Supporting Information

Two-photon brightness of NIR-emitting, atomically precise DNA-stabilized silver nanoclusters

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Contents

1. Synthesis and purification of DNA-stabilized silver nanoclusters (Ag_N-DNAs).

A stoichiometric amount of $AgNO_3$ (Sigma Aldrich) was added to the ssDNA 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 to 5 days, allowing sufficient time for the Ag_N-DNA formation, followed by purification using ion-paired reverse-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 to synthesize chlorido-protected **Ag_{16}-DNA-Cl_2.**[1] The HPLC chromatograms of the four Ag_N-DNA species are previously reported in Ref. 2.[2]

Table S1. The experimental conditions used for synthesis of Ag_N-DNAs.

Name	[DNA]/ μM	[$AgNO_3$]/ μM
Ag_{15}-DNA	25	125
Ag_{21}-DNA	20	100
Ag_{16}-DNA-Cl_2	25	187.5
Ag_{19}-DNA	25	187.5

2. Mass spectrometry

HPLC-purified Ag_N-DNAs were solvent exchanged to 10 mM ammonium acetate (pH 7) 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. Unless otherwise stated, the 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.

2.1 Molecular composition determination of Ag_N-DNAs using mass spectrometry.

HPLC-purified Ag_N-DNAs were directly injected to obtain mass spectra using negative-ion mode electrospray ionization mass spectrometry (ESI-MS). The molecular composition of Ag_N-DNA such as the number of ssDNA oligomers (n_s) and the presence of additional chlorido ligands, total number of silver atoms (N) were first determined. Then the number of effective valence electrons (N_0) of each Ag_N-DNA species was determined by fitting the calculated isotopic distribution of the Ag_N-DNA to the experimental spectra. Detailed explanation formulae used for the calculation of N and N_0 have been reported previously.[1-3] The molecular formula of Ag_N-DNA is denoted as $(DNA)_{ns}\ (Ag_NCl_x)^{Qc}$, where Q_c is the nanocluster charge that matches the isotope pattern. In the absence of Cl^- ligands, N_+ equals Q_c, whereas, in the presence of Cl^- ligands, $Q_c = N_+ - x$. The nanocluster size and charge were determined by fitting the calculated isotopic distribution of the Ag_N-DNA to the experimental spectra. 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) as shown in the insets of **Fig. S1** to **S4**.

2.2 Mass spectra of Ag_N-DNAs.

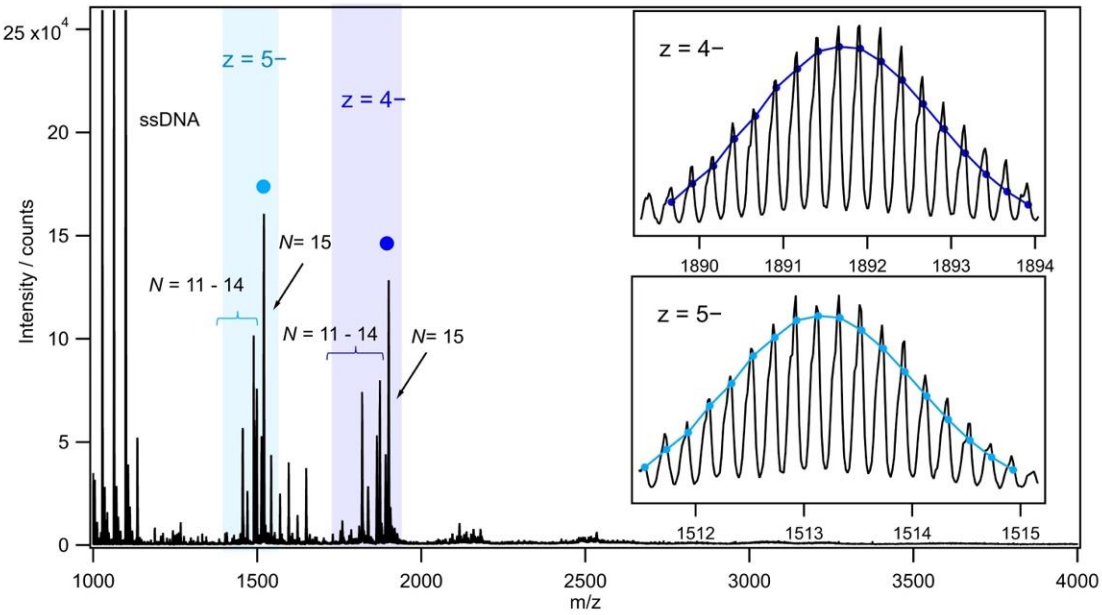


Fig. S1 Mass spectra of **Ag_{15}-DNA**. Experimental isotopic distributions (black curves) for all peaks of **Ag_{15}-DNA** mass spectra. Insets show isotopic distributions aligned with experimental peaks for $(DNA)_2[Ag_{15}]^{9+}$ at z = 5− (light blue) and z = 4− (dark blue). Isotopic distributions were calculated using the chemical formula $C_{192}H_{244}N_{78}O_{110}P_{18}Ag_{15}$.

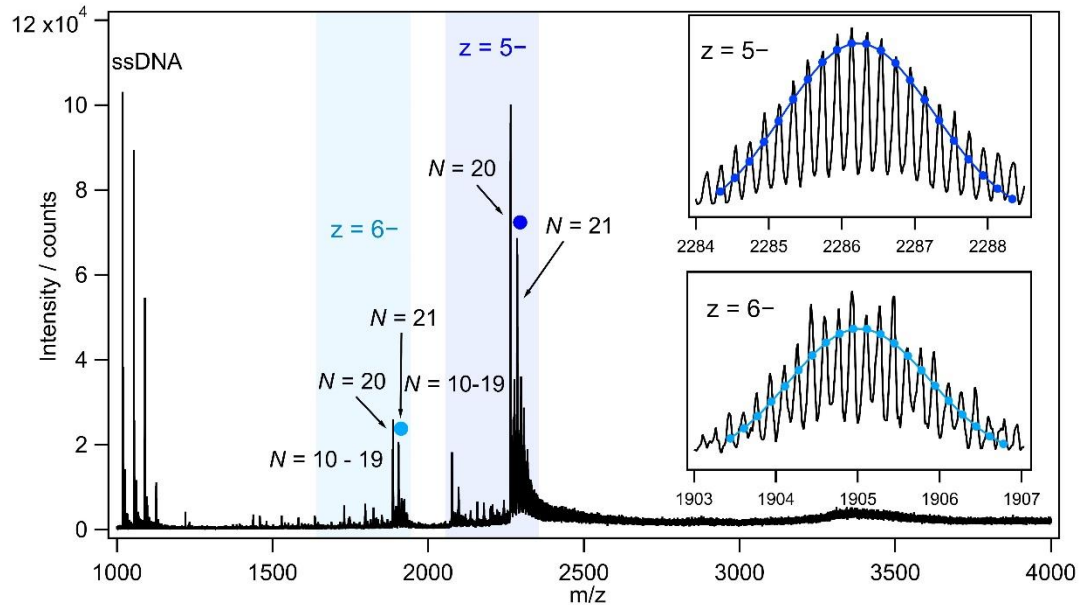


Fig. S2 Mass spectra of **Ag_{21}-DNA**. Experimental isotopic distributions (black curves) for all peaks of **Ag_{21}-DNA** mass spectra. Insets show isotopic distributions aligned with experimental peaks for $(DNA)_3[Ag_{21}]^{15+}$ at z = 6− (light blue) and z = 5− (dark blue). Isotopic distributions were calculated using the chemical formula $C_{291}H_{363}N_{132}O_{165}P_{27}Ag_{21}$.

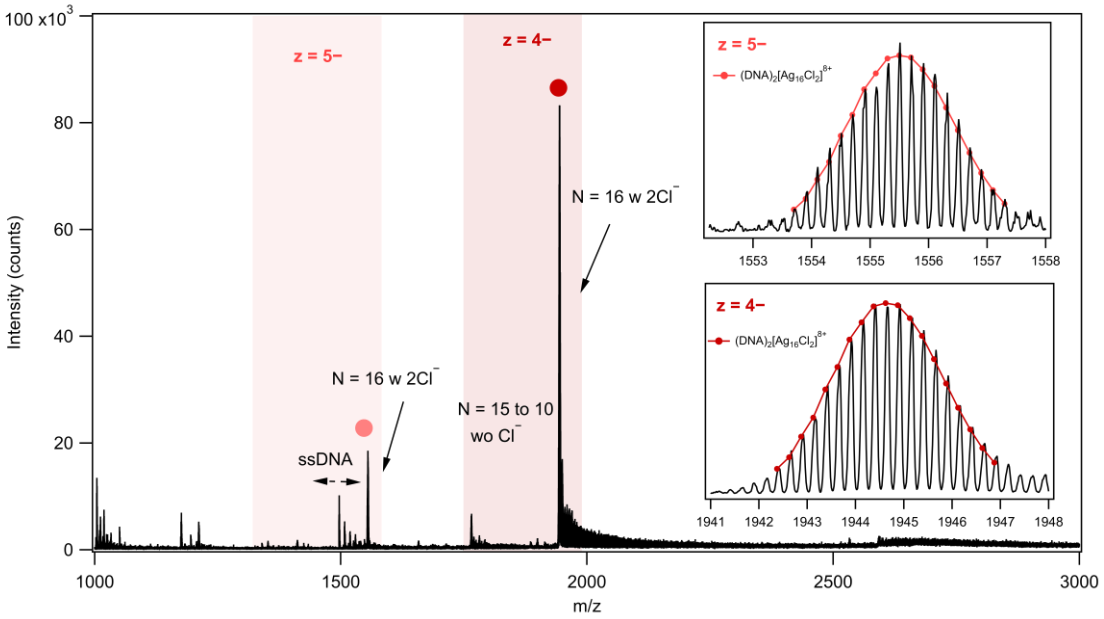


Fig. S3 Mass spectra of **Ag_{16}-DNA-Cl_2**. Experimental isotopic distributions (black curves) for all peaks of **Ag_{16}-DNA-Cl_2** mass spectra. Insets show isotopic distributions aligned with experimental peaks for $(DNA)_2[Ag_{16}Cl_2]^{8+}$ at z = 6− (light red) and z = 5− (dark red). Isotopic distributions were calculated using the chemical formula $C_{192}H_{244}N_{78}O_{112}P_{18}Cl_2Ag_{16}$.

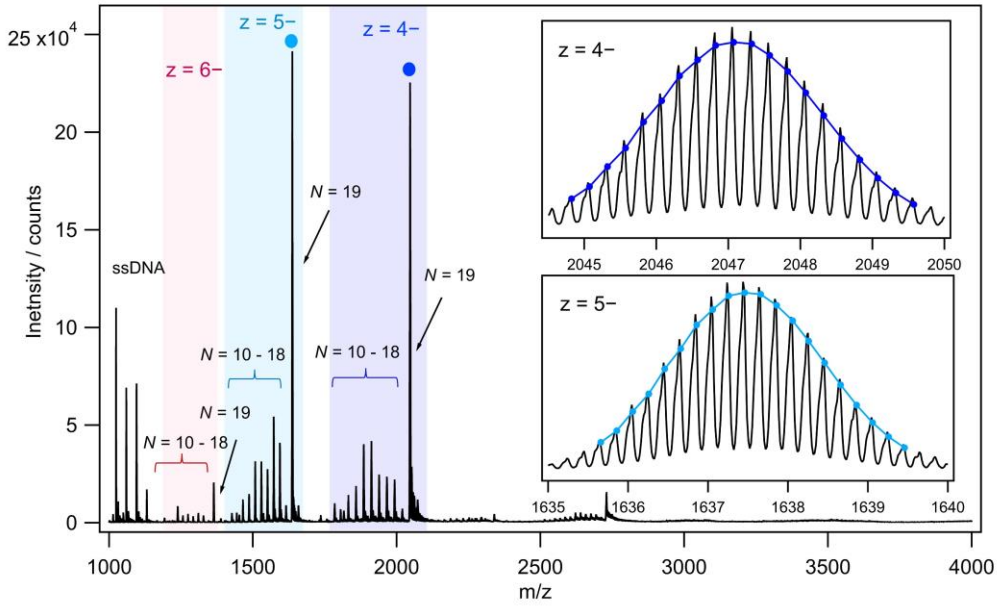


Fig. S4 Mass spectra of **Ag_{19}-DNA**. Experimental isotopic distributions (black curves) for peaks of **Ag_{19}-DNA** mass spectra. Insets show isotopic distributions aligned with experimental peaks for $(DNA)_2[Ag_{19}]^{11+}$ at z = 5− (light blue) and z = 4− (dark blue), as indicated by circles. Isotopic distributions were calculated using the chemical formula $C_{196}H_{244}N_{86}O_{112}P_{18}Ag_{19}$.

3. Two-photon spectra measurements of Ag_N-DNAs.

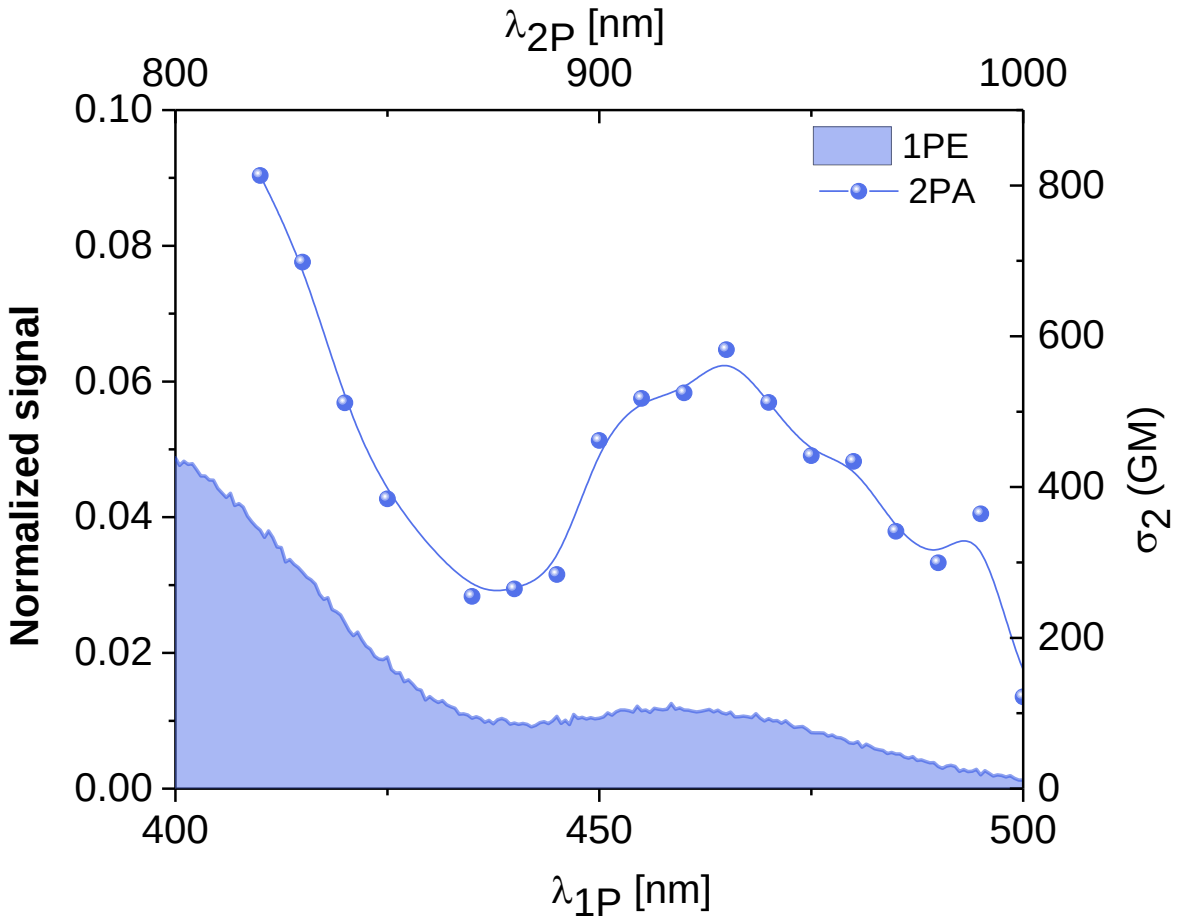


Fig. S5 Zoomed-in comparison of one-photon excitation (1PE, filled blue band) and two-photon absorbance (2PA, blue circles and lines) for **Ag_{21}.DNA.**

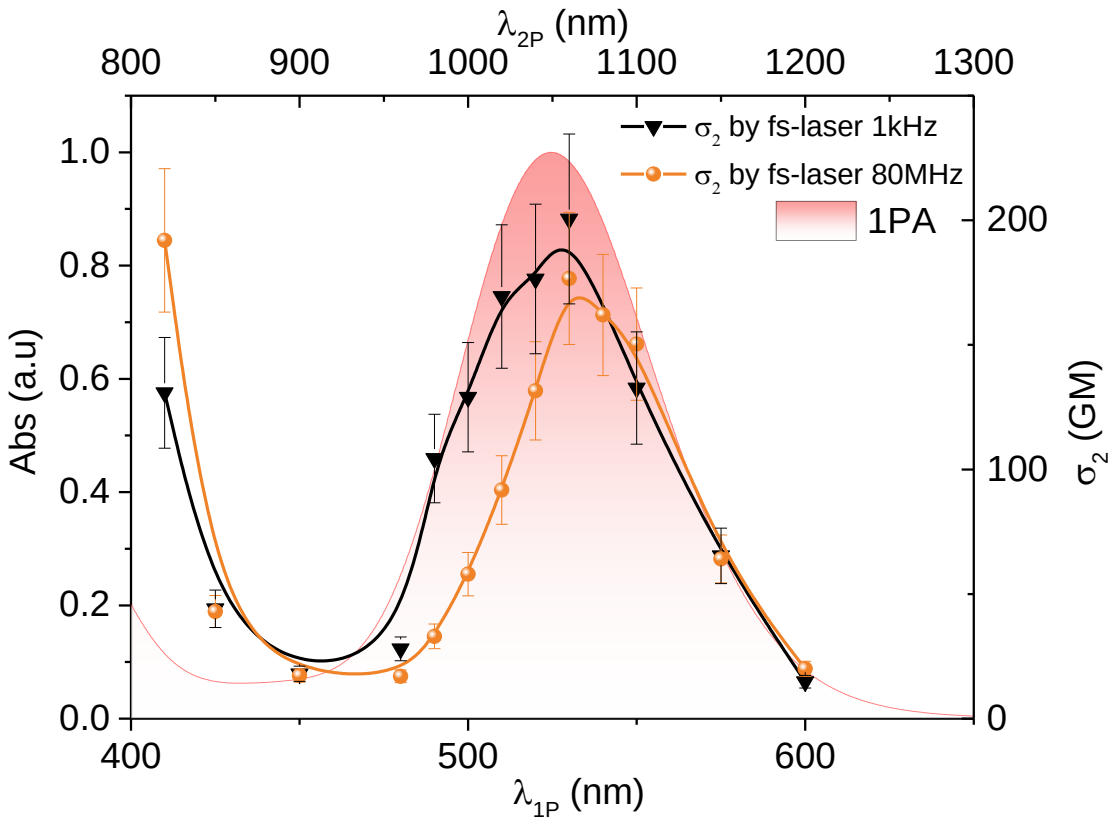
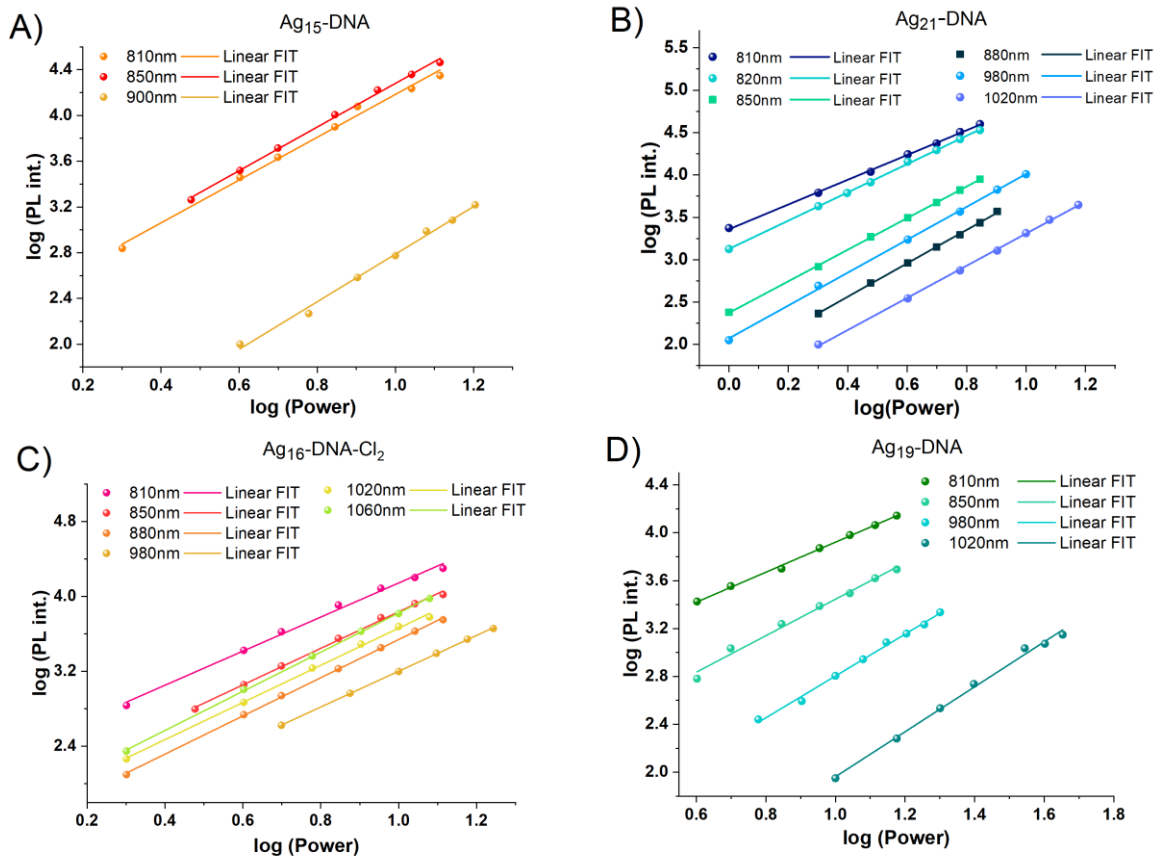


Fig. S6 Comparison of one-photon excitation (1PE, filled red band) and two-photon absorption (2PA) spectra of **Ag_{16}-DNA-Cl_2** obtained with fs lasers at repetition rates of 1 kHz (black lines with inverted triangles) and 80 MHz (orange line with circles).



Legend for Log-log plots of the PL intensity. Power exponent (n) is shown for all measured wavelengths.			
Ag_{15}-DNA	**Ag_{21}-DNA**	**Ag_{16}-DNA-Cl_2**	**Ag_{19}-DNA**
n_{810}: 1.87±0.07 n_{850}: 1.91±0.04 n_{900}: 2.08±0.07	n_{810}: 1.48±0.02 n_{820}: 1.67±0.02 n_{850}: 1.86±0.01 n_{880}: 1.97±0.02 n_{980}: 2.07±0.07 n_{1020}: 1.90±0.01	n_{810}: 1.82±0.06 n_{850}: 1.95±0.04 n_{880}: 2.04±0.02 n_{980}: 1.91±0.01 n_{1020}: 1.98±0.04 n_{1060}: 2.09±0.02	n_{810}: 1.19±0.02 n_{850}: 1.52±0.07 n_{980}: 1.75±0.04 n_{1020}: 1.88±0.06

Fig. S7 Log-log plot of the PL intensity of (A) **Ag_{15}-DNA**, (B) **Ag_{21}-DNA**, (C) **Ag_{16}-DNA-Cl_2**, and (D) **Ag_{19}-DNA**. Slopes indicate power exponent (n).

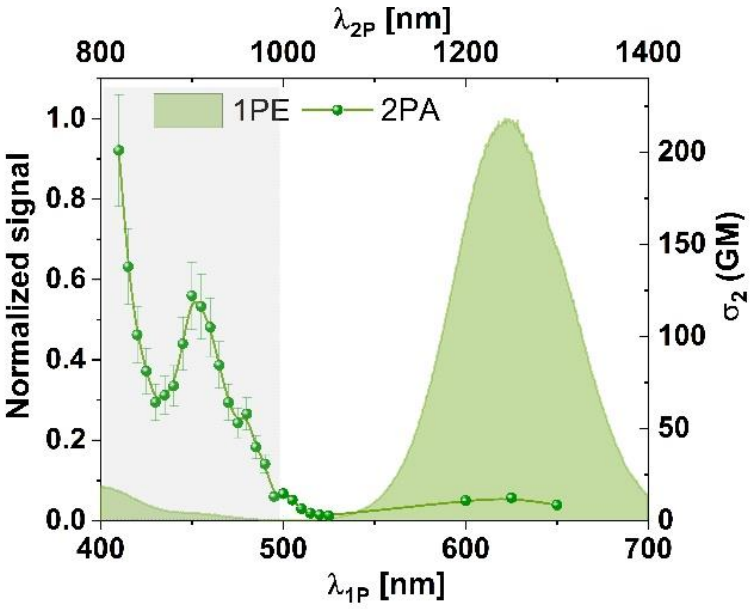


Fig. S8 Comparison between one-photon excitation (1PE, filled bands) and two-photon absorption (2PA, points and lines) of **Ag_{19}-DNA**. Grey shaded area implies a high contribution of one-photon processes based on power exponent.

4. References

1. A. Gonzàlez-Rosell, S. Malola, R. Guha, N. R. Arevalos, M. F. Matus, M. E. Goulet, E. Haapaniemi, B. B. Katz, T. Vosch, J. Kondo, H. Häkkinen and S. M. Copp, *J. Am. Chem. Soc.*, 2023, **145**, 10721-10729.
2. R. Guha, A. Gonzàlez-Rosell, M. Rafik, N. Arevalos, B. B. Katz and S. M. Copp, *Chem. Sci.*, 2023, **14**, 11340-11350.
3. R. Guha, S. Malola, M. Rafik, M. Khatun, A. Gonzàlez-Rosell, H. Häkkinen and S. M. Copp, *Nanoscale*, 2024, DOI: 10.1039/D4NR03533J.