Controlled Formation of Emissive Ag Nanoclusters Using Rationally Designed Metal-binding Proteins

Vasily A. Morozov and Michael Y. Ogawa*

Department of Chemistry and Center for Photochemical Sciences, Bowling Green State University, Bowling Green, OH 43403

Peptide Synthesis and Purification

All peptides were synthesized on an Applied Biosystems 433A peptide synthesizer using Fmoc protocols. The peptides were purified by reversed-phase HPLC on a preparative C18 Vydac column in aqueous 0.1% v/v TFA with a linear gradient of organic solvent as previously described.[20] Analytical HPLC showed that the purity of the final products was > 96%. The ESI-MS data for the apo and metal containing peptides were collected on a Shimadzu 2010A single quadrupole mass spectrometer in the positive ionization mode using a 1.5 L/min flow of nebulizing

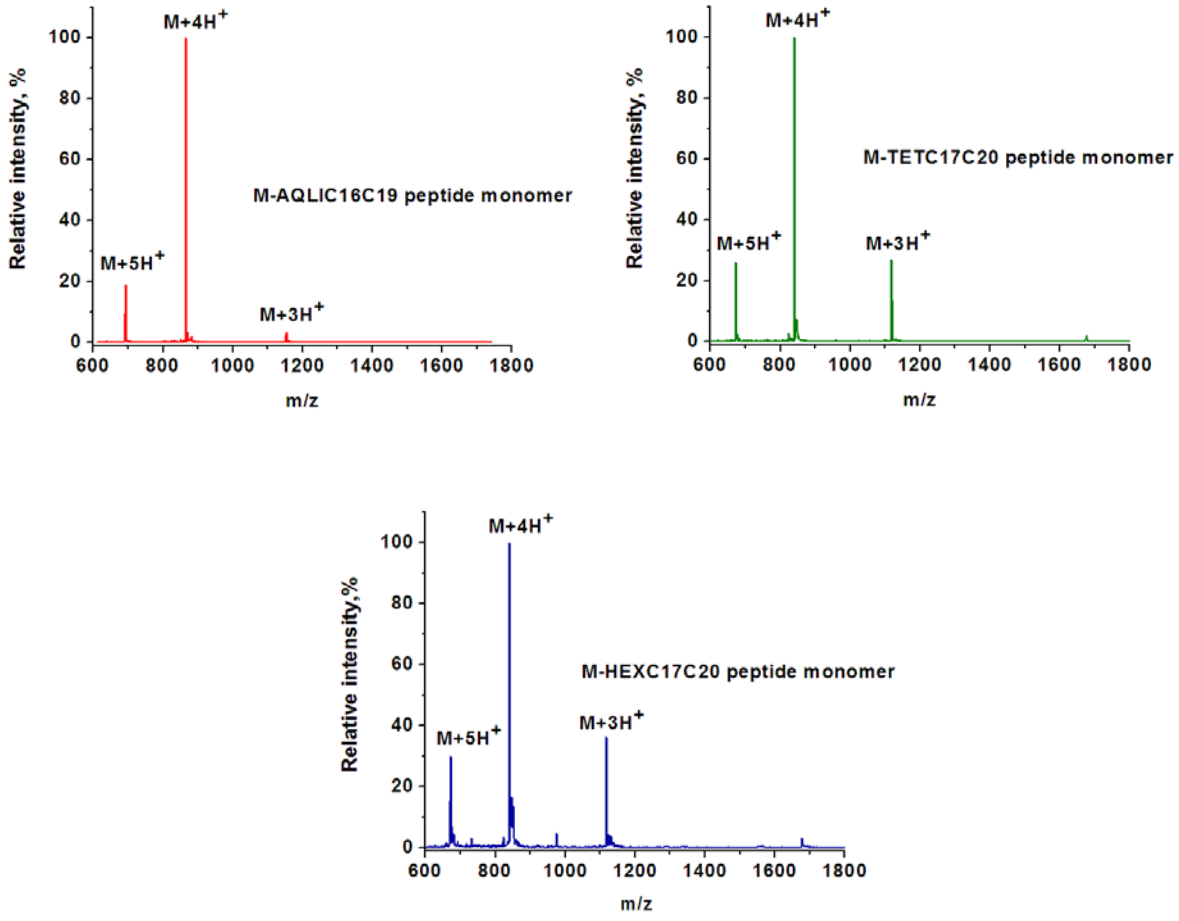

Figure S1. Electrospray ionization mass spectra of the apopeptides in 10 mM ammonium acetate buffer pH 5.5. Shown are spectra of AQLIC16C19 (red), Ag-TETC17C20 (green), and Ag-HEXC17C20 (blue).

gas and a probe voltage of 1.5 kV. The data were recorded in the scan mode in the *m*/*z* range of 600 to 2000 Da. Figure S1 shows the ESI-MS spectra of the different *apo* peptides taken from 10 mM ammonium acetate buffer pH 5.5. All spectra show m/z values corresponding to multiply charged species of the desired peptide monomer (M). For AQLIC16C19: $[M+5H^+]^{5+}$ = 692.8 (calc'd. 693.0), $[M+4H^+]^{4+}$ = 865.8 (calc'd. 866.0), and $[M+3H^+]^{3+}$ = 1154.2 (calc'd. 1154.3). For TETC17C20: $[M+5H^+]^{5+}$ = 671.7 (calc'd. 672.0), $[M+4H^+]^{4+}$ = 839.5 (calc'd. 839.8), and $[M+3H^+]^{3+}$ = 1118.9 (calc'd. 1119.3). For HEXC17C20: $[M+5H^+]^{5+}$ = 671.8 (calc'd. 672.0), $[M+4H^+]^{4+}$ = 839.5 (calc'd. 839.8), and $[M+3H^+]^{3+}$ = 1118.9 (calc'd. 1119.3).

Static Light Scattering Measurements

The peptide aggregates were first separated according to their masses on a Superdex 75 10/300 GL Tricorn size exclusion chromatography column at the flow rate of 0.3 ml/min using UV detection. The molar masses of the eluted species were determined on a Wyatt miniDAWN instrument using a quasi-elastic light scattering (QELS) detector. The apparent weight average molar mass M_w was calculated in the Astra software using the Zimm equation (eq S1):

$$\frac{K \cdot c}{R_\Theta} = \frac{1}{M_w \cdot P(\Theta)} + 2A_2 \cdot c \qquad \text{(S1)}$$

where R_Θ is the excess intensity of scattered light at angle Θ, c is the mass concentration of solute molecules in the solvent, A_2 is the second virial coefficient, $P(\Theta)$ is the function describing the angular dependence of scattered light, and K is an optical constant. The absolute value of the refractive index of the buffer used was directly measured using the Optilab rEX instrument. The *dn/dc* values, required for the mass analysis, were determined from the signal of the in-line UV-Vis spectrometer by recalculation of the UV signal into the sample concentration. The results are shown in Figure S2 and are described in the text.

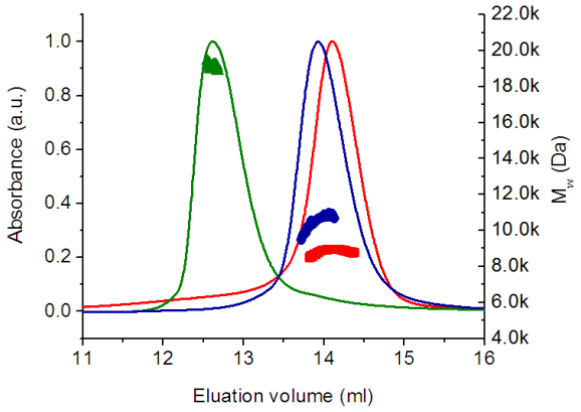

Figure S2. Size-exclusion chromatograms of the apo AQLIC16C19 (red traces), TETC16C19 (blue traces), and HEXC17C20 (green traces) obtained in 50 mM MES buffer/100 mM KCl buffer pH 5.5. Data traces across the peaks indicate the apparent molecular weight at the given time of elution.

Circular Dichroism Spectroscopy

Circular dichroism (CD) spectra were recorded on an Aviv 202DS spectrometer equipped with a thermoelectric temperature controller using a 0.1-cm path length cuvette. The spectra were obtained as an average of 3-5 scans recorded in 1 nm steps. The observed ellipticities, $[\theta]_{obs}$, were recorded and then converted to mean residue molar ellipticities ($[\theta]$) by the equation:

$$[\theta] = [\theta]_{obs}/(10*l\ c\ n) \qquad \text{(S2)}$$

where l is the path length of the cuvette in centimeters, c is the molar concentration of the peptides, and n is the number of amino acid residues in the peptide sequences. Figure S3 shows the CD spectra of the apopeptides taken at different pH values as described in the text.

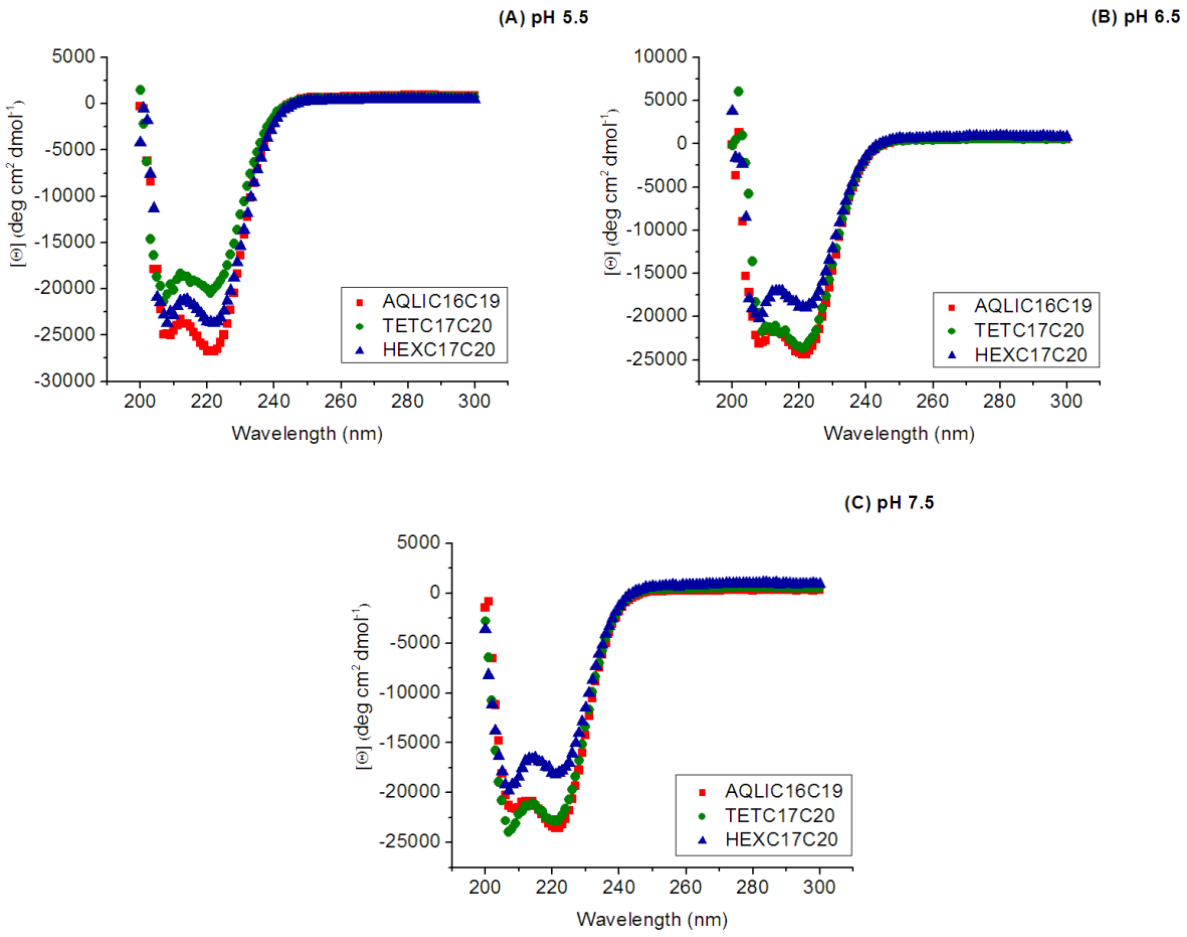

Figure S3. Circular dichroism spectra of the apo AQLIC16C19 (red squares), TETC17C20 (blue circules), HEXC17C20 (green triangles) peptides in (**A**) 100mM NaCl, 50 mM MES buffer pH 5.5 solution (**B**), 100mM NaCl, 50 mM MES buffer pH 6.5 solution (**C**) 100mM NaCl, 50 mM TRIS buffer pH 7.5 solution.

ESI-MS of Holopeptides

The degree of metal loading of the Ag^+/peptide complexes was also studied by ESI-MS. The solutions were prepared by the treating with an excess of $AgNO_3$ and further purification on a size-exclusion column with 10 mM ammonium acetate buffer pH 5.5 as an eluant. The results are shown in Figure S4 and are described in the text.

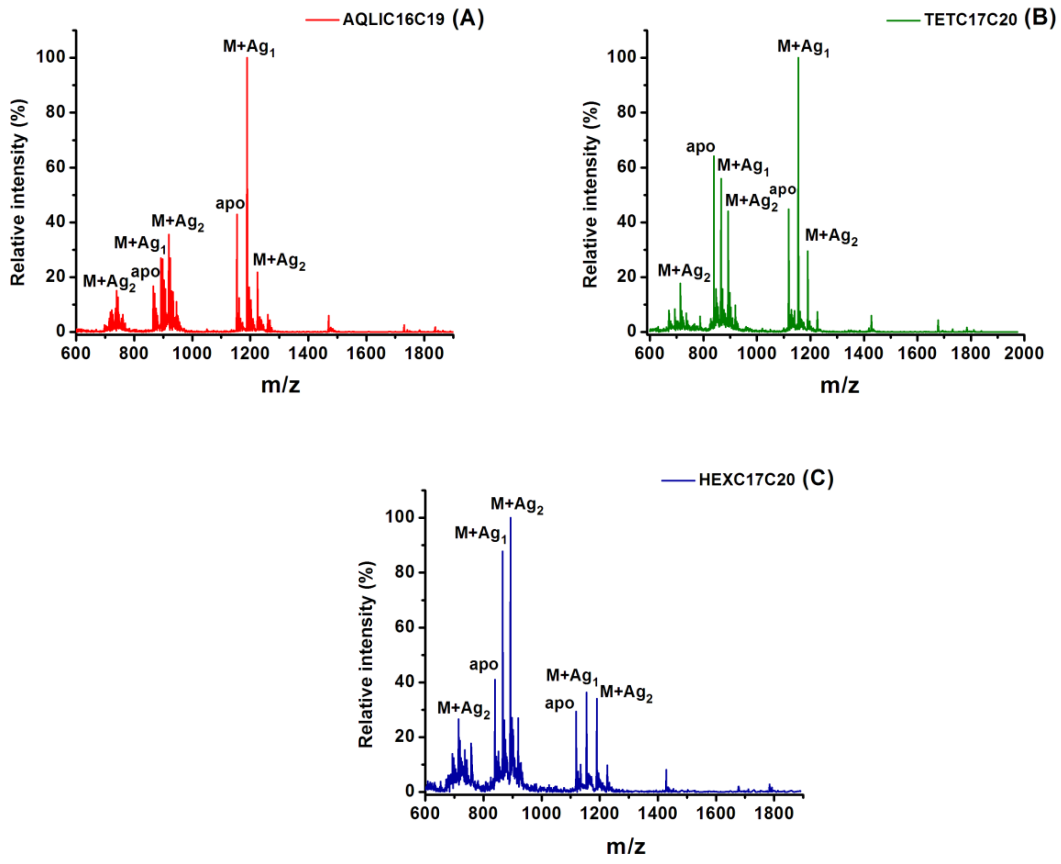

Figure S4. Electrospray ionization mass spectra of the Ag adducts of (A) AQLIC16C19, (B) TETC17C20, and (C) HEXC17C20.

Circular Dichroism Spectroscopy of Holopeptides

Circular dichroism measurements showed that the addition of Ag^+ to solutions of the various peptides produces a new signal in the near UV region of the spectrum. Figure S4 shows that the CD spectra of all three Ag^+/AQLIC16C19, Ag^+/TETC17C20 and Ag^+/HEXC17C20 complexes exhibit strong negative CD signals at ca. 260 nm and positive signals at ca. 290 nm. The results are shown in Figure S5 and are described in the text.

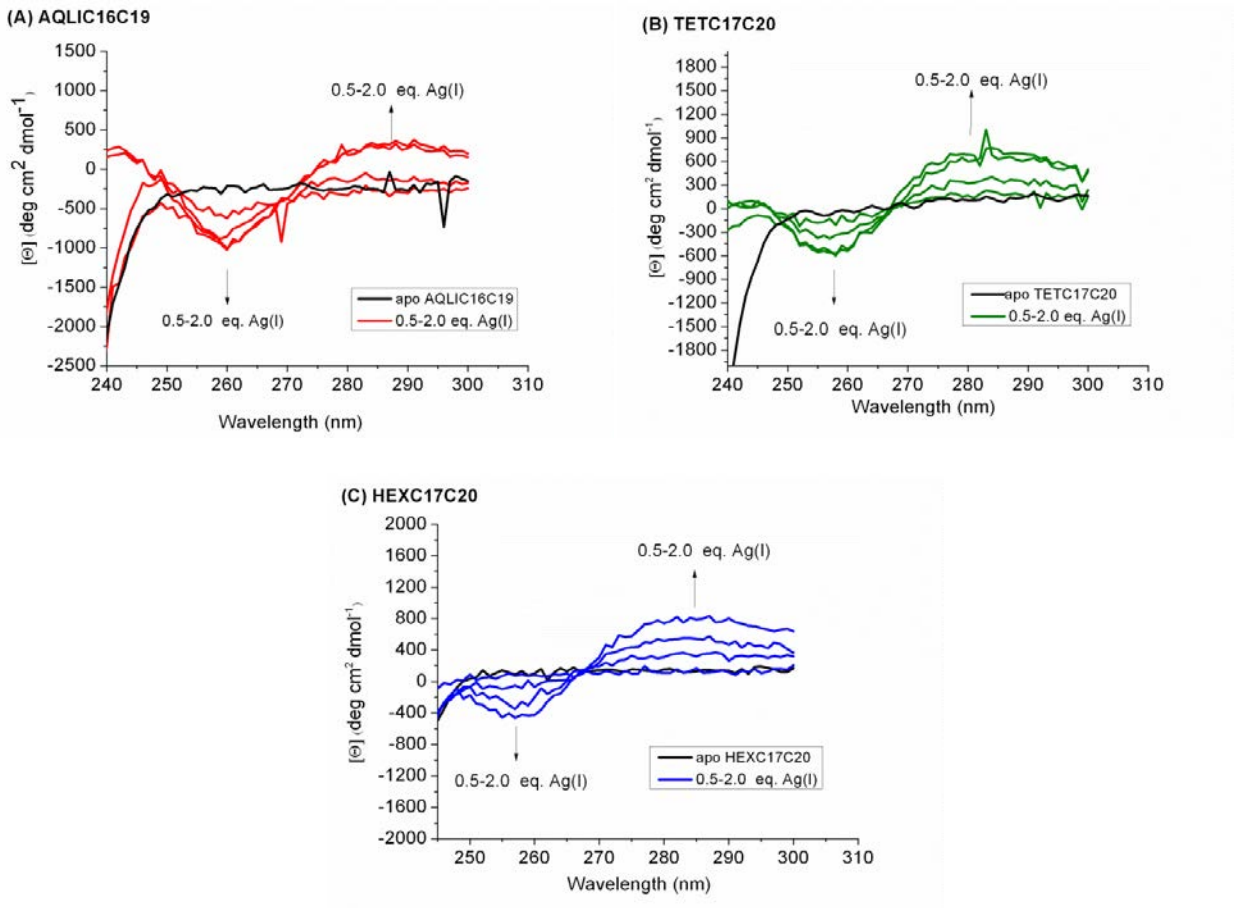

Figure S5. Circular dicroism difference spectra resulted from gradual addition of Ag^{+} into (A) AQLIC16C19, (B) TetC17C20, (C) HexC17C20 performed in 50 mM MES/100 mM NaCl solution at pH 5.5 and 25° C.

Preparation of Fluorescent Ag^{0} NC's

In a typical procedure for preparing the fluorescent metal-clusters, 300 μM of $AgNO_3$ solution were mixed with 150 μM of peptide in 50 mM MES buffer/100 mM KCl buffer pH 5.5. After vigorous stirring at room temperature for 20 min, the binding of Ag^{+} ions to the peptides was confirmed by an increased absorbance at 230 nm upon which the mixtures were treated with excess $NaBH_4$. Formation of the Ag NC's was monitored by observing the growth in emission intensity at ca. 450 nm and the reaction was seen to be complete within 24 hours. The results are described in the text.

Absorbance Spectra of the Reduced Clusters

Figure S6 shows the absorption spectra of the *apo*-peptides AQLIC16C19 (red dotted line/panel A), TETC17C20 (green dotted line/panel B), and HEXC17C20 (blue dotted line/panel C).

The addition of Ag^{+} to the peptide solutions produced an increased absorption between 250-300 nm

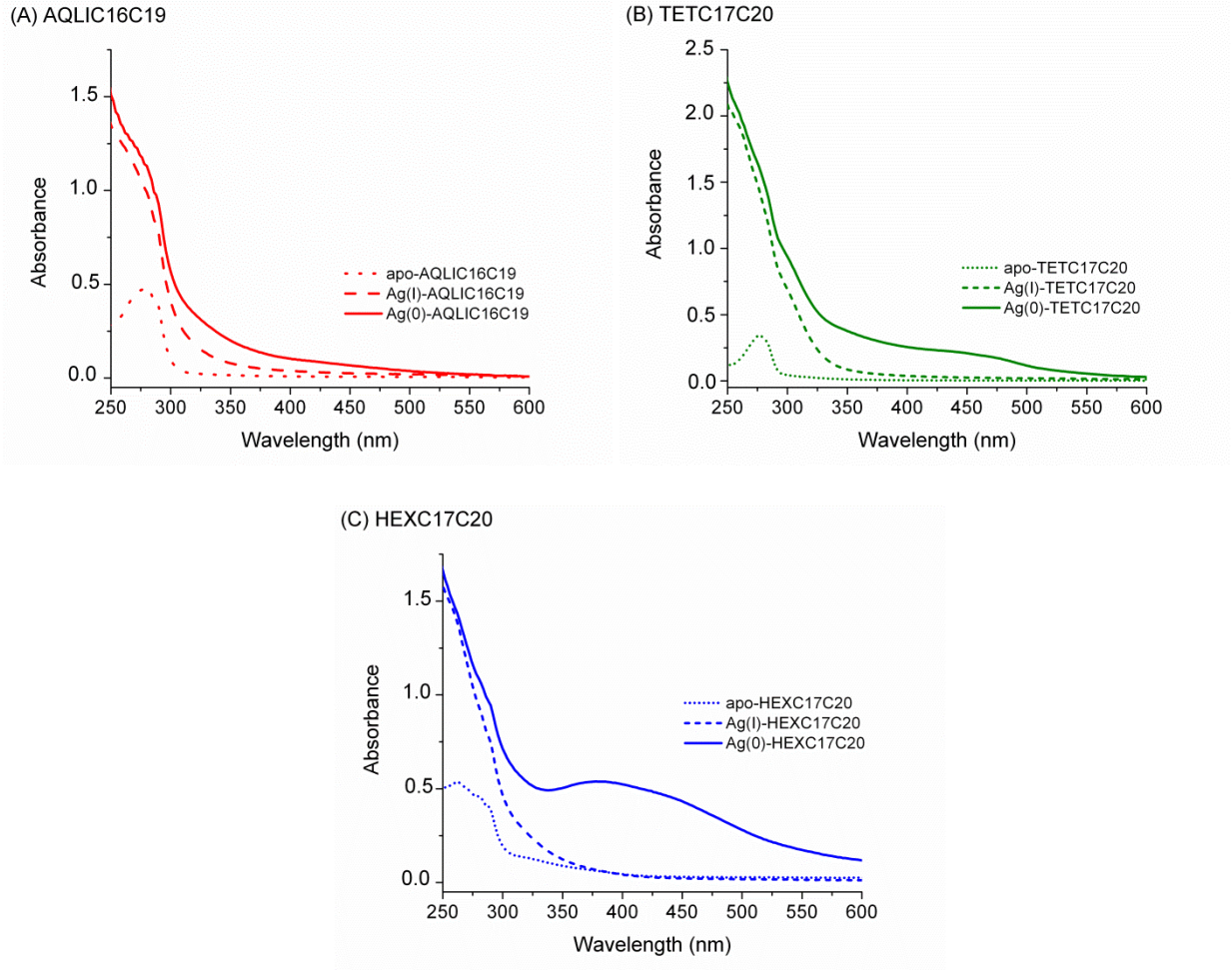

Figure S6. Absorption spectra of (A) AQLIC16C19 (red), TETC16C19 (green), and HEXC16C19 (blue) recorded in 50 mM MES buffer pH 5.5/ 100 mM NaCl.

(dashed lines) and reduction by $NaBH_4$ produced new features at ca. 350-400 nm (solid lines). The extinction coefficients of the Ag^0 NC's clusters were calculated from the concentrations of the apo coiled coils assuming complete metal loading and using the molar extinction coefficient of tyrosine

ε_{276} = 1450 M^{-1} cm^{-1} or tryptophan ε_{280} = 5540 M^{-1} cm^{-1}. The values are: $\varepsilon_{340\ nm}$ (AQLIC1619) = 2.5 x 10^4 cm^{-1} M^{-1}, $\varepsilon_{370\ nm}$(TETC17C20) = 16 x 10^4 cm^{-1} M^{-1}, and $\varepsilon_{290\ nm}$ (HEXC17C20) = 40 x 10^4 cm^{-1} M^{-1}.

Circular Dichroism Spectra of the Reduced Clusters

Circular dicroism spectra recorded after the reduction of the cluster showed negative signals at around 260 nm and positive signals at 290 nm for Ag^o/AQLIC16C19 and Ag^o/TETC17C20; and a positive band at 290 nm for Ag^o/HEXC17C20. These results suggest that the reduced Ag^0 nanoclusters remain within the peptide environment (Figure S7).

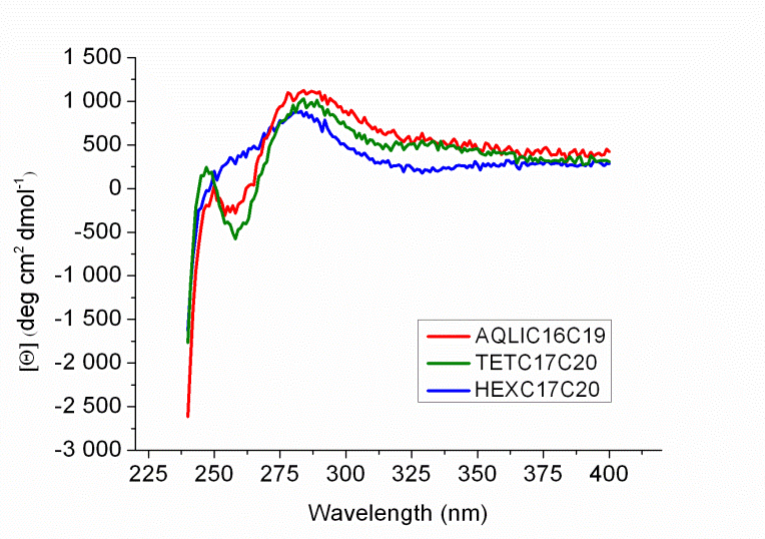

Figure S7. Circular dicroism spectra recorded for the reduced Ag^0 NC's after complete reduction with $NaBH_4$ (A) AQLIC16C19, (B) TetC17C20, (C) HexC17C20.

Steady-State Fluorescence Measurements

Fluorescence excitation and emission spectra were recorded by a single photon counting spectrofluorometer from Edinburgh Analytical Instruments (FL/FS 920). The range of excitation wavelength was from 250 nm to 525 nm nm using 2 nm steps, and the range of emission

wavelength was from 325 nm to 800 nm taken in 1 nm steps. The results are shown in Figure S8 and are described in the text.

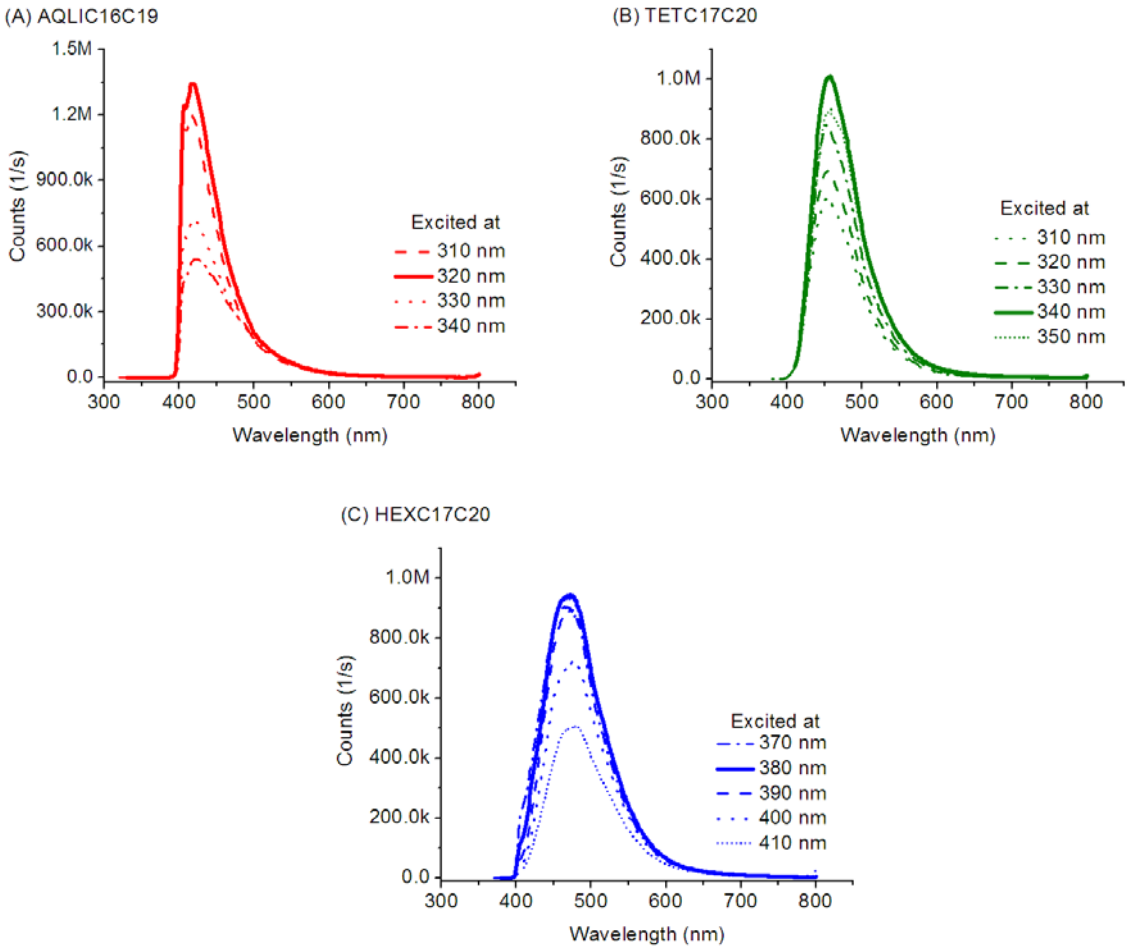

Figure S8. Photoluminescence spectra of Ag^0 nanoclusters formed within (**A**) AQLIC16C19 (red), (**B**) TETC16C19 (green), and (**C**) HEXC16C19 (blue) recorded in 50 mM MES buffer pH 5.5/ 100 mM NaCl.

Fluorescence Quantum Yield Calculations

The fluorescence quantum yields (Φ) presented in the text were measured using quinine hemisulfate monohydrate in 0.1M H_2SO_4 as a reference agent (Φ_{ref} = 0.54) according to the comparative method using the following equation:

$$\Phi_{sample} = \Phi_{ref} \times \frac{F_{sample}}{F_{ref}} \times \frac{A_{ref}}{A_{sample}} \times \frac{n^2_{sample}}{n^2_{ref}} \quad \text{(S3)}$$

In this equation, Φ_{ref} is a quantum yield of the references solution, F_{sample} and F_{ref} are areas under emission curves of the sample and reference solutions, respectively, A_{ref} and A_{sample} are the absorbance of the same reference and sample solutions, and n_{sample} and n_{ref} are refractive indexes of the solutions.

Time-resolved Fluorescence Measurements.

The steady-state lifetime measurements were recorded in a time-correlated single photon counting spectrofluorimeter from Edinburgh Analytical Instruments (FL/FS 900) with a photomultiplier tube in a Peltier-cooled housing. The samples were excited using a 375 nm laser diode from PicoQuant

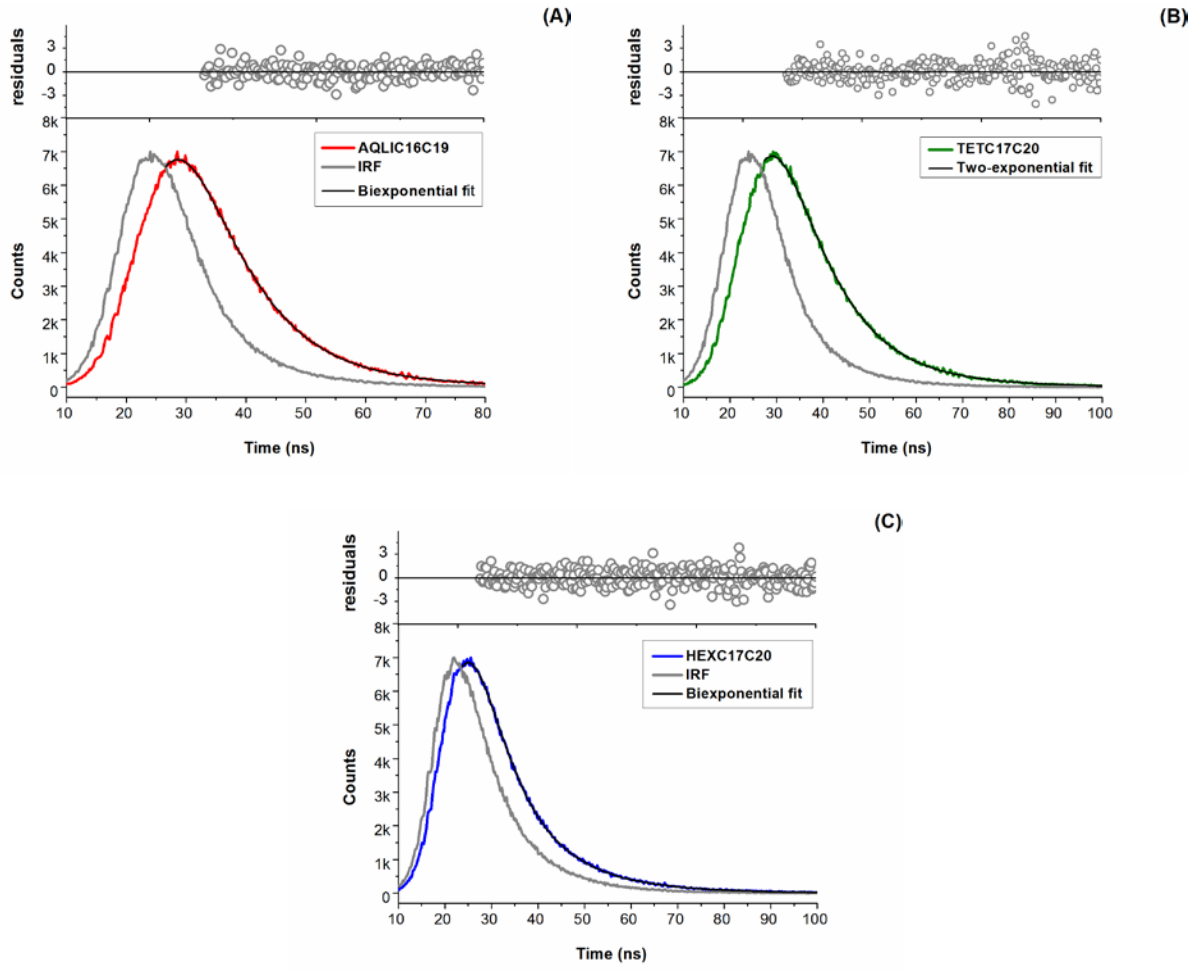

Figure S9. Fluorescence lifetime analysis of Ag nanoclusters formed within (**A**) AQLIC16C19 (**B**) TETC17C20 (**C**) HEXC17C20.

(LDH-D-C-375B) and a 355 nm laser diode from Crystal Laser (QC 355-050). For precise measurement an iterative reconvolution fit was performed. The emission intensities (I) were best fit to a biexponential decay over time (t): $I(t) = A_1 \exp(t/\tau_1) + A_2 \exp(t/\tau_2)$ in which A_1, $A_{2,}$ τ_1 and τ_2 are respectively the amplitudes and lifetimes of the two emission components. Reduced chi-square values (χ^2) of 1.14, 1.30, and 1.21 were obtained for AQLIC16C19, TETC17C20, and HEXC17C20, respectively. The results are shown in Figure S9 and are described in the text.