SUPPORTING INFORMATION

Strong fluorescence-detected two-photon circular dichroism of chiral gold nanoclusters

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Experimental

Materials and methods

6-Aza-2-thiothymine (ATT) was purchased from Alfa Aesar Chemicals. $HAuCl_4 \cdot 3H_2O$, L-arginine, D-arginine, NaOH, fluoresceine were purchased from Sigma Aldrich. All materials were used without additional purification. Aqueous solutions were prepared using milli-Q water. All glassware was cleaned with aqua regia and rinsed with water prior to use.

Synthesis of AuATT NCs

ATT (5 mL, 80 mM) was dissolved with 0.2 M NaOH. $HAuCl_4 \cdot 3H_2O$ water solution (5 mL, 10 mg/mL) was added and continuously stirred at room temperature for 1h. Solution colour was changing from dark brown, red, orange to light yellow. The as-synthesized ATT-Au NCs were purified by ultrafiltration (Millipore, 50 kDa) at 7 000 rpm for 10min. Purified nanoclusters were dissolved in 10mL Milli-Q water and stored at 4 °C in the dark prior to use.

Synthesis of AuATT-L/D-Arg NCs

2mL of purified ATT-AuNCs diluted in Milli-Q water (pH=10) was mixed with 230µL, 40 mM L- and D-Arg solutions (pH=10) in two separate vials. Both solutions were allowed to react at 37 °C for 24 h. The Arg/ATT-AuNCs solution could be stored at 4 °C in the dark for one year with negligible changes in its optical properties.

Characterization of AuNCs

The sizes and morphologies of AuNCs were determined using a FEI Tecnai G2 20 X-TWIN transmission electron microscope (TEM). UV/VIS spectra were measured in solutions, therefore all synthesized nanoclusters were freshly dissolved in ultra-pure mili-Q water before analysis. Absorption and circular dichroism were recorded using a Jasco V-670 spectrophotometer and a JASCO J-815 CD spectropolarimeter, respectively in 10-mm quartz cells. One-photon anisotropy factor, denoted as g_{CD} or g_{abs} was calculated with the equation:

$$g_{abs} = \frac{\Delta\varepsilon}{\varepsilon} = \frac{\Delta A}{A} = \frac{\theta}{32\,980 \cdot A} \quad (1)$$

Photoluminescence (PL) and photoluminescence excitation (PLE) were determined using a HORIBA FluoroMax 4 and FS5 Spectrofluorometer (Edinburgh Instruments) equipped with a Xenon lamp. Fluorescence quantum yield was determined using integrating sphere in the FS5 Spectrofluorometer (Edinburgh Instruments) and by comparative method with the following equation:

$$\Phi_f^s = \Phi_f^r \frac{f_r(\lambda_{ex})}{f_s(\lambda_{ex})} \frac{\int_{\lambda_{em}} F^s(\lambda_{em}) d\lambda_{em}}{\int_{\lambda_{em}} F^r(\lambda_{em}) d\lambda_{em}} \frac{n_s^2}{n_r^2} \quad (2)$$

$$f_x(\lambda_{ex}) = 1 - 10^{-A_x(\lambda_{ex})} \quad (3)$$

where Φ_f^r is fluorescence quantum yield of reference sample, $\int_{\lambda_{em}} F^s(\lambda_{em})\ d\lambda_{em}$ and $\int_{\lambda_{em}} F^r(\lambda_{em}) d\lambda_{em}$ correspond to integrated areas of fluorescence intensity of sample and reference, respectively and n_s and n_r correspond to refractive index of sample and

reference, respectively. $f_x(\lambda_{ex})$ refers to the corresponding absorption correction factor and $A_x(\lambda_{ex})$ to the absorbance of sample ($x=s$) or reference ($x=r$) at excitation wavelength.

Time-correlated single-photon counting (TCSPC) method was applied to determine PL lifetime using the FS5 Spectrofluorometer (Edinburgh Instruments) equipped with a 475nm diode laser as excitation source. Fluoracle software was used to determine the decay curve fitting with biexponential function:

$$\mathrm{I} = \mathrm{A} + \mathrm{B}_1 \mathrm{e}^{-\frac{\mathrm{t}}{\tau_1}} + \mathrm{B}_2 \mathrm{e}^{-\frac{\mathrm{t}}{\tau_2}} \quad (4)$$

The average lifetime was calculated with the formula:

$$< \tau_{\mathrm{int}} >= \frac{\mathrm{B}_1\tau_1^2 + \mathrm{B}_2\tau_2^2}{\mathrm{B}_1\tau_1 + \mathrm{B}_2\tau_2} \quad (5)$$

where B_1 and B_2 are the contributions of particular lifetime. The χ^2 value determined the quality of fit.
The radiative ($\mathrm{k_r}$) and nonradiative ($\mathrm{k_{nr}}$) rate constants were calculated from the determined fluorescence quantum yield (Φ) and average fluorescence lifetime (τ_{int}) using the following equations:

$$\mathrm{K_r} = \frac{\phi}{\tau_{\mathrm{int}}} \quad (6)$$

$$\mathrm{K_{nr}} = \frac{1}{\tau_{\mathrm{int}}} - \mathrm{k_r} \quad (7)$$

Two-photon optical characterization of AuNCs

Two-photon excited luminescence was measured using a custom-built set-up consisting of a femtosecond mode-locked Ti:Sapphire laser (~140 fs, 80 MHz, Chameleon, Coherent Inc.) with incident wavelength range tuneable within λ = 680-1080 nm. Luminescence was collected in parallel shortpass filter (cut-off wavelength 650 nm). In order to exclude the potential excitation at lower wavelength range 700 nm long-pass filters were applied. The two-photon excited emission spectra were measured with a spectrograph (Shamrock 303i Andor). Measurement conditions were set the same for each nanoclusters sample and reference sample. Solutions in the same cuvettes were illuminated in the range of λ=700-930nm excitation. Since the Babinet-Soleil compensator is wavelength dependent, taking a set of measurements at a particular excitation wavelength required a new calibration. The exciting laser power was set in the range 22-30 mW in such a manner as to obtain exactly the same luminescence intensity signal from the nonchiral, reference sample at each wavelength. The experimental conditions were chosen to prevent photobleaching and achieve a high signal to noise ratio. Two-photon absorption cross sections were calculated with the equation:

$$\sigma_{2,s} = \frac{F_{2,s(\lambda_{exc})} C_r \varphi_r}{F_{2,r(\lambda_{exc})} C_s \varphi_s} \sigma_{2,r} \quad (8)$$

Fluorescein dye was used in this work as an optically inactive (non-chiral), fluorescent reference sample. The reference dye was carefully chosen to have a similar range of emission as the cluster samples examined in this work to exclude possible FDCD shifting. [1]
The Θ_{TPCD}, defined according to Eq. 1 is consistent with the Θ_{TPCD} definitions adopted in other Z-scan[2] and polarization modulated Z-scan techniques of measuring two-photon circular dichroism.[3]

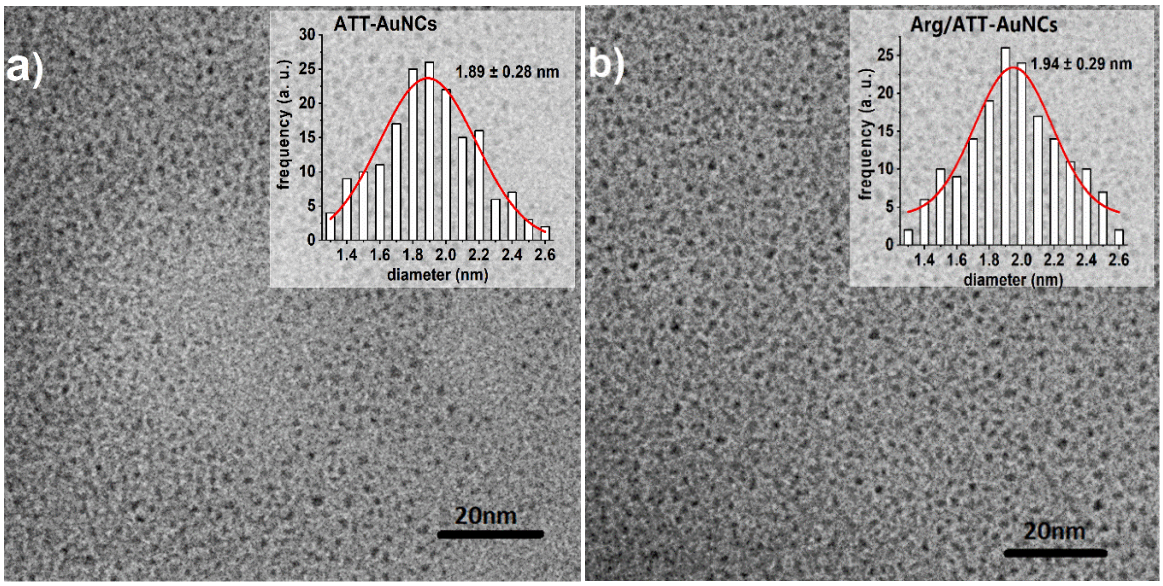

Figure S1. TEM images of and a) ATT-AuNCs and b)L-Arg/ATT-AuNCs. The inset of each figure presents corresponding size distribution histogram of NCs. FWHM of given curves of fitted size distribution are equal to 0.57 nm and 0.55 nm, giving 1.61-2.18 nm and 1.67-2.22 nm for ATT-AuNCs and Arg/ATT-AuNCs.

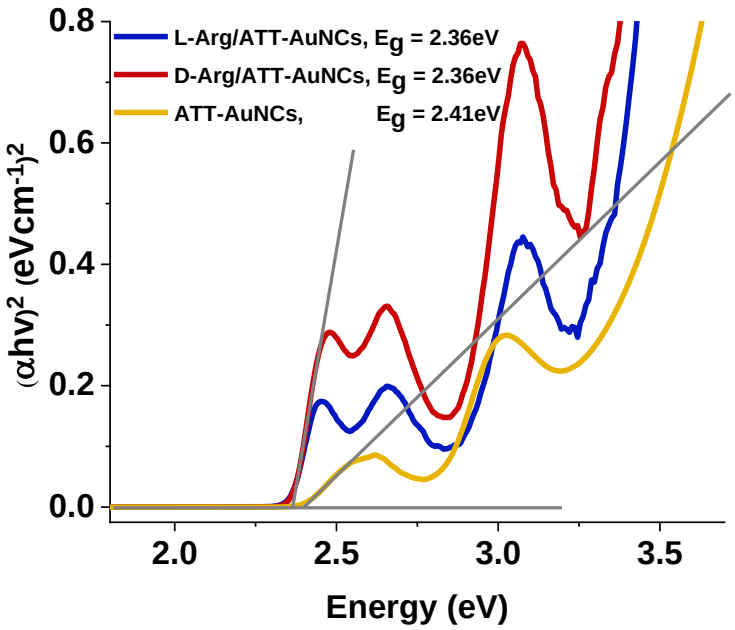

Figure S2. Determination of bandgap energies of ATT-AuNCs, Arg-L/ATT-AuNCs, Arg-D/ATT-AuNCs.

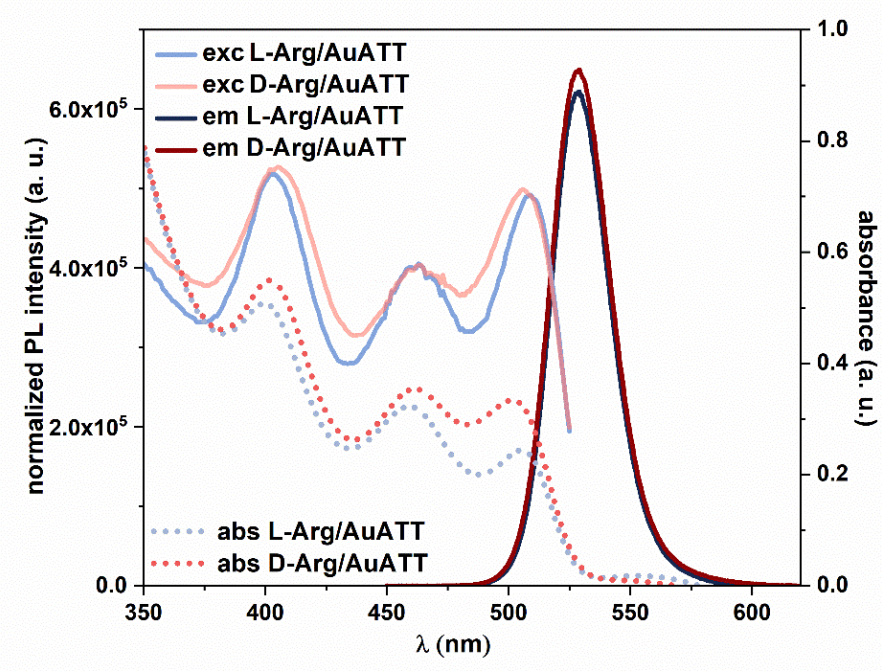

Figure S3. Absorption, excitation and emission spectra of L-Arg/ATT-AuNCs (blue) and D-Arg/ATT-AuNCs (red).

Table S1. Quantum yield (Φ), fluorescence lifetimes (τ_1 , τ_2) and their relative contributions, average lifetime (τ_{int}), radiative (k_r) and nonradiative rate (k_{nr}) constants determined for ATT-AuNCs, L-Arg/ATT-AuNCs and D-Arg/ATT-AuNCs.

	Φ [%]	τ_1 [ns]	τ_2 [ns]	τ_{int} [ns]	k_r [10^7 s^{-1}]	k_{nr} [10^7 s^{-1}]
ATT-AuNCs	**1.4 ± 0.3**	**3.9 ± 0.1** (99.4%)	**50.0 ± 5.4** (0.6%)	**7.1 ± 0.8** (χ^2=1.2)	**0.20**	**13.9**
L-Arg/ATT-AuNCs	**63.2 ± 2.0**	**24.2 ± 0.5** (58.9%)	**54.5 ± 0.6** (41.1%)	**42.7 ± 0.5** (χ^2=1.26)	**1.48**	**0.86**
D-Arg/ATT-AuNCs	**58.4 ± 2.0**	**27.5 ± 0.5** (67.5%)	**58.0 ± 0.8** (32.5%)	**42.9 ± 0.7** (χ^2=1.37)	**1.36**	**0.97**

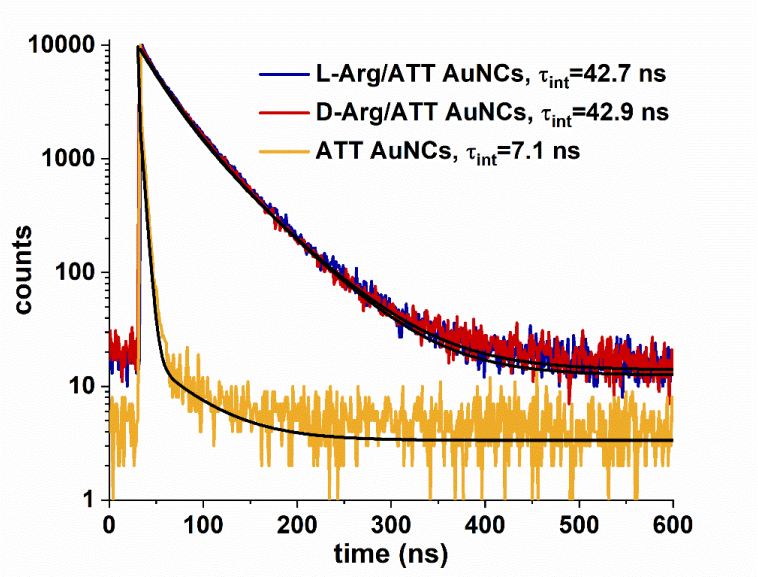

Figure S4. Fluorescence decay curves of L-Arg/ATT-AuNCs (blue) and D-Arg/ATT-AuNCs (red) compared to ATT-AuNCs (orange).

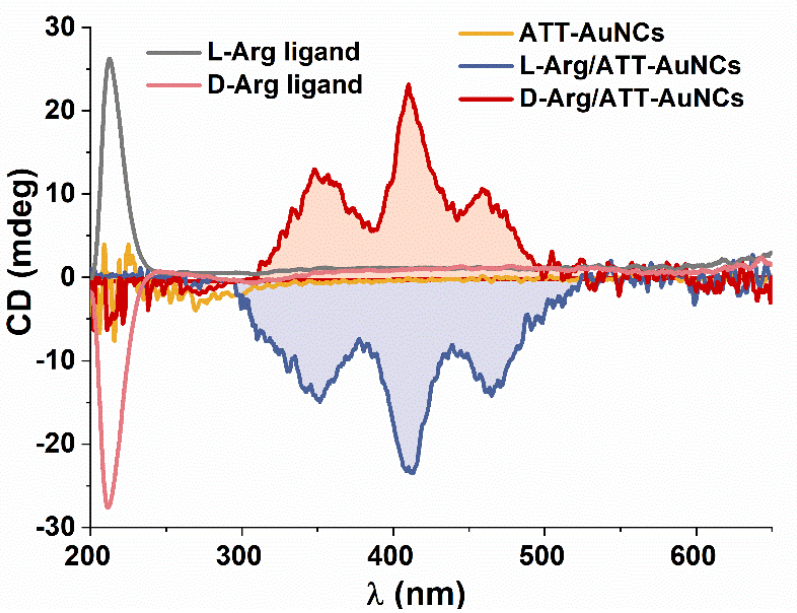

Figure S5. A comparison of CD spectra of L- and D- Arg/ATT-AuNCs with CD spectra of respective chiral ligands L-Arg and D-Arg, as well as nonchiral nanoclusters without arginine coating: ATT-AuNCs.

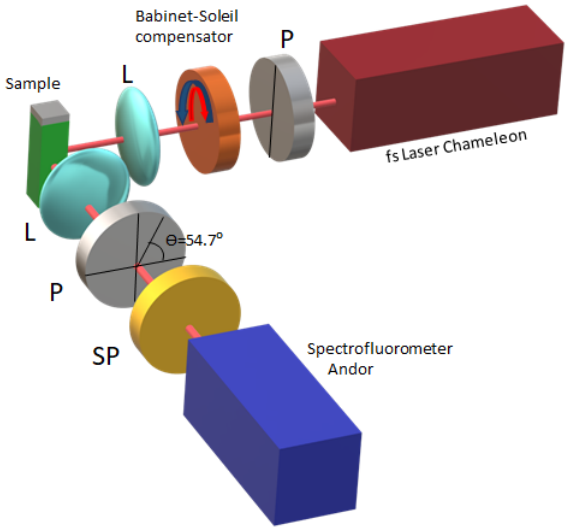

Figure S6. A scheme of the FDCD optical set-up (P=polarizer, L=lens, SP=shortpass filter).

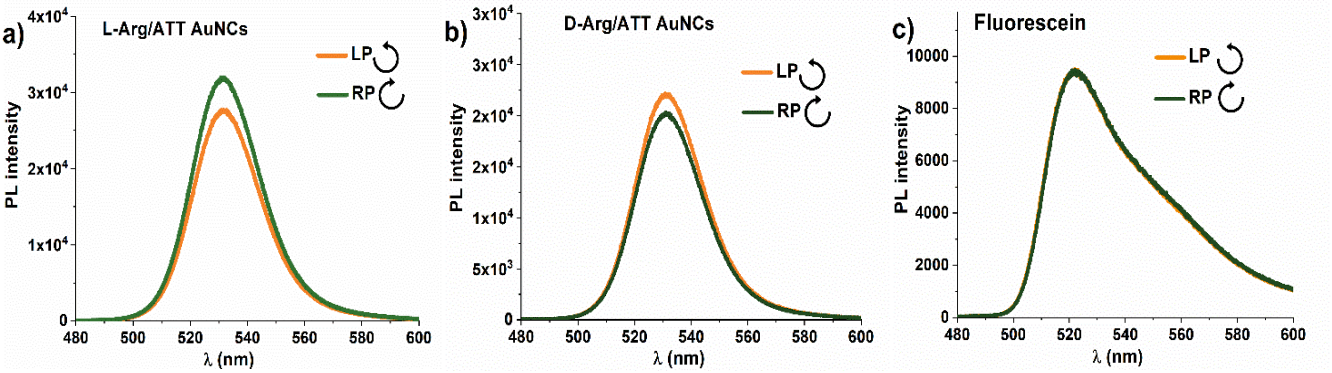

Figure S7. Two-photon excited luminescence of a) L-Arg/ATT-AuNCs, b) D-Arg/ATT-AuNCs and c) fluorescein, recorded at magic angle polarization of emission. Arrows in labels indicate direction of circularly polarized excitation, LP=left-handed polarization, RP= right-handed polarization.

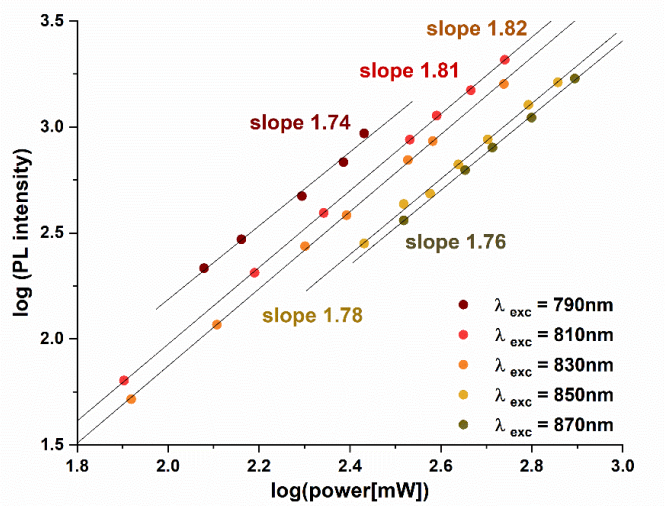

Figure S8. Log-log plot of the PL intensity of L-Arg/ATT-AuNCs as a function of average incident laser power measured at several excitation wavelengths. The slope of the linear fit corresponds roughly to the expected order of multiphoton processes.

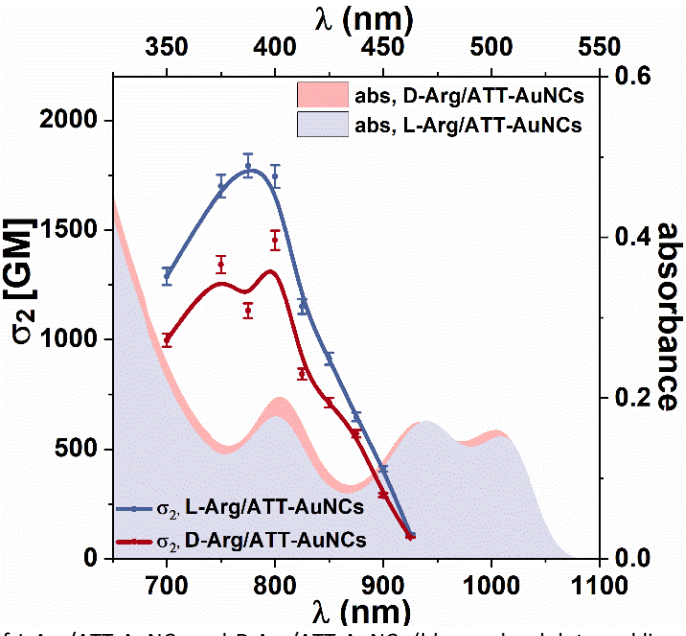

Figure S9. Two-photon absorption of *L*-Arg/ATT-AuNCs and *D*-Arg/ATT-AuNCs (blue and red dots and lines, left and bottom axes) correlated with absorption of those nanoclusters (blue and red areas, right and upper axes).

1. Turner, D. H.; Tinoco, I.; Maestre, M., Fluorescence detected circular dichroism. *Journal of the American Chemical Society* **1974,** *96* (13), 4340-4342.
2. Olesiak-Banska, J.; Waszkielewicz, M.; Samoc, M., Two-photon chiro-optical properties of gold Au25 nanoclusters. *Physical Chemistry Chemical Physics* **2018,** *20* (38), 24523-24526.
3. Samoc, M.; Samoc, A.; Miniewicz, A.; Markowicz, P.; Prasad, P.; Grote, J., *Cubic nonlinear optical effects in deoxyribonucleic acid (DNA) based materials containing chromophores*. SPIE: 2007; Vol. 6646.