Supporting information

Electron count and ligand composition influence the optical and chiroptical signatures of far-red and NIR-emissive DNA-stabilized silver nanoclusters

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1 Experimental section.

1.1 General experimental details.

Reagents such as silver nitrate, sodium borohydride, ammonium acetate, triethylamine, and acetic acid were purchased from Sigma Aldrich and Fisher Chemicals. Methanol used for high performance liquid chromatography (HPLC) was ultrapure HPLC grade (>99.8%). MilliQ water from Millipore was used to prepare samples. HPLC purification was performed on an Agilent 1260 Infinity system.

1.2 Preparation and purification methods

1.2.1 Synthesis of Ag_N-DNAs.

At first, a stoichiometric amount of $AgNO_3$ (Sigma Aldrich) was added to the single-stranded DNA oligomer (Integrated DNA Technologies, standard desalting) in 10 mM ammonium acetate (pH 7.0) to form the Ag^+–DNA complex. After 15 minutes, a freshly prepared aqueous solution of $NaBH_4$ ($[BH_4^-]/[Ag^+] = 0.5$) was added to the Ag^+–DNA complex. Samples were stored at 4 °C in the dark for 3-5 days, allowing sufficient time for the Ag_N-DNA formation, followed by purification using ion-paired reversed-phase high-performance liquid chromatography (RP-HPLC). The stoichiometry for Ag^+:DNA was optimized for each Ag_N-DNA to achieve maximum chemical yield (**Table S1**). No additional chloride source was added for synthesis of chlorido-protected ***Group* III** Ag_N-DNAs.[1] The increase in the storage temperature after reduction strongly increased the chemical yield of NIR-emissive ***IV*.4**.[2]

It is crucial to purify the synthesized Ag_N-DNA before any characterization to remove synthesis byproducts such as excess DNA oligomers, Ag^+–DNA complexes, larger silver nanoparticles, and other fluorescent and/or nonfluorescent nanoclusters that can influence the photophysical properties and obstruct the accurate determination of the composition of the Ag_N-DNA. As evident in the HPLC chromatogram presented in section **1.2.3**, multiple byproducts (excess DNA, fluorescent Ag_N-DNA byproducts) formed during synthesis can be separated by optimizing the HPLC gradient.

1.2.2 Purification of Ag_N-DNAs.

Purification was performed on an Agilent 1260 Infinity system. The original fluorescence detector (FLD) was replaced with a Hamamatsu R13456 photomultiplier tube (PMT) to achieve higher sensitivity > 600 nm, providing 250x signal enhancement at 750 nm. We used a Kinetex C18 column with 100 Å pore diameter, 5 μm particle size, and 50 mm × 46 μm dimensions (Phenomenex). The

solvents used were MilliQ H_2O and MeOH containing 35 mM triethyl ammonium acetate (TEAA, pH = 7.0) as an ion-pairing agent. The *in-situ* absorbance spectra recorded by the diode-array detector (DAD) and the fluorescence spectra (FLD) confirm the collection of the fluorescent product of interest. After purification, samples were solvent exchanged into 10 mM ammonium acetate using 3 kDa spin filters (Amicon, Millipore Sigma).

1.2.3 HPLC chromatograms of Ag_N-DNAs.

The HPLC method generally involves a 5 min pre-injection at 95% of the aqueous solvent that contains 35 mM TEAA in H_2O (**Solvent A**) and 5% of 35 mM TEAA in MeOH (**Solvent B**). Elution steps were the following: 0 – 2 min: 95% to **x**% solvent A, 2 – 12 min: **x**% – **y**% of solvent A (See **Table S1**), 12 – 14 min: **y**% – 5 % of solvent A, 14 – 19 min: 95% of solvent A. The flow rate and the gradient (**x**% to **y**% of solvent A in 10 minutes, see **Table S1**) used for purification are optimized for each Ag_N-DNA to ensure maximum separation from synthesis byproducts. The collection of pure fractions of Ag_N-DNAs is based on the absorbance (DAD) and emission (FLD) signals collected for the absorbance of the DNA at 260 nm (red), absorbance specific to Ag_N-DNA (yellow) and emission at peak emission wavelength (shown in dark blue) by universally exciting all the Ag_N-DNAs at 260 nm. The time during which the aliquots were collected is mentioned in Table **S1** and shown in the dashed box in the following HPLC chromatograms (**Figure S1** to **S11**). HPLC chromatograms of ***Group III*** are previously reported here.[1] HPLC chromatograms of ***IV*.1** and ***IV*.2** are reported here.[3]

The signals that appear before 2 minutes and after 12 minutes are never collected. After 12 minutes, the change in gradient is sharp and goes to a higher percentage of solvent B (35 mM TEAA in MeOH) which is generally used to wash the column after each HPLC run.

Table S1. The experimental conditions used for the synthesis of Ag_N-DNAs and the solvent gradient used and retention time for HPLC purification. (NOTE: **Solvent A** is 35 mM TEAA in H_2O and **solvent B** is 35 mM TEAA in methanol)

	DNA Sequence (5' to 3')	**[DNA] /μM**	**$[Ag^+]$/[DNA]**	**HPLC Gradient x% to y% of solvent A**	**Retention time/min**
***I*.1**	GTCCGGGCCA	25	5	88% to 75% 1.0 mL/min	6.7 – 8.2
***I*.2**	ACCAATGACC	25	5	80% to 60% 1.0 mL/min	11.4 – 11.8
***I*.3**	CCAGCCCGGA	30	5	88% to 70% 1.0 mL/min	3.5 – 3.8
***I*.4**	GTAGTCCCTA	20	5	88% to 73% 1.0 mL/min	3.2 –3.5
***I*.5**	ATCCCCTGTC	25	5	90% to 80% 1.0 mL/min	2.5 – 2.9
***I*.6**	AGTCACGACA	30	5	88% to 73% 1.0 mL/min	5.2 – 5.7
***II*.1**	CCCGGCCGAA	25	5	82% to 79% 1.0 mL/min	5.4 – 5.9
***II*.2**	CCCGGAGAAG	20	5	85% to 70%, 1.3 mL/min	3.3 – 3.7
***II*.3**	CCTGGGGAAA	25	5	78% to 68% 1.3 mL/min	7.2 – 7.8
***III*.1**	AACCCCACGT	25	7.5	See reference[1]	–
***III*.2**	CACCTAGCGA	25	7.5	See reference[1]	–
***III*.3**	CACCAAGCGA	25	7.5	See reference[1]	–
***III*.4**	CACCCAGCGA	25	7.5	See reference[1]	–
***III*.5**	CACCGAGCGA	25	7.5	See reference[1]	–
***III*.6**	CACCTAGCG_	25	7.5	See reference[1]	–
***IV*.1**	GCGCAAGATG	25	5	88% to 75% 1.0 mL/min	3.2 – 3.5
***IV*.2**	GACGACGGAT	35	5	See reference[3]	–
***IV*.3**	ATCTCCACAG	25	4	See reference[3]	–
***IV*.4**	AGGCGATCAT	25	7.5	80% to 65% 1.3 mL/min	10.4 – 11.0

Group I: *Ag_N-DNAs containing N_0 = 6 and n_s =2*

1. *I*.1

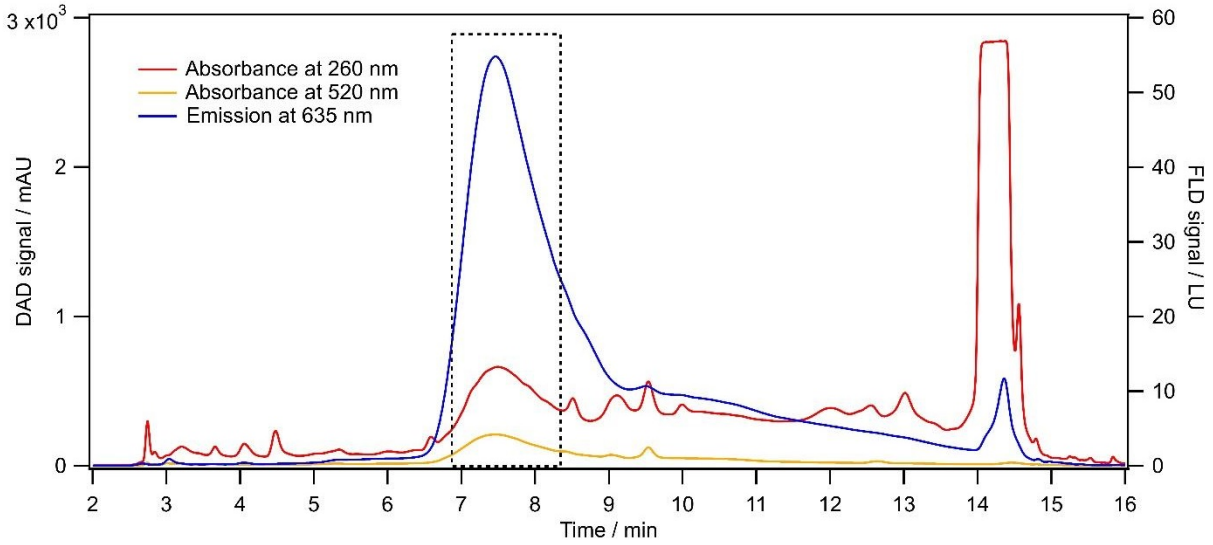

Figure S1. HPLC chromatograms of ***I*.1**. The dashed line shows the collected fraction. DAD signals correspond to absorbance through the DNA nucleobases (260 nm, red), and absorbance through the nanocluster of interest (520 nm, orange). FLD signals (635 nm, blue) correspond to the emission wavelengths of the product of interest (635 nm).

2. *I*.2

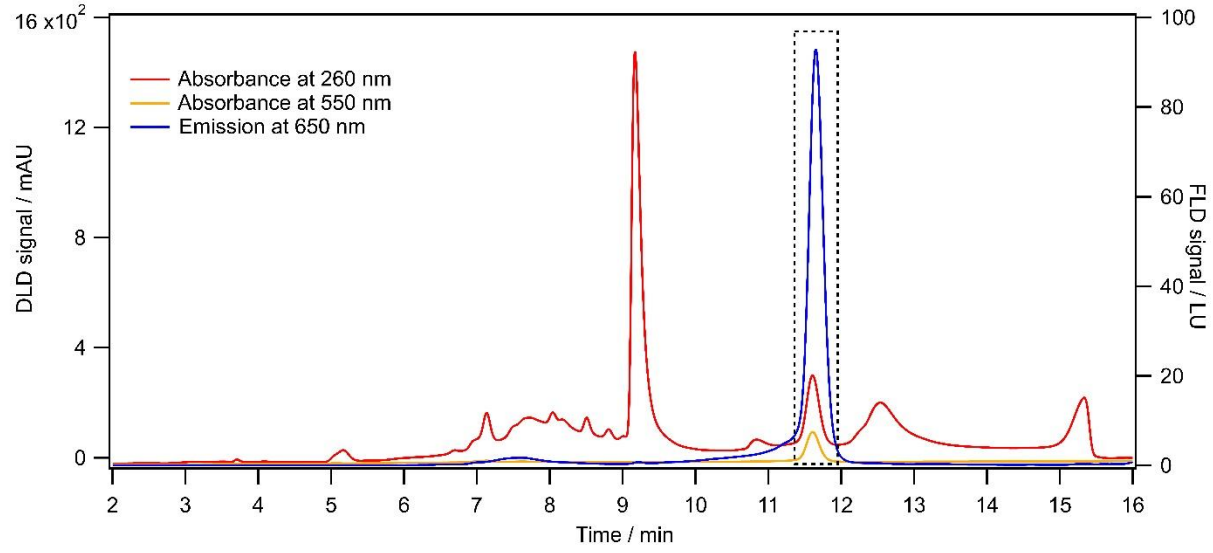

Figure S2. HPLC chromatograms of ***I*.2**. The dashed line shows the collected fraction. DAD signals correspond to absorbance through the DNA nucleobases (260 nm, red), and absorbance through the nanocluster of interest (550 nm, orange). FLD signals (650 nm, blue) correspond to the emission wavelengths of the product of interest (650 nm).

3. *I*.3

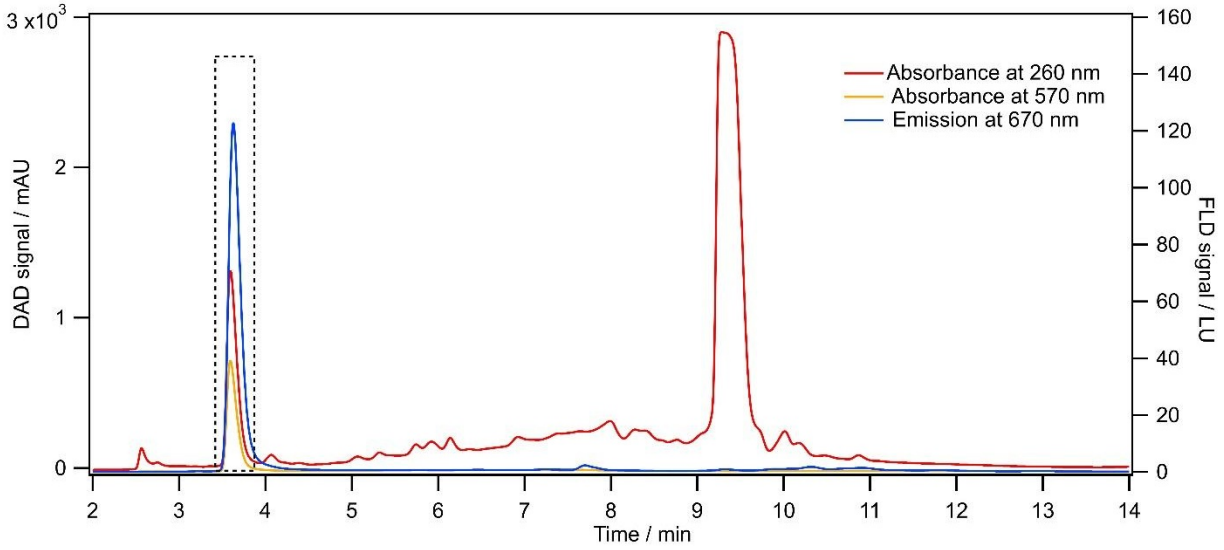

Figure S3. HPLC chromatograms of ***I*.3**. The dashed line shows the collected fraction. DAD signals correspond to absorbance through the DNA nucleobases (260 nm, red), and absorbance through the nanocluster of interest (570 nm, orange). FLD signals (blue) correspond to the emission wavelengths of the product of interest (670 nm).

4. *I*.4

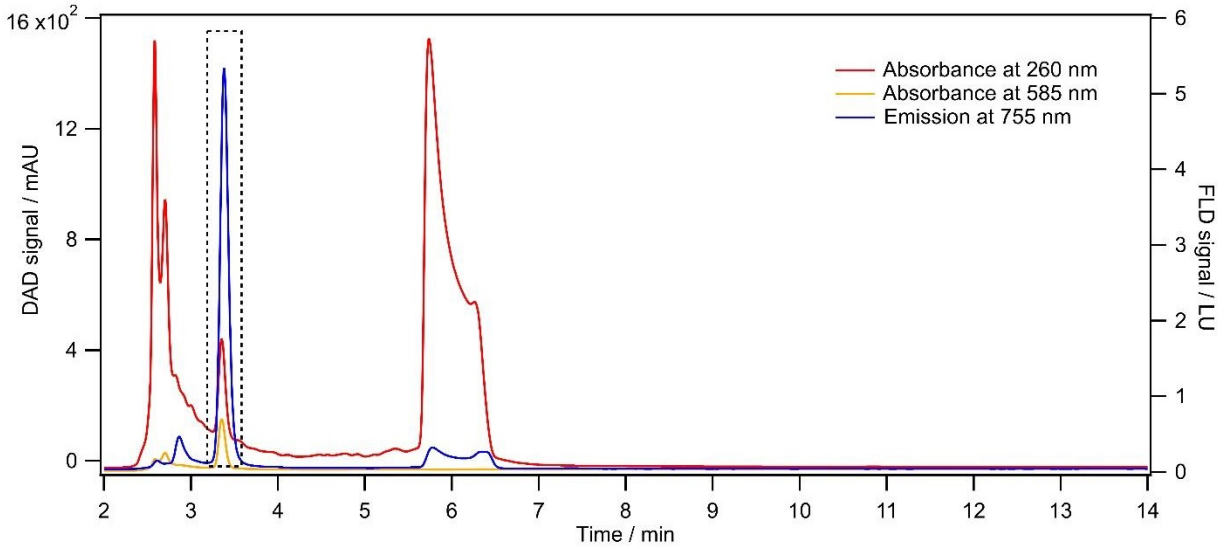

Figure S4. HPLC chromatograms of ***I*.4**. The dashed line shows the collected fraction. DAD signals correspond to absorbance through the DNA nucleobases (260 nm, red), and absorbance through the NIR nanocluster of interest (585 nm, orange). FLD signals correspond to the emission wavelengths of the NIR product of interest (755 nm, blue). The fraction eluting at 5.8 to 6.5 min is not collected since no absorbance at 585 nm was observed.

5. *I*.5

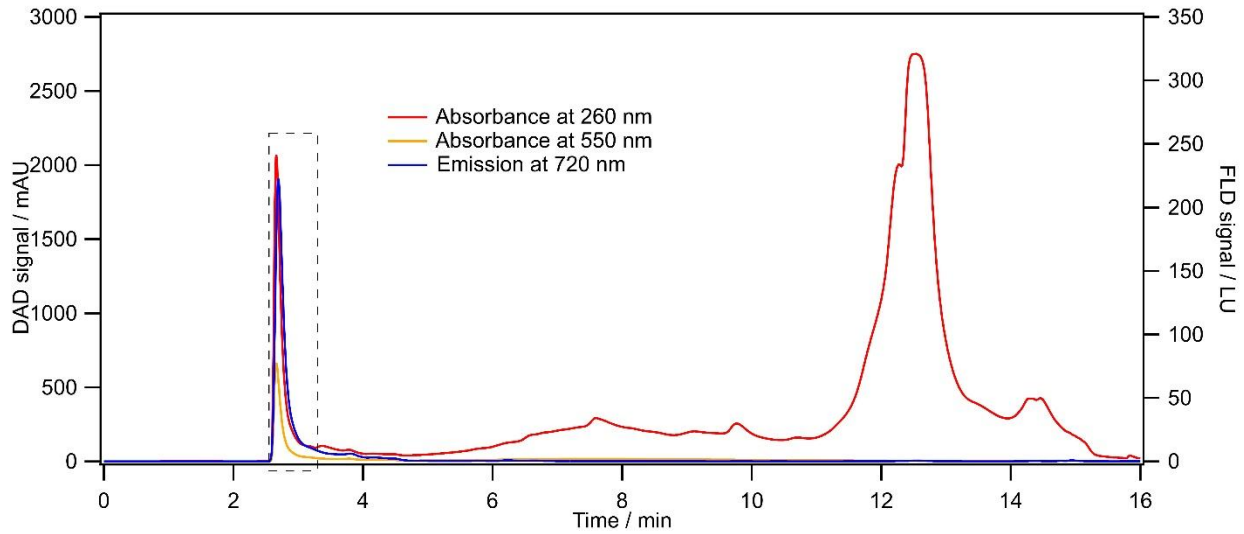

Figure S5. HPLC chromatograms of ***I*.5**. The dashed line shows the collected fraction. DAD signals correspond to absorbance through the DNA nucleobases (260 nm, red), and absorbance through the NIR nanocluster of interest (550 nm, orange). FLD signals correspond to the emission wavelengths of the NIR product of interest (720 nm, blue).

6. *I*.6

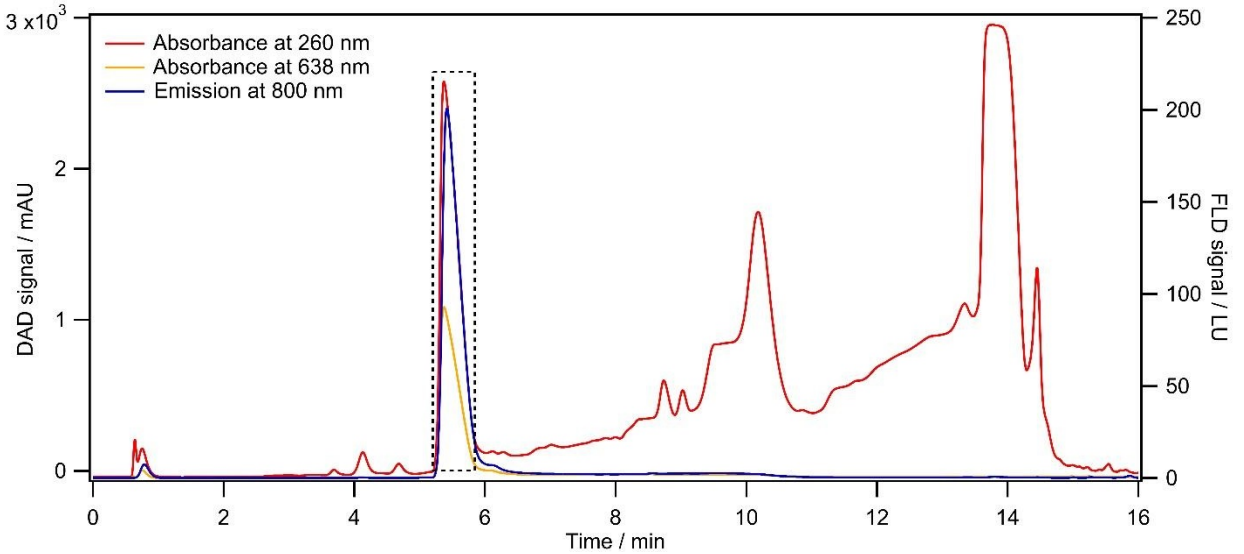

Figure S6. HPLC chromatograms of ***I*.6**. The dashed line shows the collected fraction. DAD signals correspond to absorbance through the DNA nucleobases (260 nm, red), and absorbance through the NIR nanocluster of interest (638 nm, orange). FLD signals correspond to the emission wavelengths of the NIR product of interest (800 nm, blue). The fraction eluting at 0.9 min is not collected because fractions eluting before 2 minutes are generally not pure.

Group II. *Ag_N-DNAs containing N_0 = 6 and n_s =3*

1. *II*.1

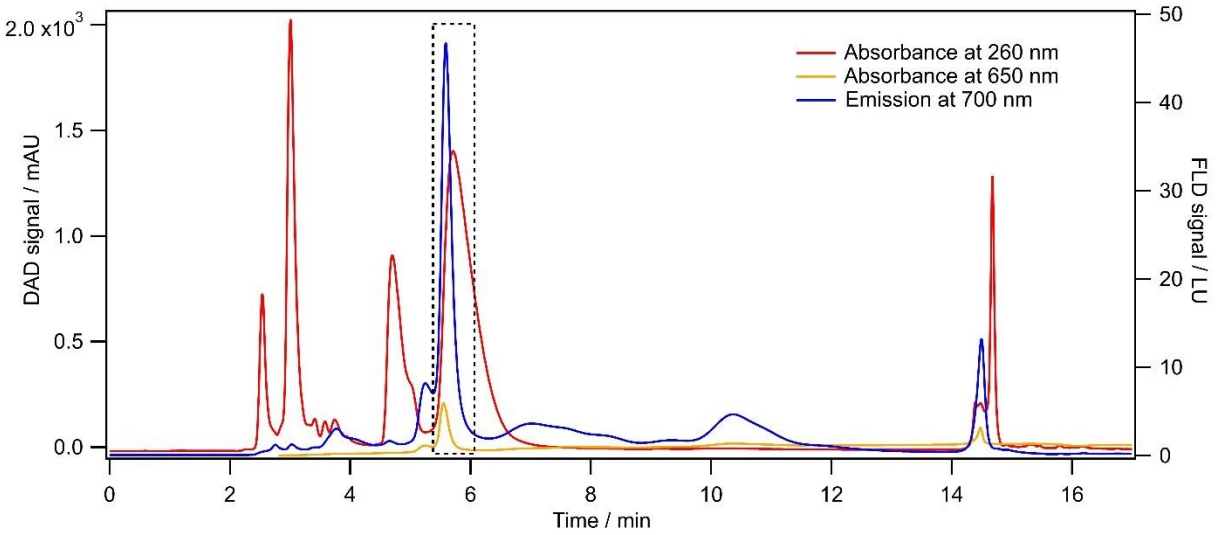

Figure S7. HPLC chromatograms of ***II*.1**. The dashed line shows the collected fraction. DAD signals correspond to absorbance through the DNA nucleobases (260 nm, red), and absorbance through the nanocluster of interest (650 nm, orange). FLD signals (700 nm, blue) correspond to the emission wavelengths of the product of interest (700 nm). Fractions eluting between 2.3 to 5.5 min are not collected since no absorbance at 650 nm (orange) was not observed.

2. *II*.2

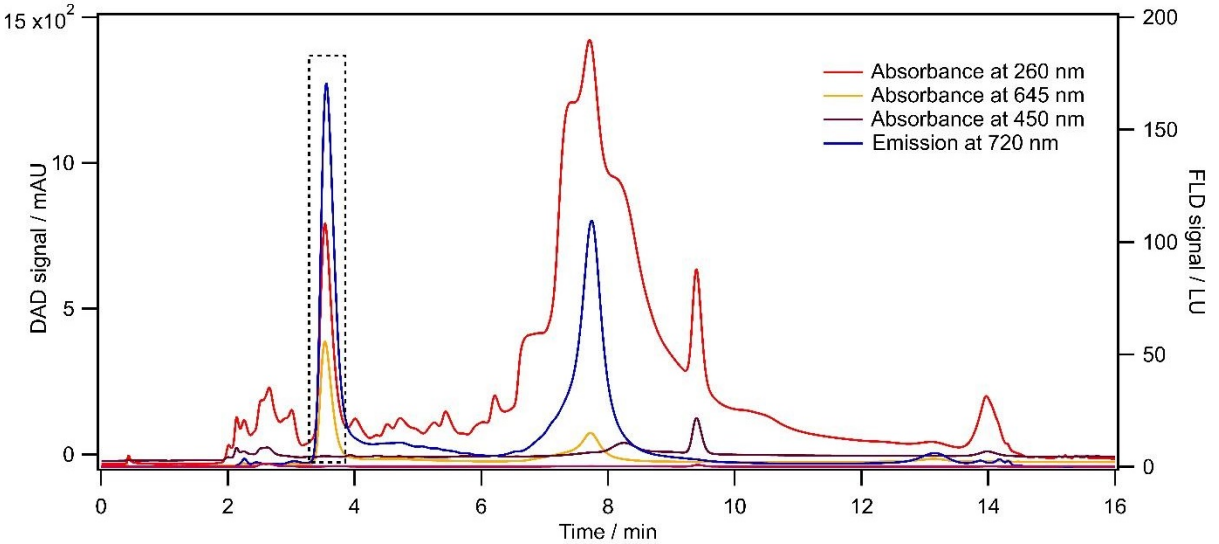

Figure S8. HPLC chromatograms of ***II*.2**. The dashed line shows the collected fraction. DAD signals correspond to absorbance through the DNA nucleobases (260 nm, red), and absorbance through the nanocluster of interest (645 nm, orange). FLD signals (blue) correspond to the emission wavelengths of the product of interest (720 nm).

3. *II*.3

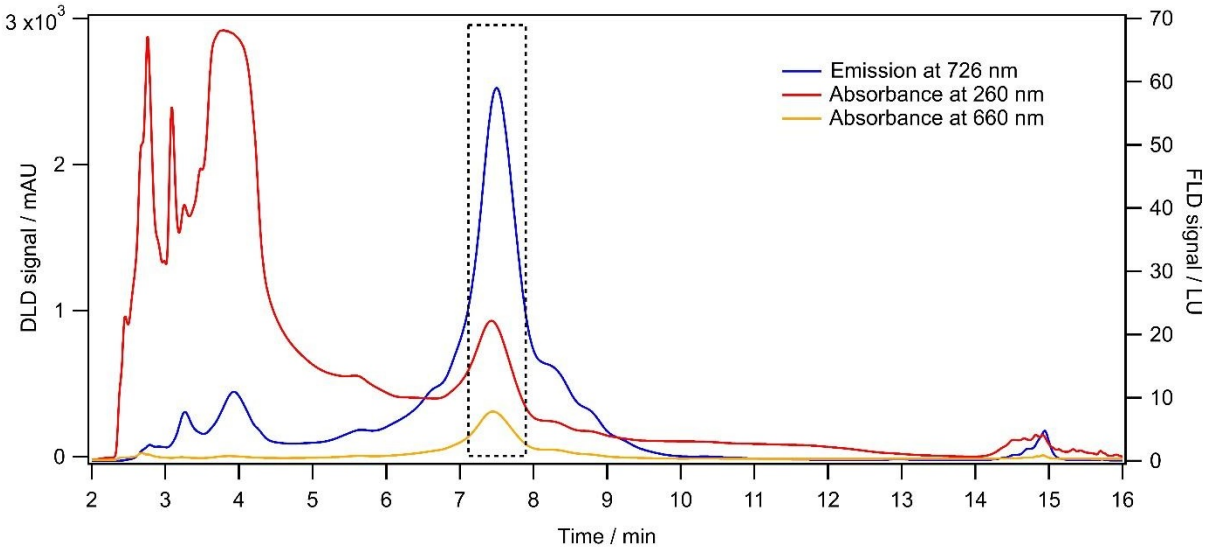

Figure S9. HPLC chromatograms of ***II*.3**. The dashed line shows the collected fraction. DAD signals correspond to absorbance through the DNA nucleobases (260 nm, red), and absorbance through the nanocluster of interest (660 nm, orange). FLD signals (blue) correspond to the emission wavelengths of the product of interest (726 nm). The fractions eluting at 3 to 4 min are not collected since no absorbance at 660 nm was observed.

Group III: *Ag_N-DNAs containing N_0 = 6 with additional chloride ligands (see reference 1)*[1]

Group IV: *Ag_N-DNAs containing N_0 = 8 and n_s =2*

1. *IV*.1

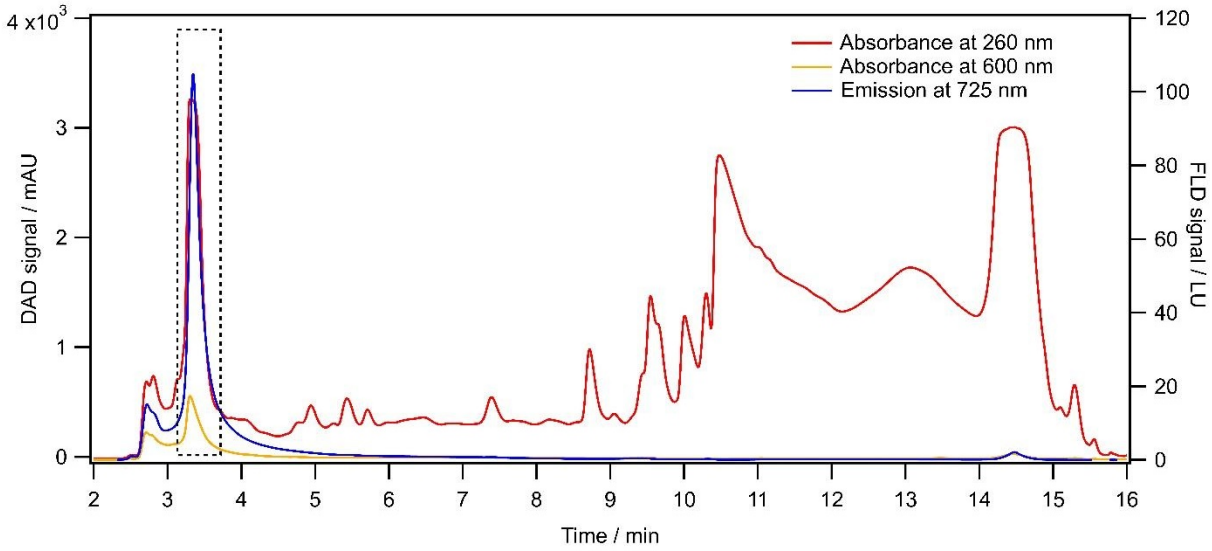

Figure S10. HPLC chromatograms of ***IV*.1**. The dashed line shows the collected fraction. DAD signals correspond to absorbance through the DNA nucleobases (260 nm, red) and absorbance of the nanocluster of interest (600 nm, orange). FLD signals (blue) correspond to the emission wavelengths of the product of interest (725 nm).

2. *IV*.4

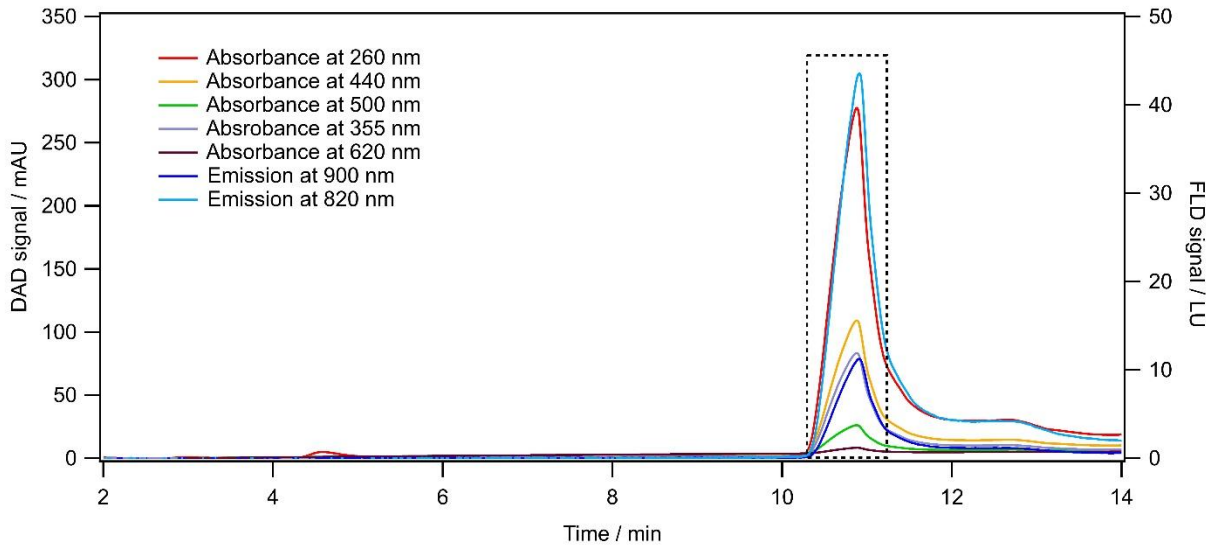

Figure S11. HPLC chromatograms of ***IV*.4**. The dashed line shows the collected fraction. DAD signals correspond to absorbance through the DNA nucleobases (260 nm, red) and absorbance of the nanocluster of interest (355, 440, 500 and 620 nm). FLD signals (900 nm: blue and 820 nm: light blue) correspond to the emission wavelengths of the NIR product of interest.

2 Characterization methods.

2.1 Steady-state absorbance and fluorescence measurements.

Absorbance and emission spectra of HPLC purified Ag_N-DNAs were measured using a thermoelectrically cooled, fiber-coupled spectrometer (Ocean Optics QE65000). Absorbance spectra were measured using a DH-Mini (Ocean Insight) deuterium & tungsten halogen UV-Vis-NIR light source. Fluorescence spectra were collected using a UV LED as a light source for universal UV-excitation of the nanoclusters.[4]

2.2 Circular dichroism (CD) spectroscopy.

Circular dichroism spectra were measured in Chirascan V100 from Applied Photophysics. The concentration of samples was maintained such that the absorbance of the Ag_N-DNA ranged between 0.8 to 1.0. The CD spectra of Ag_N-DNA in 10 mM ammonium acetate were recorded from 200 nm to 800 nm in a quartz cuvette (Starna Cells) of 0.5 mm optical path length at 20 °C with a scanning rate of 1.0 nm interval per 1.0 s. The CD spectrum of each Ag_N-DNA is the average of three scans with a manual baseline correction (contribution from 10 mM ammonium acetate).

2.3 Mass spectrometry.

HPLC-purified Ag_N-DNAs were solvent exchanged to 10 mM ammonium acetate and were directly injected at 100 µL/min in negative ion mode with a 2 kV capillary voltage, 30 V cone voltage and no collision energy. Spectra were collected from 1000 to 4000 *m/z* and integrated for 1 s. Source and desolvation temperatures were 80 and 150 ºC, respectively. Gas flows were 45 L/h for the cone, and 450 L/h for the desolvation. Samples were injected with 50 mM NH_4OAc – MeOH (80:20) solution at pH 7**.**

2.3.1 Determination of the molecular formula of Ag_N-DNAs.

Determination of N_0, N and n_s:

High resolution ESI-MS allows the determination of the ion mass to charge ratio, *m/z*, as well *m* and *z* by resolving the isotopic distribution of the product. For compositionally pure Ag_N-DNAs, the experimentally measured ESI-MS isotope pattern is compared to the calculated distribution of the cluster and the numbers of silver atoms *N,* and the number of DNA strands *ns* are determined. to separate *N* into effective neutral (N_0) and cationic (N_+) silver content *i.e.,* $N = N_0 + N_+$.[1,5–8]
The charge state *z*– of a *m/z* peak is determined by the spacing between adjacent peaks of the isotope pattern, which are spaced by 1/*z*. The total charge of the complex corresponding to this *m/z* peak is equal to the charge of the number of silver cations, eN_+, minus the charge of the number of protons removed from the DNA, en_{pr}, to reach the total charge of $-eZ$ observed experimentally:

$$-ez = eN_+ - en_{pr} \qquad (1)$$

Then, because n_{pr} protons have been removed from the Ag_N-DNA complex, the measured total mass *m* (in amu) is:

$$m = m_{DNA}\, n_s + m_{Ag}\,(N_+ + N_0) - n_{pr} \qquad (2)$$

where m_{DNA} is the DNA template strand mass, n_s is the number of DNA strands in the complex, and m_{Ag} is the silver atom mass (the mass of a proton is treated here as 1 amu). N_+ and N_0 may be determined by calculating the isotope distribution pattern for varying values of N_+, and thus n_{pr}, to determine the cluster charge (Q_c) that best matches the isotope pattern.

The nanocluster size and charge were determined by fitting the calculated isotopic distribution of the Ag_N-DNA to the experimental spectra (Figure **S12-S19**). Calculated isotopic distributions were obtained from MassLynx using the chemical formula and corrected for the nanocluster's overall positive charge (oxidation state) cluster. The Ag_N-DNA composition and charge were determined by fitting the calculated isotopic distribution of the Ag_N-DNA to the experimental spectra. To confirm the overall charge of the nanocluster (Q_c), we compared the best fit with the two observed charge states peaks, z = 4− (dark blue curve) and z = 5− (light blue curve).

Determination of the overall charge of the nanocluster (Q_c) to deduce the N_0.

To determine the overall charge of the nanocluster (Q_c), single Gaussians were fitted to the peaks that appeared in experimental mass spectra and compared the centers (x_0) of these Gaussian fits. The centers the Gaussian fits of the isotopic distributions calculated for a range of Q_c values that generally includes $Q_c = 0$, best fitted Q_c and ± 1 values of centers fitted Q_c.[1,3,9]

Once the Q_c is determined, N_0 is determined using the formula:

$$N_0 = N - Q_c \qquad (3)$$

Table S2 summarizes the chemical composition and the N, N_0, n_s and Q_c values for each Ag_N-DNAs. **Table S3** provides the values of gaussian fitting of the isotopic distribution to determine the Q_c.

Table S2. Summary of molecular formula of the Ag_N-DNAs reported. The ns denotes the total number of strands that stabilizes the Ag_N-DNA, N denotes the total number of silver atoms present in the Ag_N-DNA, N_0 denotes the total effective valence electrons, N_{Cl^-} denotes the number of chlorido ligands, and Q_c denotes the oxidation state of the Ag_N core.

Ag_N-DNA	Molecular formula	No. of DNA strands (n_s)	N	N_0	N_{Cl^-}	Q_c
Group I: *Ag_N-DNAs containing N_0 = 6 and n_s =2*						
***I*.1**	$C_{192}H_{244}N_{78}O_{116}P_{18}Ag_{20}$	2	20	6	0	+14
***I*.2**	$C_{192}H_{244}N_{78}O_{110}P_{18}Ag_{15}$	2	15	6	0	+9
***I*.3**	$C_{190}H_{242}N_{80}O_{112}P_{18}Ag_{18}$	2	18	6	0	+12
***I*.4**	$C_{194}H_{248}N_{70}O_{118}P_{18}Ag_{16}$	2	16	6	0	+10
***I*.5**	$C_{190}H_{248}N_{62}O_{120}P_{18}Ag_{17}$	2	17	6	0	+11
***I*.6**	$C_{194}H_{244}N_{82}O_{110}P_{18}Ag_{16}$	2	16	6	0	+10
Group II: *Ag_N-DNAs containing N_0 = 6 and n_s =3*						
***II*.1**	$C_{285}H_{363}N_{120}O_{168}P_{27}Ag_{18}$	3	18	6	0	+12
***II*.2**	$C_{291}H_{363}N_{132}O_{165}P_{27}Ag_{21}$	3	21	6	0	+15
***II*.3**	$C_{294}H_{366}N_{129}O_{168}P_{27}Ag_{16}$	3	16	6	0	+10
Group III: *Ag_N-DNAs containing N_0 = 6 with additional chlorido ligands*						
***III*.1**	$C_{190}H_{244}N_{74}O_{112}P_{18}\ ClAg_{15}$	2	15	6	1	+8
***III*.2**	$C_{192}H_{244}N_{78}O_{112}P_{18}Cl_2Ag_{16}$	2	16	6	2	+8
***III*.3**	$C_{192}H_{242}N_{84}O_{108}P_{18}Cl_2Ag_{16}$	2	16	6	2	+8
***III*.4**	$C_{190}H_{242}N_{80}O_{110}P_{18}Cl_2Ag_{16}$	2	16	6	2	+8
***III*.5**	$C_{192}H_{242}N_{84}O_{110}P_{18}Cl_2Ag_{16}$	2	16	6	2	+8
***III*.6**	$C_{172}H_{220}N_{68}O_{102}P_{16}Cl_2Ag_{16}$	2	16	6	2	+8
Group IV: *Ag_N-DNAs containing N_0 = 8 and n_s =2*						
***IV*.1**	$C_{196}H_{244}N_{86}O_{112}P_{18}Ag_{19}$	2	19	8	0	+11
***IV*.2**	$C_{196}H_{244}N_{86}O_{112}P_{18}Ag_{17}$	2	17	8	0	+9
***IV*.3**	$C_{129}H_{246}N_{72}O_{114}P_{18}Ag_{16}$	2	16	8	0	+8
***IV*.4**	$C_{196}H_{246}N_{80}O_{114}P_{18}Ag_{20}$	2	20	8	0	+12

Table S3. Summary of center of Gaussian fits, x_0, to the dominant peaks in the experimentally measured mass spectra of Ag_N-DNAs (Figure S12 to S19) to the calculated mass distributions for different overall nanocluster charges Q_c. The rightmost column compares x_0 for the experimental mass spectral peak to x_0 for the calculated mass distribution at the specific Q_c. Bold text corresponds to experimental values and the corresponding best matching Q_c.

Ag_N-DNA	Charge state	Fit	x_0	Error	Experimental x_0 − Calculated x_0
Group I: *Ag_N-DNAs containing $N_0 = 6$ and $n_s = 2$*					
***I*.1**	z = 4− *N* = 16 **$N_0 = 6$**	*Experimental*	**1942.4**	**0.0667**	**0**
		$Q_c = 0$	1944.9	0.00426	−2.5
		$Q_c = 9$	1942.7	0.00426	−0.3
		$Q_c = 10$	**1942.4**	**0.00374**	**0**
		$Q_c = 11$	1942.2	0.00426	0.2
	z = 5− *N* = 16 **$N_0 = 6$**	*Experimental*	**1553.7**	**0.0573**	**0**
		$Q_c = 0$	1555.8	0.00349	−2.1
		$Q_c = 9$	1554	0.00349	−0.3
		$Q_c = 10$	**1553.8**	**0.00349**	**−0.1**
		$Q_c = 11$	1553.5	0.00349	0.2
	z = 4− *N* = 20 **$N_0 = 6$**	***Experimental***	**2049.3**	**0.0762**	**0**
		$Q_c = 0$	2052.8	0.00427	−3.5
		$Q_c = 13$	2049.6	0.00427	−0.3
		$Q_c = 14$	**2049.3**	**0.00427**	**0**
		$Q_c = 11$	2049.1	0.00427	0.2
	z = 5− *N* = 20 **$N_0 = 6$**	***Experimental***	**1639.2**	**0.0663**	**0**
		$Q_c = 0$	1642	0.00342	−2.8
		$Q_c = 13$	1639.4	0.00341	−0.2
		$Q_c = 14$	**1639.2**	**0.00341**	**0**
		$Q_c = 15$	1639	0.00341	0.2
***I*.2**	z = 4− **$N_0 = 6$**	*Experimental*	**1891.8**	**0.0696**	**0**
		$Q_c = 0$	1894	0.00455	−2.2
		$Q_c = 8$	1892	0.00455	−0.2
		$Q_c = 9$	**1891.7**	**0.00455**	**0.1**
		$Q_c = 10$	1891.5	0.00455	0.3
	z = 5− **$N_0 = 6$**	*Experimental*	**1513.2**	**0.0572**	**0**
		$Q_c = 0$	1515	0.00364	−1.8
		$Q_c = 8$	1513.4	0.00364	−0.2
		$Q_c = 9$	**1513.2**	**0.00364**	**0**

		$Q_c = 10$	1513	0.00364	0.2
***I*.3**	z = 4– $N = 15$ **$N_0 = 6$**	*Experimental*	**1900.2**	**0.0656**	**0**
		$Q_c = 0$	1902.5	0.00463	−2.3
		$Q_c = 8$	1900.5	0.00463	−0.3
		$Q_c = 9$	**1900.2**	**0.00463**	**0**
		$Q_c = 10$	1900	0.00463	0.2
	z = 5– $N = 15$ **$N_0 = 6$**	*Experimental*	**1519.9**	**0.0555**	**0**
		$Q_c = 0$	1521.8	0.00371	−1.9
		$Q_c = 8$	1520.2	0.00371	−0.3
		$Q_c = 9$	**1520**	**0.00371**	**−0.1**
		$Q_c = 10$	1519.8	0.00371	0.1
	z = 4– $N = 18$ **$N_0 = 6$**	*Experimental*	**1980.4**	**0.0717**	**0**
		$Q_c = 0$	1983.4	0.00439	−3.0
		$Q_c = 11$	1980.6	0.00439	−0.2
		$Q_c = 12$	**1980.4**	**0.00439**	**0**
		$Q_c = 13$	1980.1	0.00439	0.3
	z = 5– $N = 18$ **$N_0 = 6$**	*Experimental*	**1584.1**	**0.0591**	**0**
		$Q_c = 0$	1586.5	0.00351	−2.4
		$Q_c = 11$	1584.3	0.00351	−0.2
		$Q_c = 12$	**1584.1**	**0.00351**	**0**
		$Q_c = 13$	1583.9	0.00351	0.2
***I*.4**	z = 4– $N = 18$ **$N_0 = 6$**	*Experimental*	**1929.4**	**0.103**	**0**
		$Q_c = 0$	1931.9	0.00431	−2.5
		$Q_c = 11$	1929.2	0.00431	−0.2
		$Q_c = 12$	**1929.4**	**0.00431**	**0**
		$Q_c = 13$	1929.7	0.00431	0.3
	z = 5– $N = 18$ **$N_0 = 6$**	*Experimental*	**1543.3**	**0.0775**	**0**
		$Q_c = 0$	1545.4	0.00344	−2.1
		$Q_c = 11$	1543.6	0.00344	−0.3
		$Q_c = 12$	1543.4	0.00344	**0.1**
		$Q_c = 13$	1543.4	0.00344	0.1
***I*.5**	z = 4– $N = 17$ **$N_0 = 6$**	*Experimental*	**1924.1**	**0.0668**	**0**
		$Q_c = 0$	1926.9	0.00473	−2.8
		$Q_c = 10$	1924.4	0.00473	−0.3
		$Q_c = 11$	**1924.1**	**0.00473**	**0**
		$Q_c = 12$	1923.9	0.00473	0.2

	z = 5– *N* = 17 **N_0 = 6**	*Experimental*	**1539.1**	**0.0523**	**0**
		Q_c = 0	1541.3	0.00379	−2.2
		Q_c = 10	1539.3	0.00379	−0.2
		Q_c = 11	**1539.1**	**0.00385**	**0**
		Q_c = 12	1538.9	0.00379	0.2
***I*.6**	z = 4– *N* = 16 **N_0 = 6**	*Experimental*	**1938.5**	**0.0971**	**0**
		Q_c = 0	1941	0.00406	−2.5
		Q_c = 9	1938.7	0.00406	−0.2
		Q_c = 10	**1938.5**	**0.00406**	**0**
		Q_c = 11	1938.2	0.00406	0.3
	z = 5– *N* = 16 **N_0 = 6**	*Experimental*	**1550.5**	**0.0869**	**0**
		Q_c = 0	**1552.6**	0.00325	−2.1
		Q_c = 9	**1550.8**	0.00325	−0.3
		Q_c = 10	**1550.6**	**0.00325**	**0.1**
		Q_c = 11	1550.4	0.00325	0.1
Group II: *Ag_N-DNAs containing N_0 = 6 and n_s =3*					
***II*.1**	z = 5– *N* = 18 **N_0 = 6**	*Experimental*	**2183.7**	**0.0361**	**0**
		Q_c = 0	2186.1	0.00252	−2.4
		Q_c = 11	2183.9	0.00252	−0.2
		Q_c = 12	**2183.7**	**0.00252**	**0**
		Q_c = 13	2183.5	0.00252	0.2
	z = 6– *N* = 18 **N_0 = 6**	*Experimental*	**1819.5**	**0.0294**	**0**
		Q_c = 0	1821.6	0.00211	−2.1
		Q_c = 11	1819.7	0.00211	−0.2
		Q_c = 12	**1819.6**	**0.00211**	**−0.1**
		Q_c = 13	1819.4	0.00211	−0.1
***II*.2**	z = 5– *N* = 21 **N_0 = 6**	*Experimental*	**2286.2**	**0.0624**	**0**
		Q_c = 0	2289.2	0.00222	−3.0
		Q_c = 14	2286.4	0.00222	−0.2
		Q_c = 15	**2286.2**	**0.00222**	**0**
		Q_c = 16	2286	0.00222	0.2
	z = 6– *N* = 21 **N_0 = 6**	*Experimental*	**1905**	**0.0452**	**0**
		Q_c = 0	1907.5	0.00185	−2.5
		Q_c = 14	1905.2	0.00185	−0.2
		Q_c = 15	**1905**	**0.00185**	**0**
		Q_c = 16	1904.9	0.00185	0.1

	z = 5− *N* = 20 **N_0 = 6**	*Experimental*	**2264.9**	**0.0483**	**0**
		Q_c = 0	2267.7	0.00203	−2.8
		Q_c = 13	2265.1	0.00224	−0.2
		Q_c = 14	**2264.9**	**0.00224**	**0**
		Q_c = 15	2264.7	0.00224	0.2
	z = 6− *N* = 20 **N_0 = 6**	*Experimental*	**1887.2**	**0.0488**	**0**
		Q_c = 0	1889.6	0.00187	−2.4
		Q_c = 13	1887.4	0.00187	−0.2
		Q_c = 14	**1887.2**	**0.00187**	**0**
		Q_c = 15	1887.1	0.00187	0.1
***II*.3**	z = 5− **N_0 = 6**	*Experimental*	**2188.3**	**0.0421**	**0**
		Q_c = 0	2190.4	0.00282	−2.1
		Q_c = 9	2188.6	0.00282	−0.3
		Q_c = 10	**2188.4**	**0.00282**	**−0.1**
		Q_c = 11	2188.2	0.00282	0.1
	z = 6− **N_0 = 6**	*Experimental*	**1823.4**	**0.0337**	**0**
		Q_c = 0	1825.1	0.00234	−1.7
		Q_c = 9	1823.6	0.00234	−0.2
		Q_c = 10	**1823.5**	**0.00234**	**−0.1**
		Q_c = 11	1823.3	0.00234	0.1
Group III: *Ag_N-DNAs containing N_0 = 6 with additional chloride ligands*					
***III*.1**	z = 4− **N_0 = 6**	*Experimental*	1888.8	0.0659	**0**
		Q_c = 0	1890.8	0.00493	−2.0
		Q_c = 7	1889.1	0.00476	−0.3
		Q_c = 8	**1888.8**	**0.00493**	**0**
		Q_c = 9	1888.6	0.00477	0.2
	z = 5− **N_0 = 6**	*Experimental*	**1510.9**	**0.0521**	**0**
		Q_c = 0	1512.5	0.00394	−1.6
		Q_c = 7	1511.1	0.00394	−0.2
		Q_c = 8	**1510.9**	**0.00394**	**0**
		Q_c = 9	1510.7	0.00394	0.2
Group IV: *Ag_N-DNAs containing N_0 = 8 and n_s =2*					
***IV*.1**	z = 4− *N* = 19 **N_0 = 8**	*Experimental*	**2047.1**	**0.0705**	**0**
		Q_c = 0	2049.9	0.00376	−2.8
		Q_c = 10	2047.4	0.00376	−0.3
		Q_c = 11	**2047.1**	**0.00376**	**0**

		$Q_c = 12$	2046.9	0.00376	0.2
		Experimental	**1637.5**	**0.0535**	**0**
	z = 5−	$Q_c = 0$	1639.7	0.003	−2.2
	$N = 19$	$Q_c = 10$	1637.7	0.003	−0.2
	$\boldsymbol{N_0 = 8}$	$\boldsymbol{Q_c = 11}$	**1637.5**	**0.003**	**0**
		$Q_c = 12$	1637.3	0.003	0.2
IV.4		*Experimental*	**2061.3**	**0.0745**	**0**
	z = 4−	$Q_c = 0$	2064.3	0.00386	−3.0
	$N = 20$	$Q_c = 11$	2061.6	0.00386	−0.3
	$\boldsymbol{N_0 = 8}$	$\boldsymbol{Q_c = 12}$	**2061.3**	**0.00386**	**0**
		$Q_c = 13$	2061.1	0.00386	0.1
		Experimental	**1648.8**	**0.0607**	**0**
	z = 5−	$Q_c = 0$	1651.1	0.00309	−2.3
	$N = 20$	$Q_c = 11$	1649.1	0.00309	−0.3
	$\boldsymbol{N_0 = 8}$	$\boldsymbol{Q_c = 12}$	**1648.9**	**0.00309**	**−0.1**
		$Q_c = 13$	1648.5	0.00309	0.3
		Experimental	**2034.6**	**0.0663**	**0**
	z = 4−	$Q_c = 0$	2037.4	0.00386	−2.8
	$N = 19$	$Q_c = 10$	2034.9	0.00386	−0.3
	$\boldsymbol{N_0 = 8}$	$\boldsymbol{Q_c = 11}$	**2034.6**	**0.00386**	**0**
		$Q_c = 12$	2034.4	0.00386	0.2
		Experimental	**1627.5**	**0.0546**	**0**
	z = 5−	$Q_c = 0$	1629.7	0.00309	−2.2
	$N = 19$	$Q_c = 10$	1627.7	0.00309	−0.2
	$\boldsymbol{N_0 = 8}$	$\boldsymbol{Q_c = 11}$	**1627.5**	**0.00309**	**0**
		$Q_c = 12$	1627.3	0.00309	0.2

2.3.2 Mass spectra of Ag_N-DNAs.

***Group I**: Ag_N-DNAs containing 6 neutral silvers (N_0 = 6) and two DNA strands (n_s = 2).*

1. *I*.1

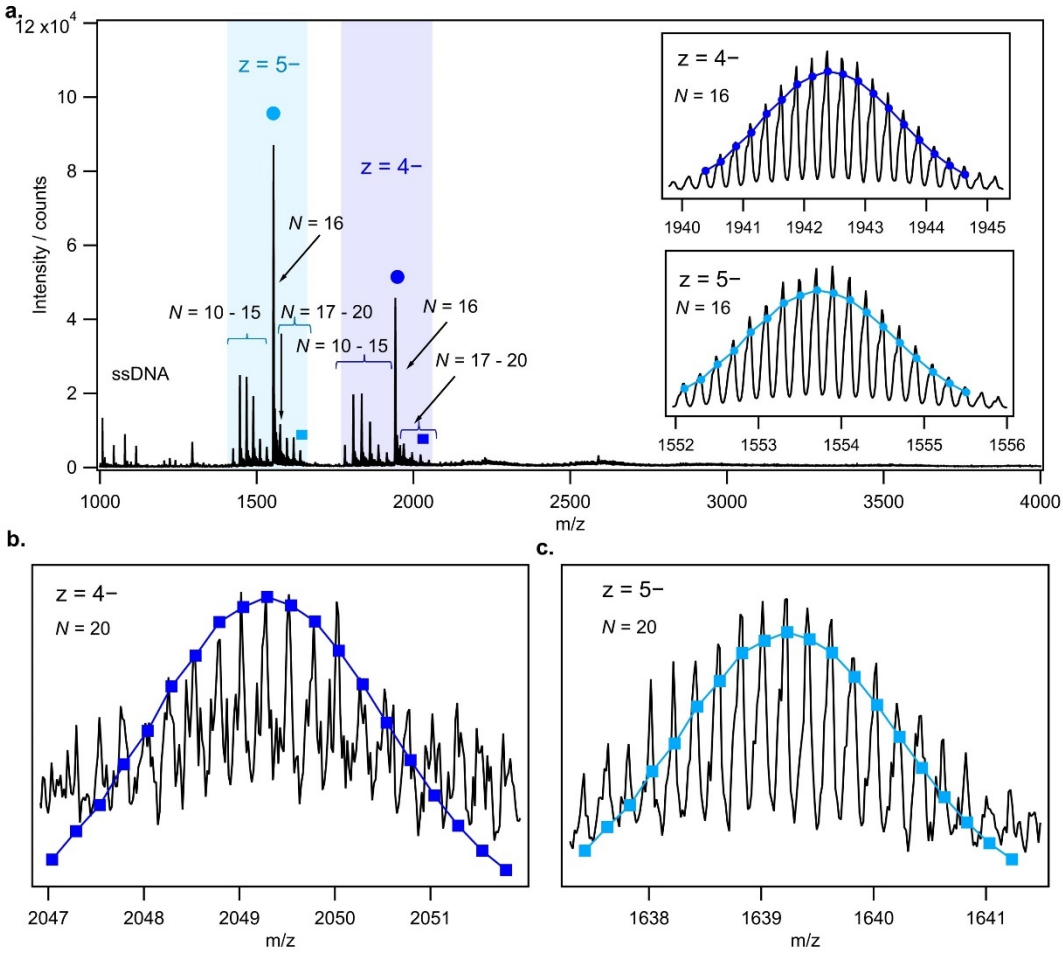

Figure S12. Mass spectrum of ***I*.1**. **a**) Experimental isotopic distributions (black curves) for all peaks of ***I*.1** mass spectra. Insets show isotopic distributions aligned with experimental peaks for $(DNA)_2[Ag_{16}]^{10+}$ at z = 5− (light blue, circled) and z = 4− (deep blue, circled) for the peaks with the highest intensity. **b**) and **c**) Isotopic distributions with experimental peaks for $(DNA)_2[Ag_{20}]^{14+}$ at z = 4− (deep blue, squares) and z = 5− (light blue, squares), respectively. Isotopic distributions were calculated using the chemical formula $C_{192}H_{244}N_{78}O_{116}P_{18}Ag_{16}$ (**a**. insets) and $C_{192}H_{244}N_{78}O_{116}P_{18}Ag_{20}$ (for **b.** and **c.**), respectively.

2. *I.2*

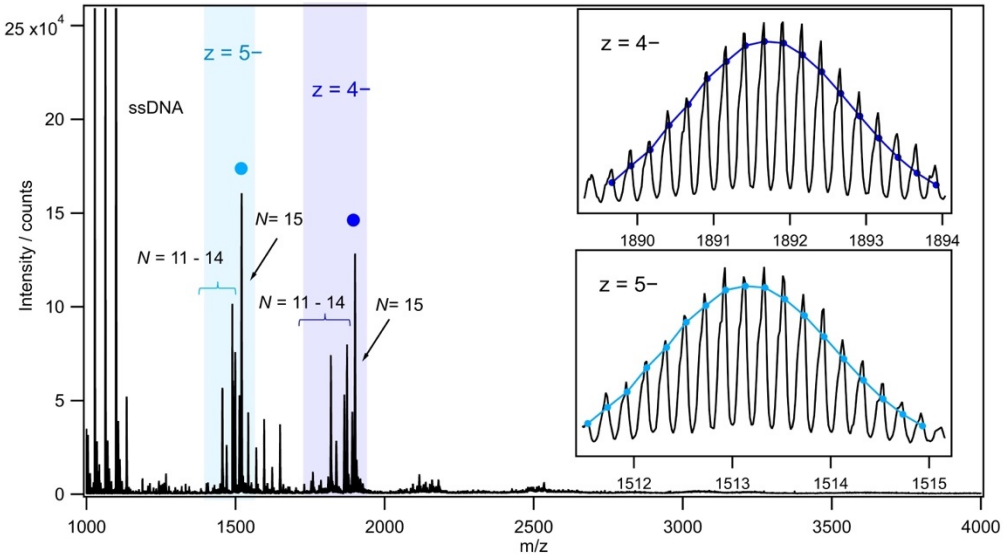

Figure S13. Mass spectrum of ***I.2***. Experimental isotopic distributions (black curves) for all peaks of ***I.2*** mass spectra. Insets show isotopic distributions aligned with experimental peaks for $(DNA)_2[Ag_{15}]^{9+}$ at z = 5− (light blue) and z = 4− (deep blue). Isotopic distributions were calculated using the chemical formula $C_{192}H_{244}N_{78}O_{110}P_{18}Ag_{15}$.

3. *I.3*

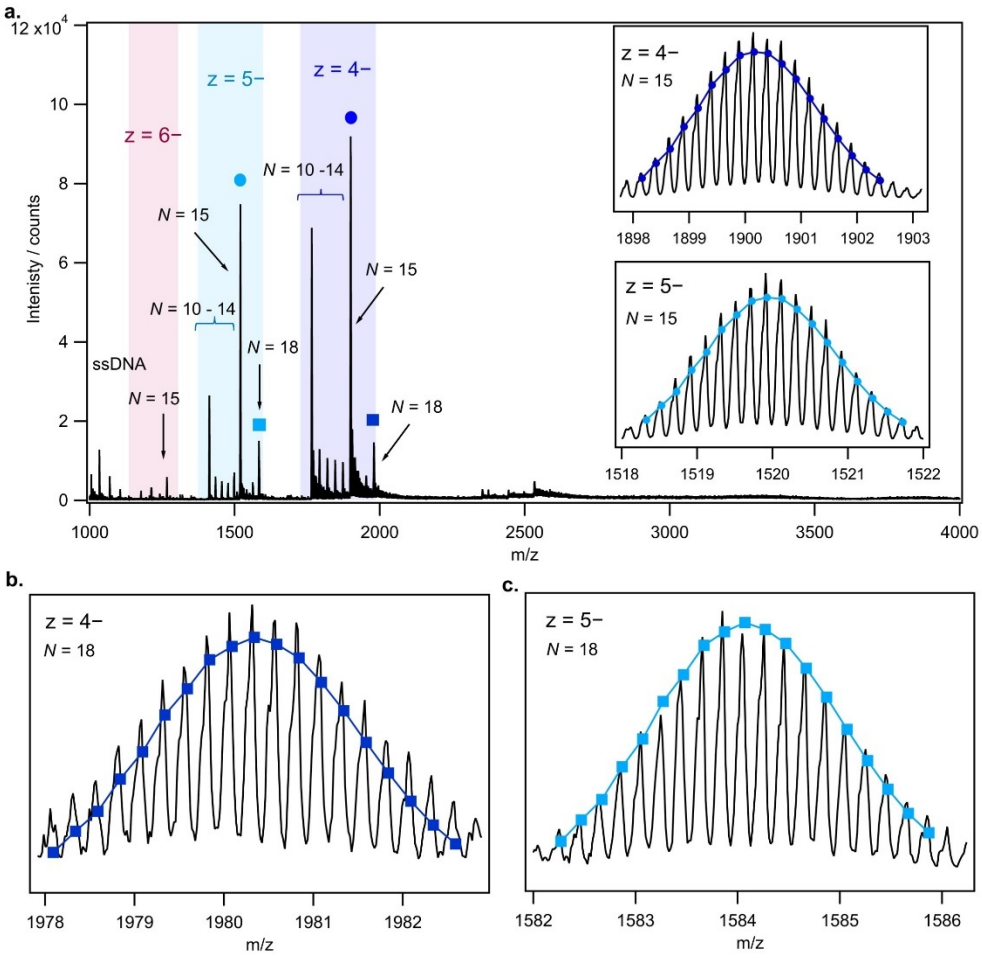

Figure S14. Mass spectrum of ***I.3***. **a.** Experimental isotopic distributions (black curves) for all peaks of ***I.3*** mass spectra. Insets show isotopic distributions aligned with experimental peaks for $(DNA)_2[Ag_{15}]^{9+}$ at z = 5− (light blue, circled) and z = 4− (deep blue, circled) for the peaks with the highest intensity. **b**) and **c**) Isotopic distributions with experimental peaks for $(DNA)_2[Ag_{18}]^{12+}$ at z = 4− (deep blue, squares) and z = 5− (light blue, squares), respectively. Isotopic distributions were calculated using the chemical formula $C_{190}H_{242}N_{80}O_{112}P_{18}Ag_{15}$ (**a**. insets) and $C_{190}H_{242}N_{80}O_{112}P_{18}Ag_{18}$ (for **b.** and **c.**), respectively.

4. *I*.4

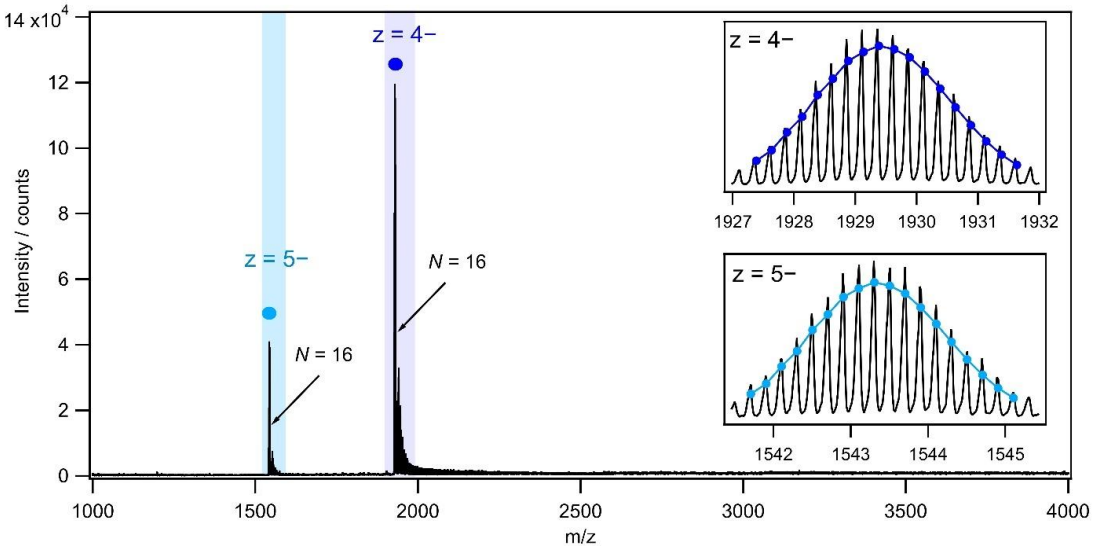

Figure S15. Mass spectrum of ***I*.4**. Experimental isotopic distributions (black curves) for all peaks of ***I*.4** mass spectra. Insets show isotopic distributions aligned with experimental peaks for $(DNA)_2[Ag_{16}]^{10+}$ at z = 5− (light blue) and z = 4− (deep blue). Isotopic distributions were calculated using the chemical formula $C_{194}H_{248}N_{80}O_{118}P_{18}Ag_{16}$.

5. *I*.5

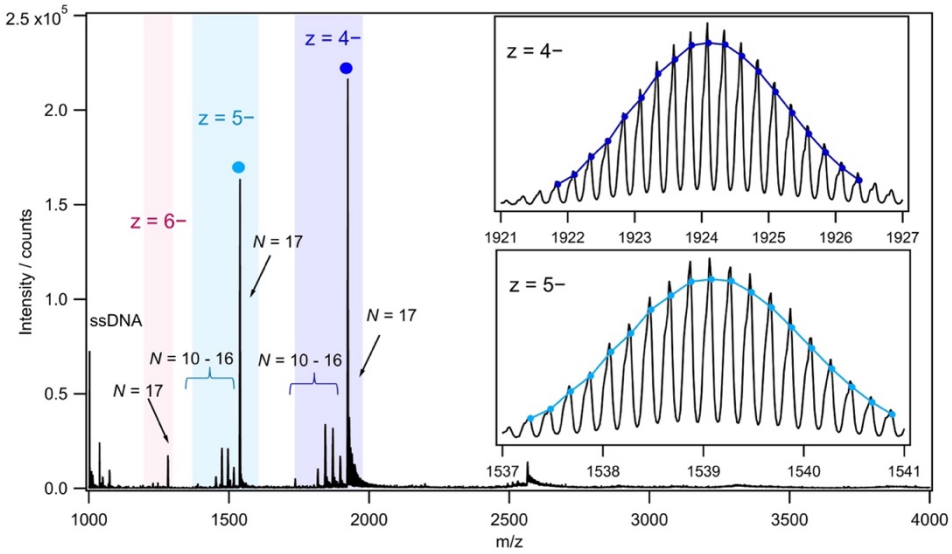

Figure S16. Mass spectrum of ***I*.5**. Experimental isotopic distributions (black curves) for all peaks of ***I*.5** mass spectra. Insets show isotopic distributions aligned with experimental peaks for $(DNA)_2[Ag_{17}]^{11+}$ at z = 5− (light blue) and z = 4− (deep blue). Isotopic distributions were calculated using the chemical formula $C_{190}H_{248}N_{62}O_{120}P_{18}Ag_{17}$.

***Group II**: 6 neutral silvers (N_0 = 6) and three DNA strands (n_s = 3).*

1. *II*.1

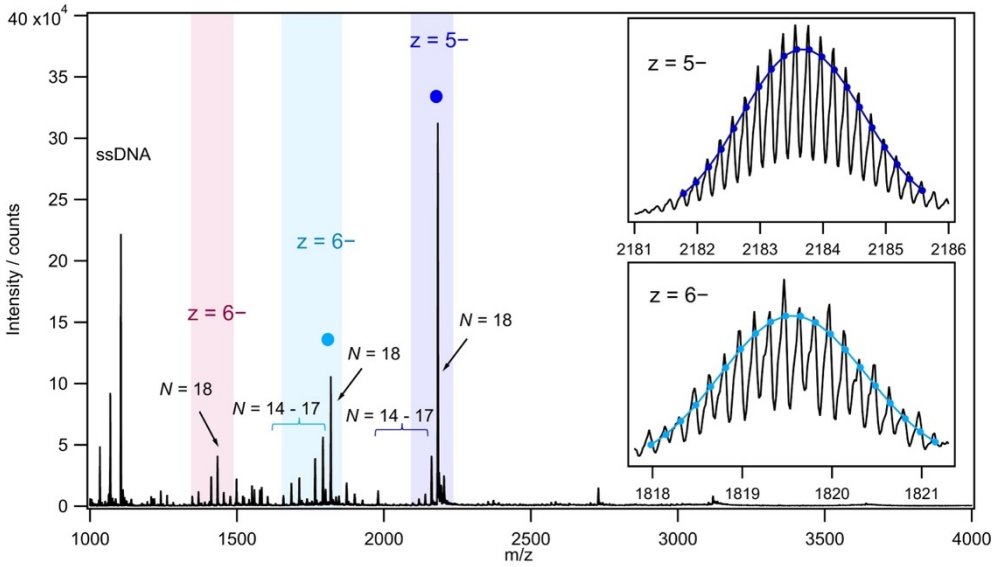

Figure S17. Mass spectrum of ***II*.1**. Experimental isotopic distributions (black) for all peaks of ***II*.1** mass spectra. Insets show isotopic distributions aligned with experimental peaks for $(DNA)_3[Ag_{18}]^{12+}$ at z = 6− (light blue) and z = 5− (deep blue). Isotopic distributions were calculated using the chemical formula $C_{285}H_{363}N_{120}O_{168}P_{27}Ag_{18}$.

2. *II*.2

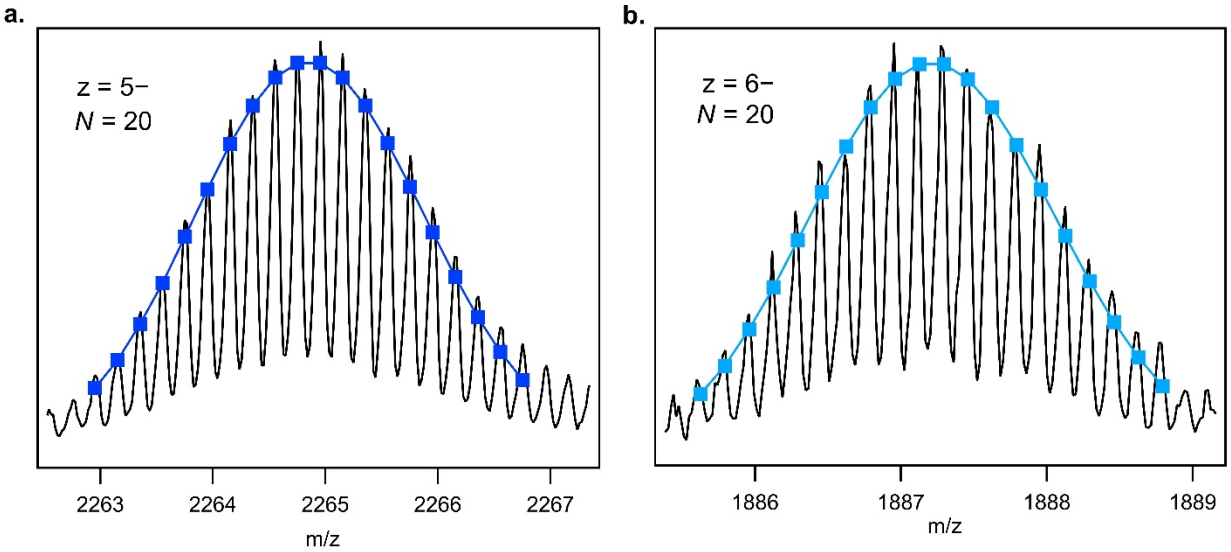

Figure S18. **a)** and **b**) Isotopic distributions with experimental peaks for $(DNA)_2[Ag_{20}]^{14+}$ at z = 5− (deep blue, squares) and z = 6− (light blue, squares), respectively. Isotopic distributions were calculated using the chemical formula $C_{291}H_{363}N_{132}O_{165}P_{27}Ag_{20}$. Isotopic distributions for chemical formula $C_{291}H_{363}N_{132}O_{165}P_{27}Ag_{21}$ are shown in main text Figure **1b**.

3. *II*.3

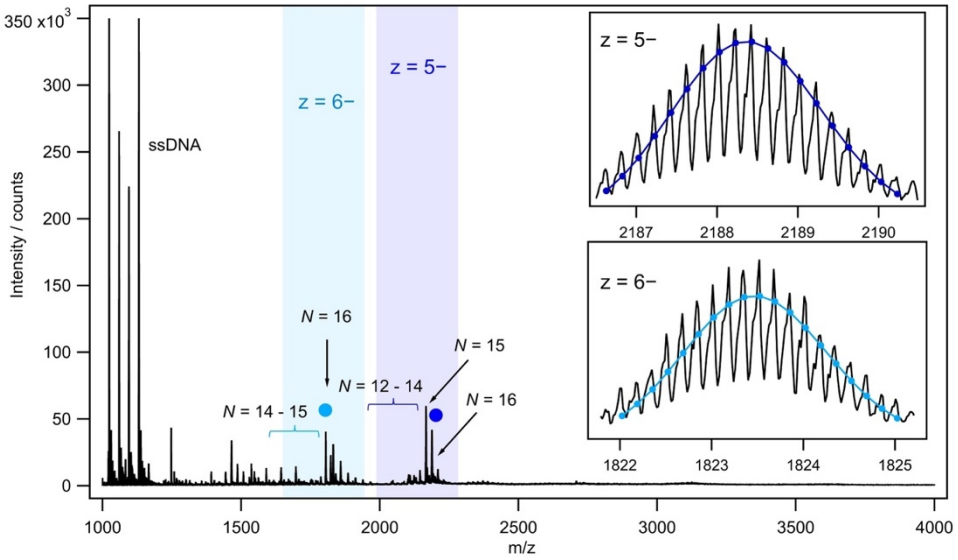

Figure S19. Mass spectrum of ***II*.3**. Experimental isotopic distributions (black curves) for all peaks of ***II*.3** mass spectra. Insets show isotopic distributions aligned with experimental peaks for $(DNA)_3[Ag_{16}]^{10+}$ at z = 6− (light blue) and z = 5− (deep blue). Isotopic distributions were calculated using the chemical formula $C_{294}H_{366}N_{129}O_{168}P_{27}Ag_{16}$.

***Group III**: Ag_N-DNAs containing 6 neutral silvers (N_0 = 6), two DNA strands (n_s = 2) and additional chlorido ligands.*

The mass spectra of ***III*.2** to ***III*.6** are reported in reference 1.[1]

***Group IV**: Ag_N-DNAs containing 8 neutral silvers (N_0 = 8) and two DNA strands (n_s = 2).*

The mass spectra of ***IV*.2** and ***IV*.3** are reported in reference 3.[3]

1. *IV*.1

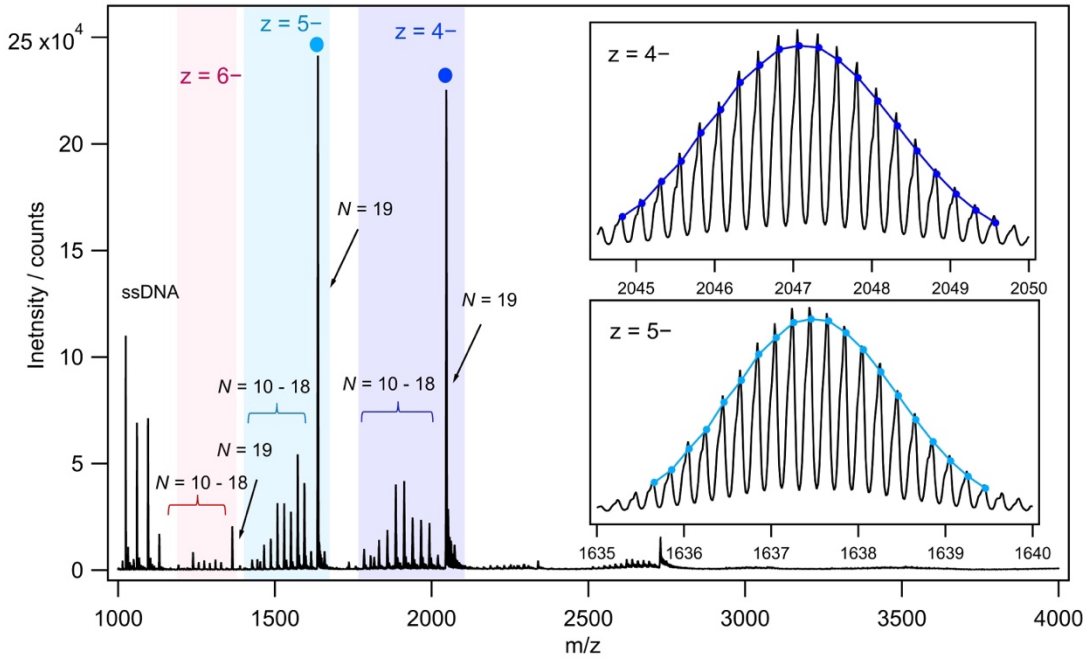

Figure S20. Mass spectrum of ***IV*.1**. Experimental isotopic distributions (black curves) for all peaks of Greengen208 mass spectra. Insets show isotopic distributions aligned with experimental peaks for $(DNA)_2[Ag_{19}]^{11+}$ at z = 5− (light blue) and z = 4− (deep blue), as indicated by circles. Isotopic distributions were calculated using the chemical formula $C_{196}H_{244}N_{86}O_{112}P_{18}Ag_{19}$.

2. *IV*.4

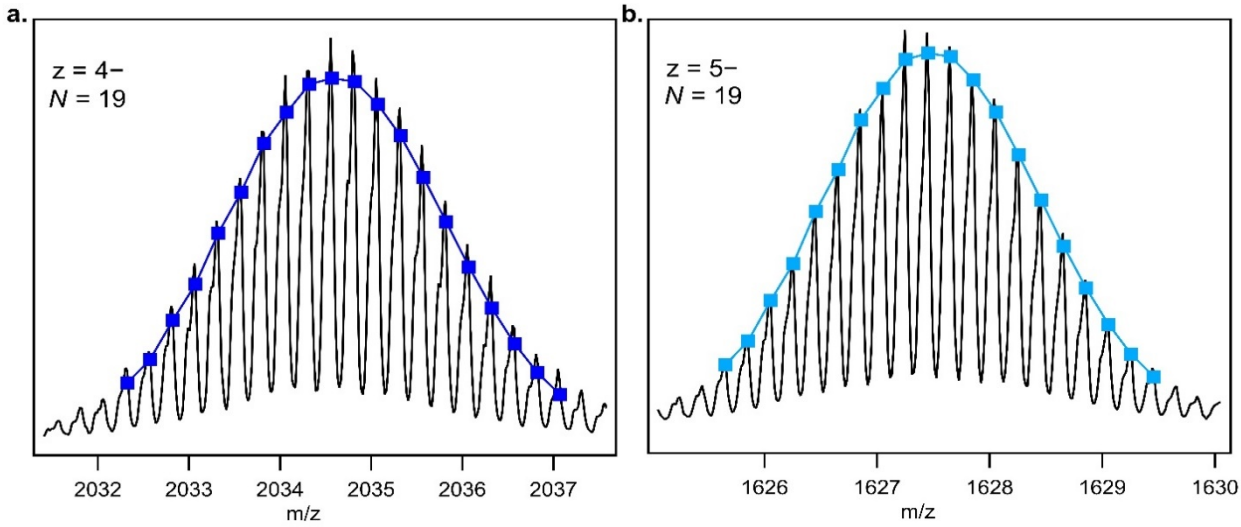

Figure S21. **a)** and **b**) Isotopic distributions with experimental peaks for $(DNA)_2[Ag_{19}]^{11+}$ at z = 4− (deep blue, squares) and z = 5− (light blue, squares), respectively. Isotopic distributions were calculated using the chemical formula $C_{196}H_{246}N_{80}O_{114}P_{18}Ag_{19}$. Isotopic distributions for chemical formula $C_{196}H_{246}N_{80}O_{114}P_{18}Ag_{20}$ are shown in main text Figure **1d**.

General correlation among peak absorbance/emission with overall nanocluster charges (Q_c) and total number of silvers (N) for the N_0 = 6 Ag_N-DNA.

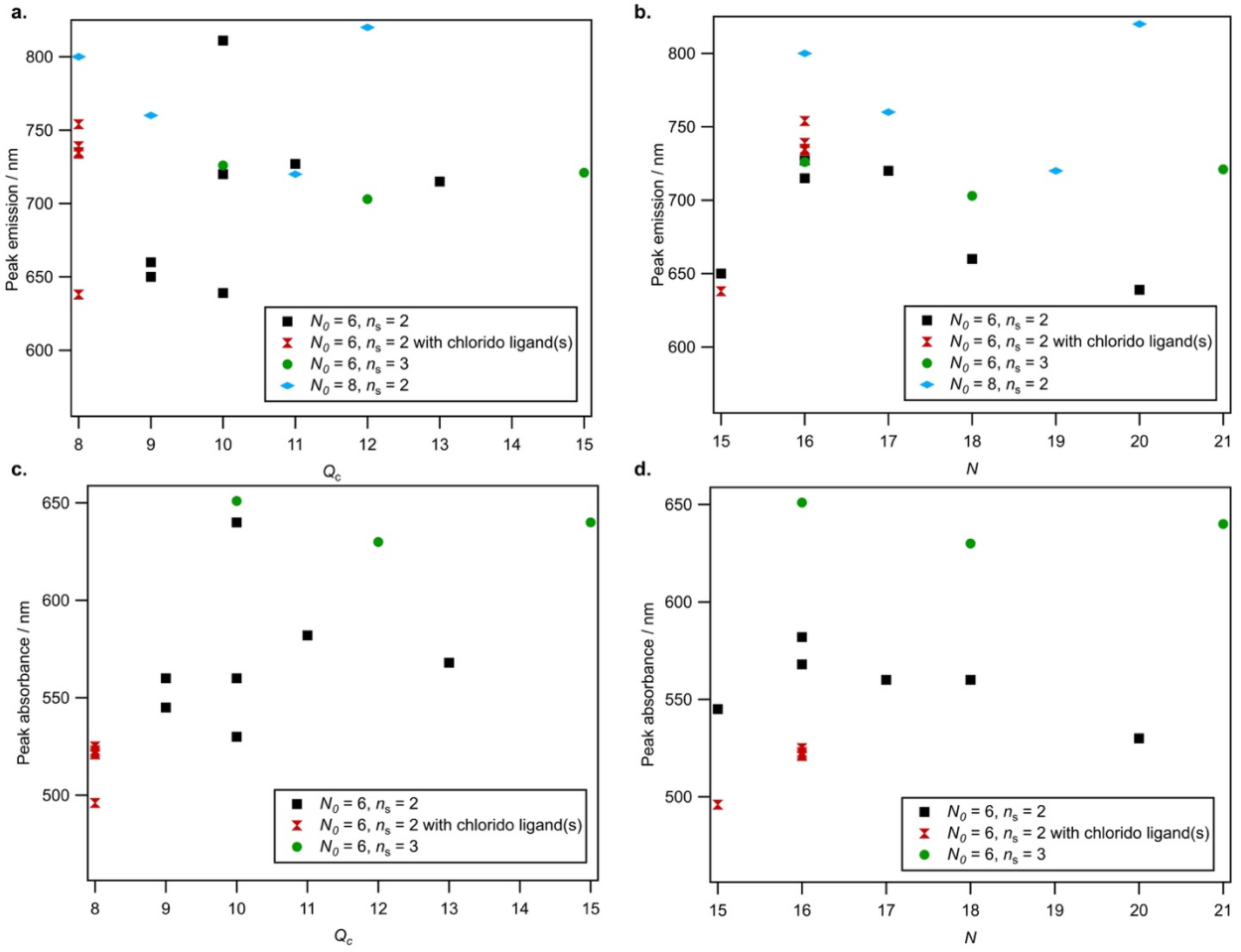

Figure S22. a) and **b)** Correlation between the peak emission with overall nanocluster charges (Q_c) and total number of silvers (N) for both N_0 = 6 and N_0 = 8 Ag_N-DNAs; **c)** and **d)** Correlation between the peak absorbance with Q_c and N for all the N_0 = 6 Ag_N-DNAs, respectively.

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