA750-AgNCs in 10 mM ammonium acetate. The dashed lines in b and e are the shape-corrected emission spectra of the reference dye and DNA750-AgNCs, respectively (*vide supra*). (c) and (f) Zero-intercept linear fits of the integrated fluorescence *vs* the fraction of absorbed light for the reference dye and DNA750-AgNCs, respectively. The slopes were used to calculate the quantum yield of the sample.

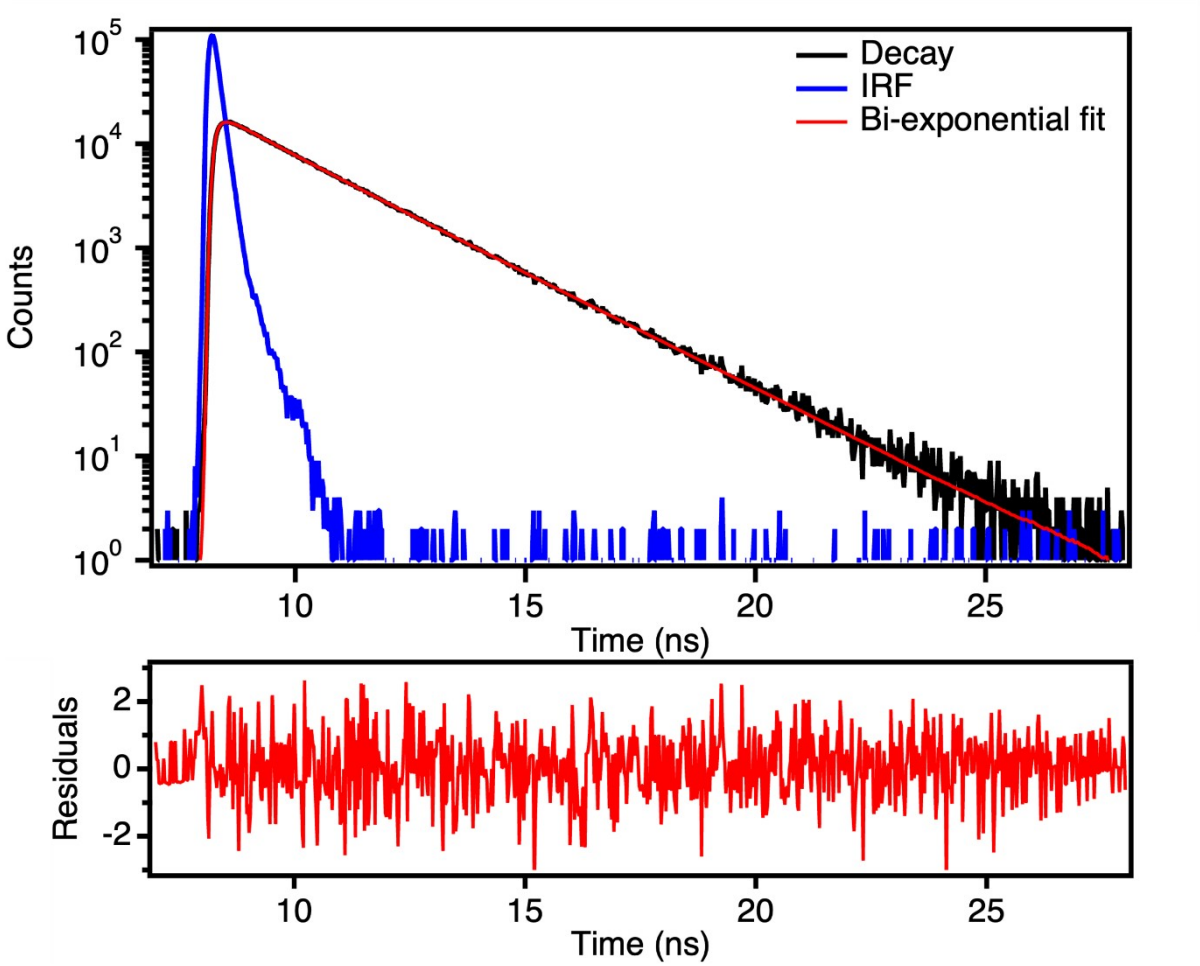

Figure S8. TCSPC data and fitting. Fluorescence decay (black) collected at 825 nm (λ_{exc}=726 nm) and instrument response function (blue), along with the bi-exponential model (red) used to fit the data. The residuals are reported in the bottom panel. The χ^2 of the fit is 0.943.

5. ICP-OES calibration curves:

DNA470-AgNC

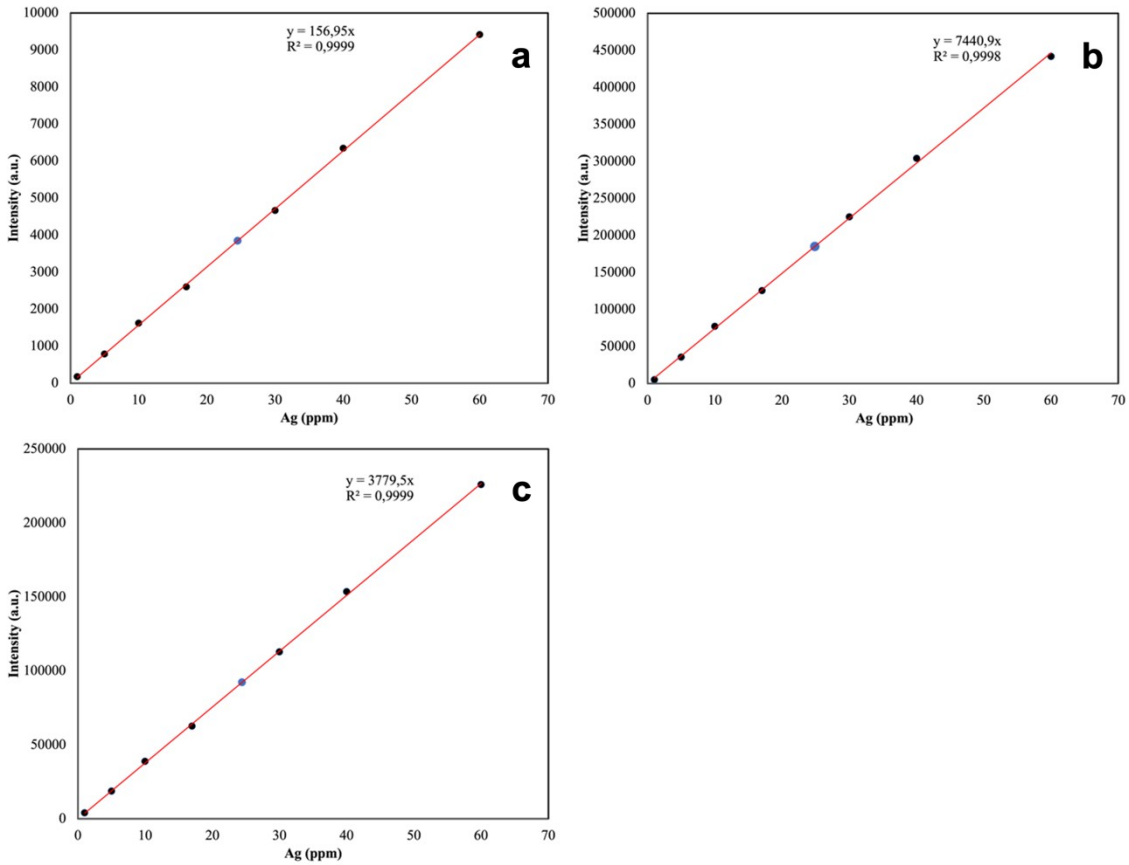

Figure S9. ICP-OES calibration curves for DNA470-AgNCs using different emission lines of Ag: a) 243.8 nm, b) 328 nm, and c) 338 nm. The blue dot indicates the amount of Ag in the DNA470-AgNC sample.

DNA525-AgNC

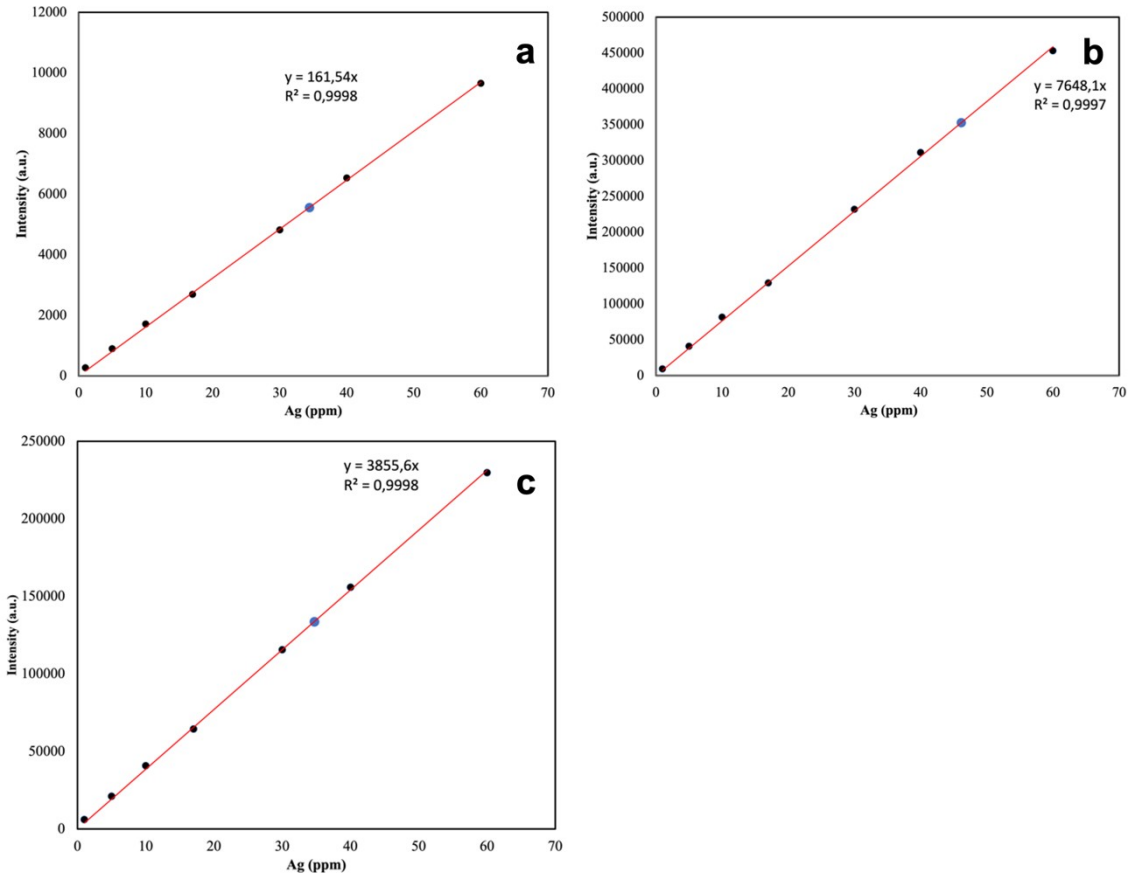

Figure S10. ICP-OES calibration curves for DNA525-AgNCs using different emission lines of Ag: a) 243.8 nm, b) 328 nm, and c) 338 nm. The blue dot indicates the amount of Ag in the DNA525-AgNC sample.

DNA540-AgNC

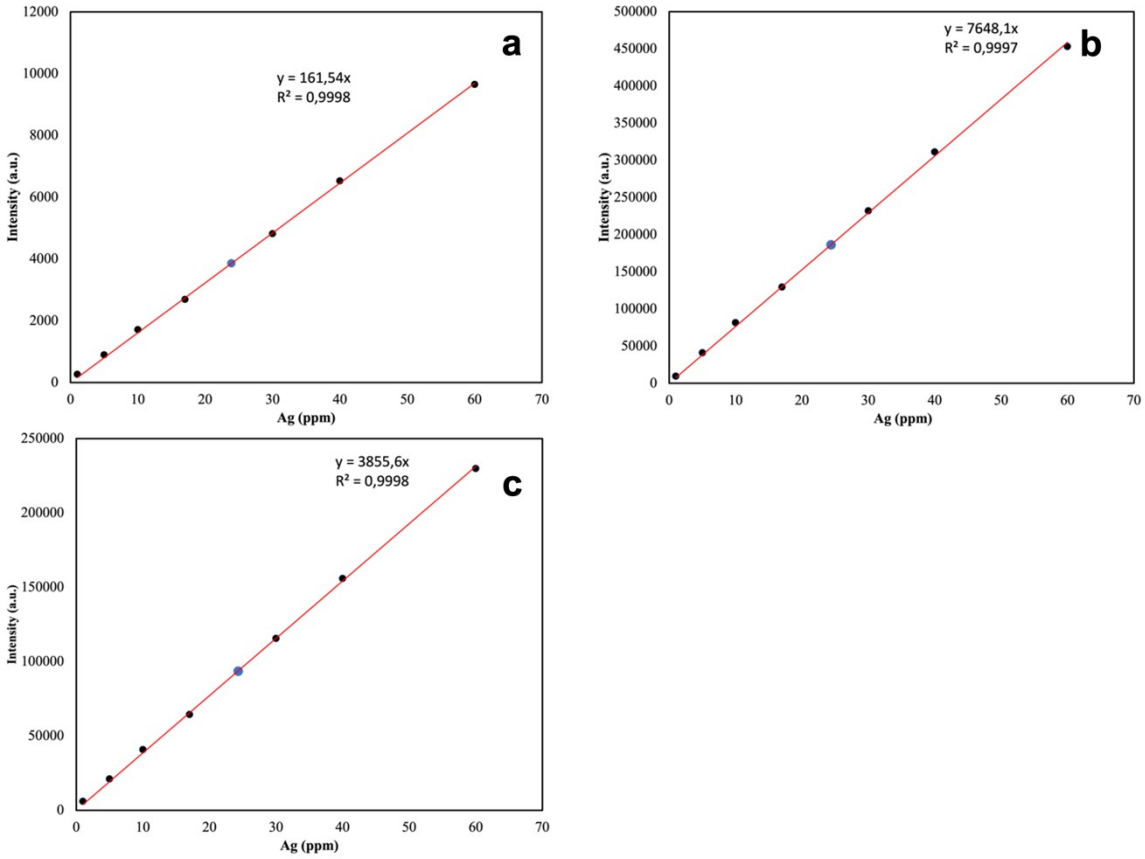

Figure S11. ICP-OES calibration curves for DNA540-AgNCs using different emission lines of Ag: a) 243.8 nm, b) 328 nm, and c) 338 nm. The blue dot indicates the amount of Ag in the DNA540-AgNC sample.

DNA575-AgNC

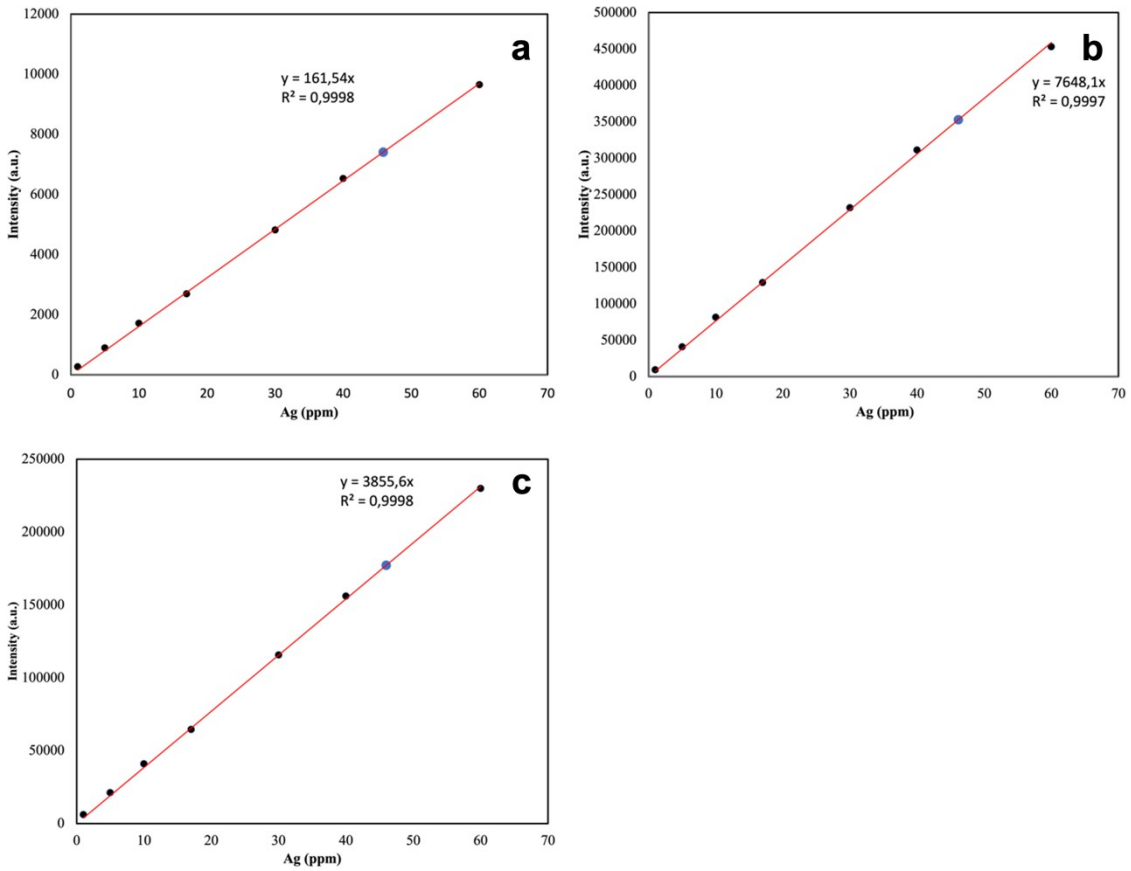

Figure S12. ICP-OES calibration curves for DNA575-AgNCs using different emission lines of Ag: a) 243.8 nm, b) 328 nm, and c) 338 nm. The blue dot indicates the amount of Ag in the DNA575-AgNC sample.

DNA600-AgNC

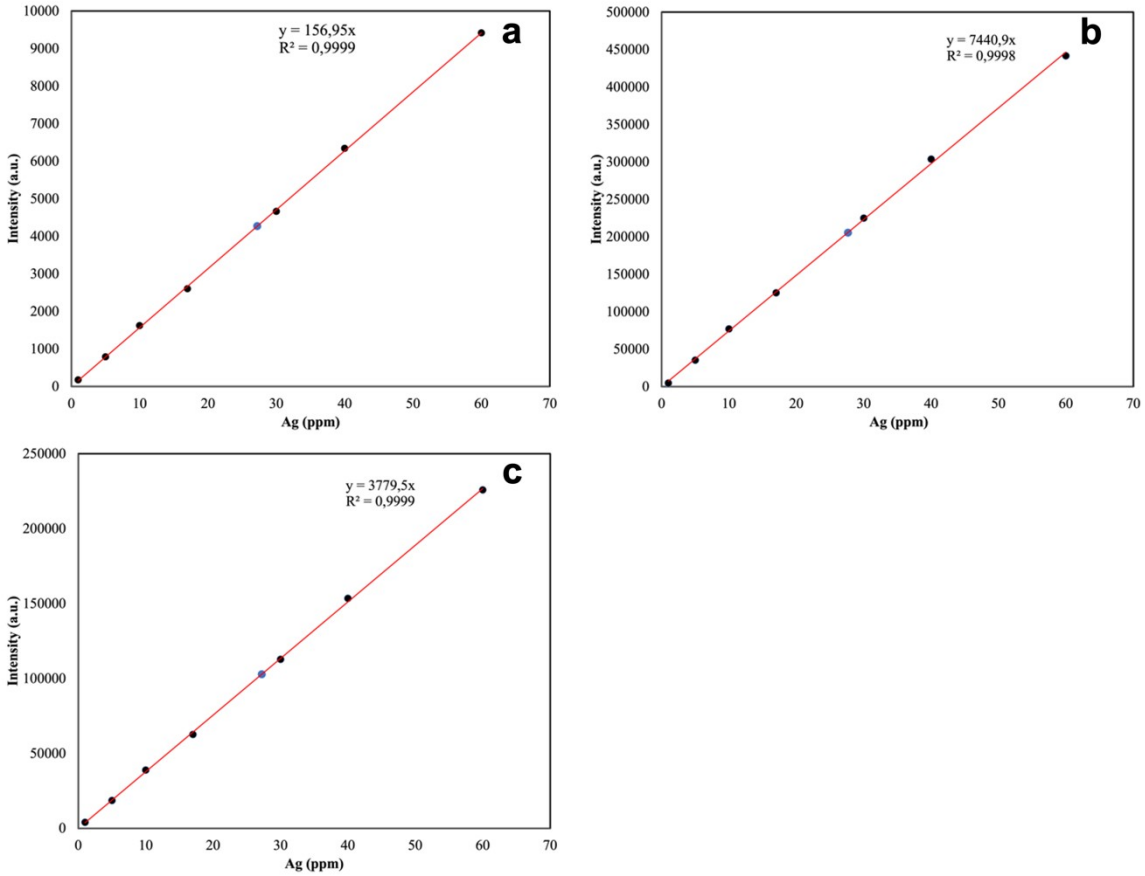

Figure S13. ICP-OES calibration curves for DNA600-AgNCs using different emission lines of Ag: a) 243.8 nm, b) 328 nm, and c) 338 nm. The blue dot indicates the amount of Ag in the DNA600-AgNC sample.

DNA750-AgNC

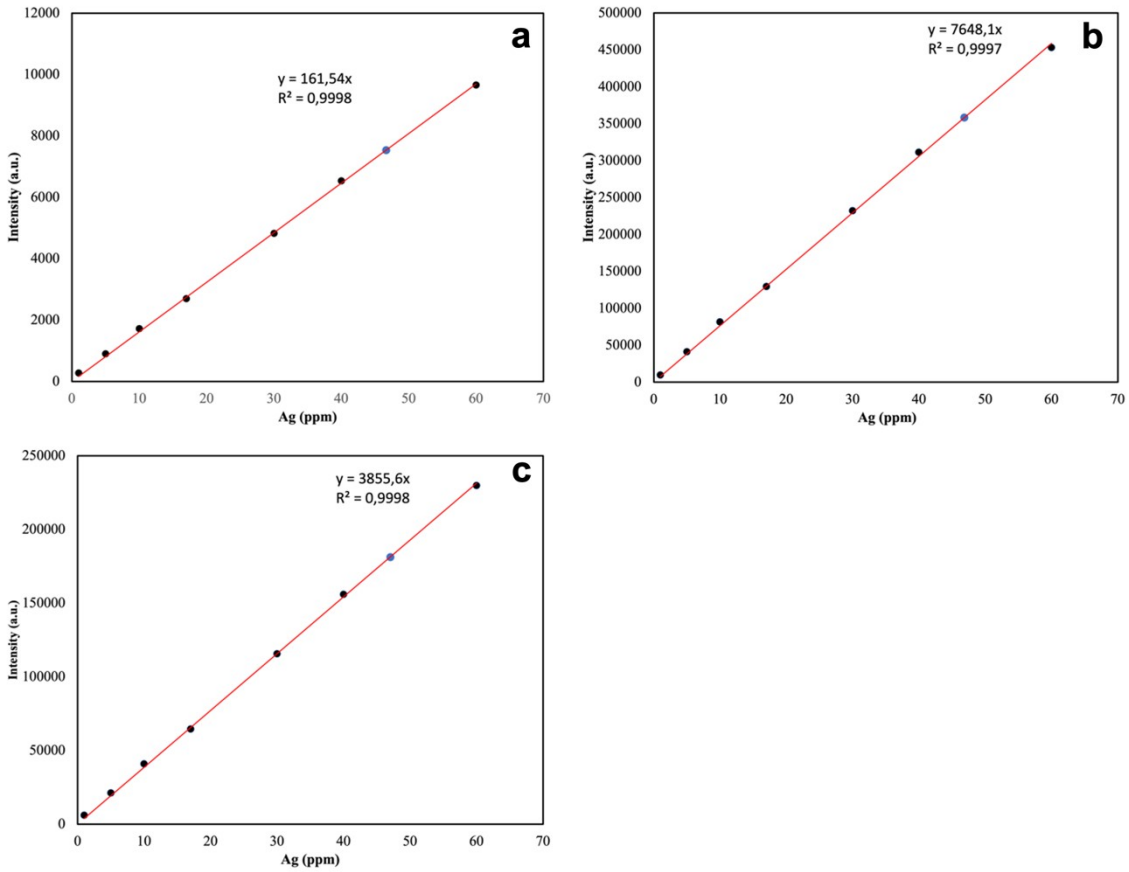

Figure S14. ICP-OES calibration curves for DNA750-AgNCs using different emission lines of Ag: a) 243.8 nm, b) 328 nm, and c) 338 nm. The blue dot indicates the amount of Ag in the DNA750-AgNC sample.

6. Comparison of fresh and aged DNA750-AgNCs

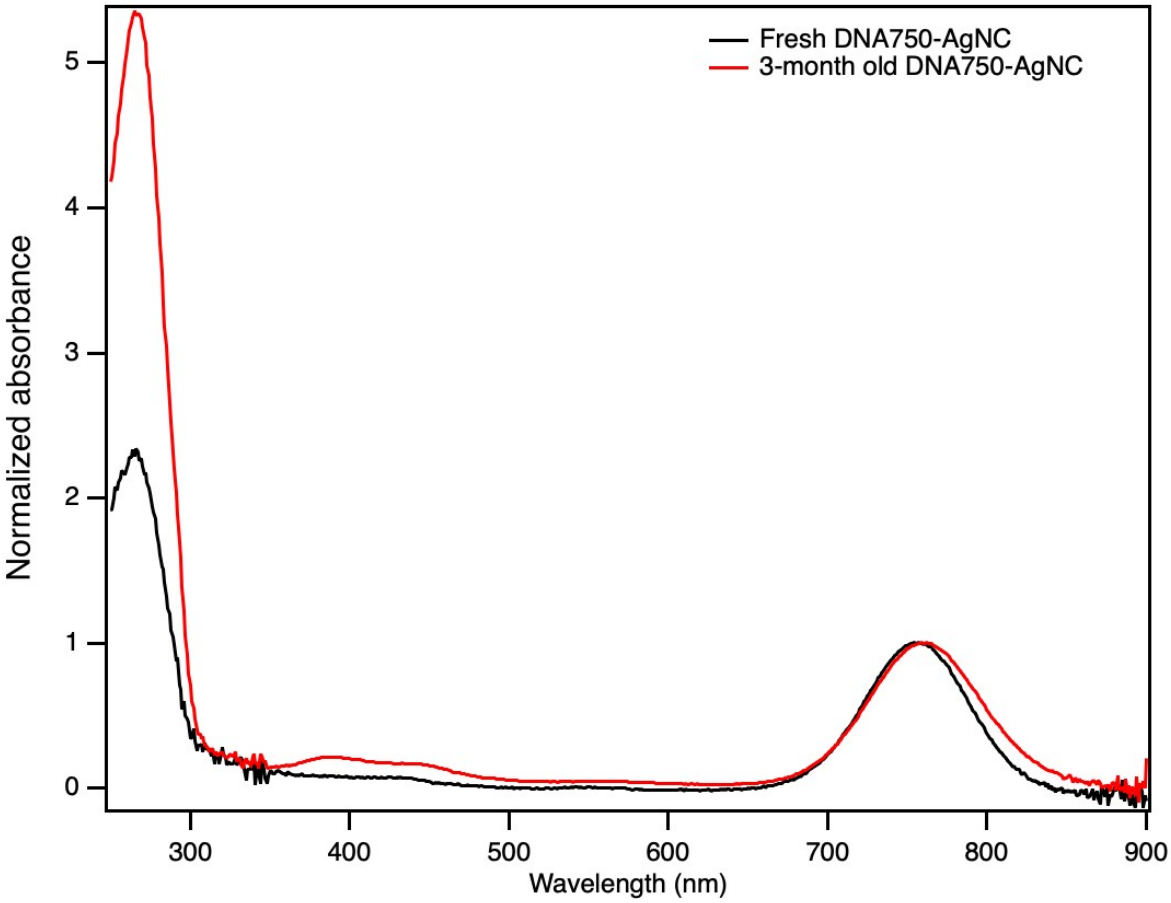

Figure S15. Comparison of absorption spectra (normalized to the AgNC peak) measured for a fresh DNA750-AgNC sample and a 3-month old DNA750-AgNC solution.

References:

[1] D. Schultz, K. Gardner, S. S. R. Oemrawsingh, N. Markešević, K. Olsson, M. Debord, D. Bouwmeester, E. Gwinn, *Advanced Materials* **2013**, *25*, 2797-2803.
[2] J. T. Petty, C. Fan, S. P. Story, B. Sengupta, A. St. John Iyer, Z. Prudowsky, R. M. Dickson, *The Journal of Physical Chemistry Letters* **2010**, *1*, 2524-2529.
[3] D. Schultz, E. G. Gwinn, *Chemical Communications* **2012**, *48*, 5748-5750.
[4] M. B. Liisberg, Z. Shakeri Kardar, S. M. Copp, C. Cerretani, T. Vosch, *The Journal of Physical Chemistry Letters* **2021**, *12*, 1150-1154.