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Controlled Formation of Emissive Silver Nanoclusters Using Rationally Designed Metal-Binding Proteins

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Supporting Information

ABSTRACT: The metal-binding properties of rationally designed, synthetic proteins were used to prepare a series of emissive silver nanoclusters having predictable sizes and emission energies. Metal-binding α-helical coiled coils were designed to exist as peptide trimers, tetramers, and hexamers and found to uniquely bind 6, 8, and 12 Ag^+ ions, respectively. Subsequent treatment with a chemical reducing agent produced a series of peptide-bound Ag^0 nanoclusters that display a strong visible fluorescence whose emission energies depend on the number of bound metal ions in excellent agreement with theory.

Fluorescent metal nanoclusters (NCs) consist of 10^0−10^2 atoms of a noble metal, usually gold (Au) or silver (Ag). Their strong emission intensities, small sizes (<2 nm), extreme photostability, and overall biocompatibility make them attractive candidates for use as biological probes.[1−4] In recent years, Ag NCs have attracted particular attention because of their strong fluorescence. These systems have been typically prepared by the chemical reduction of Ag^+ ions that have been incorporated within frameworks of polymers,[5−7] dendrimers,[8−10] DNA,[11−14] proteins,[15] or peptides.[16,17] However, the preparation of Ag NCs having predictable sizes, and thus tunable emission properties, remains a challenge because of the difficulty in precisely regulating the degree of metal loading into these frameworks. The work presented herein describes a new approach to this problem in which rationally designed, synthetic metal-binding proteins (Figure 1) were used to prepare a series of Ag NCs whose emission energies were systematically varied in a predictable manner. This approach extends previous work from our group that has studied the metal-binding properties of α-helical coiled coils.[18−20]

The coiled-coil peptide structures used in these studies are easily prepared and result from the supercoiling of two or more α-helices stabilized by the interchain packing of their hydrophobic surfaces. Our group has shown that a Cys-X-X-Cys metal binding site can be incorporated into the hydrophobic surface of a coiled-coil peptide and that its subsequent self-assembly into an n-stranded coiled coil will produce a thiolate-rich metal-binding domain within its hydrophobic core. Indeed, we recently showed that the binding of Cd^{2+} ions to a three-stranded coiled coil resulted in the formation of an adamantane-like $Cd_4S_6O_4$ metal-ion cluster that was characterized by X-ray crystallography.[20] From these results, we speculated that (1) appropriate control over the oligomerization states of these coiled coils might produce a series of metal-binding proteins able to bind different numbers of metal ions in a discrete and well-defined manner because larger peptides would contain more metal binding sites and (2) these systems might then be used as new frameworks in the preparation of Ag NCs having predictable sizes and thus tunable emission energies. To test these hypotheses, three different coiled-coil peptides were prepared: AQLIC16C19, TETC17C20, and HEXC17C20. The sequence of the AQLIC16C19 peptide is based on one previously shown to exist as a three-stranded coiled coil[20] and the sequences of TETC17C20 and HEXC17C20 were modified from those developed by Woolfson and co-workers to form four- and six-stranded coiled coils, respectively.[21]

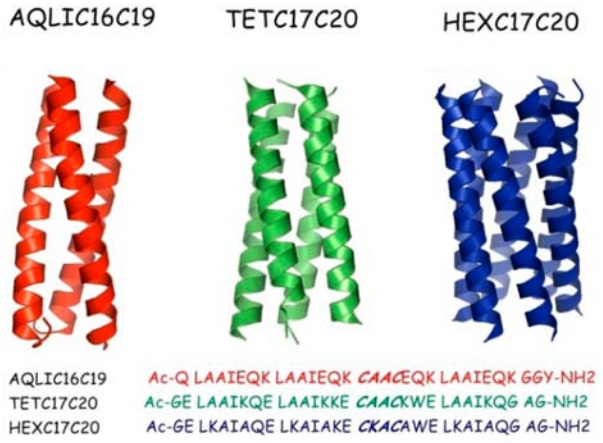

Figure 1. Coiled-coil peptides employed in the synthesis of Ag NCs. AQLIC16C19, TETC17C20, and HEXC17C20 are shown as being self-assembled into a peptide trimer, tetramer, and hexamer, repectively. The sequences of the parent monomer peptides are shown at the bottom of the figure.

All peptides were prepared by solid-phase methods, purified by reverse-phase high-performance liquid chromatography (HPLC), and analyzed by both electrospray ionization mass spectrometry (ESI-MS) and matrix-assisted laser desorption/ionization mass spectrometry. Size-exclusion chromatography with multiangle laser light scattering detection (SEC-MALS) was used to determine the oligomerization state of the apopeptides. The chromatograms obtained for AQLIC16C19, TETC17C20, and HEXC17C20 in 50 mM 2-(N-morpholino)ethanesulfonic

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acid (MES) buffer/100 mM KCl buffer (pH 5.5) each consisted of a single band, and the eluted species were found to have molecular weights of 9.6, 10.6, and 19.1 kDa, respectively. As anticipated, these results show that AQLIC16C19 exists as a peptide trimer, TETC17C20 as a peptide tetramer, and HEXC17C20 as a peptide hexamer. The circular dichroism (CD) spectrum of each peptide was measured at pH 5.5−7.5 and consists of minima at 208 and 222 nm, which are assigned to the $\pi-\pi^*$ and $n-\pi^*$ transitions of an α-helix (Supporting Information, SI). The molar ellipticities observed at 222 nm were compared with that calculated for a completely helical 32-residue peptide,[22] which showed that the AQLIC16C19, TETC17C20, and HEXC17C20 peptides were 75%, 65%, and 55% helical, respectively. In addition, ratios of $\theta_{222}/\theta_{208} > 1.0$ were observed for all peptides, indicating that they exist as coiled coils.

The metal-binding properties of the peptides were examined by UV−vis titration experiments (Figure 2). In all cases, the addition of Ag^+ to a peptide solution produced a broad absorption band centered at ca. 230 nm with a shoulder at ca. 290 nm (inset, Figure 2), which are respectively assigned to a CysS-Ag^+ ligand-to-metal charge-transfer transition and a cluster-centered Ag^+ (d−s) transition.[23] Figure 2 presents the molar absorptivity at 230 nm for AQLIC16C19, TETC17C20, and HEXC17C20 measured as increasing amounts of Ag^+ were added to the peptide solution. Note that each plot displays a change in the slope after the addition of ca. 2 mol equiv of Ag^+ per peptide monomer. Because AQLIC16C19, TETC17C20, and HEXC17C20 exist as a peptide trimer, tetramer, and hexamer, respectively, these results show that AQLIC16C19 binds 6 Ag^+ ions, TETC17C20 binds 8 Ag^+ ions, and HEXC17C20 binds 12 Ag^+ ions. The continued addition of Ag^+ produced further increases in absorption arising from the added Ag salt, as verified by suitable controls.

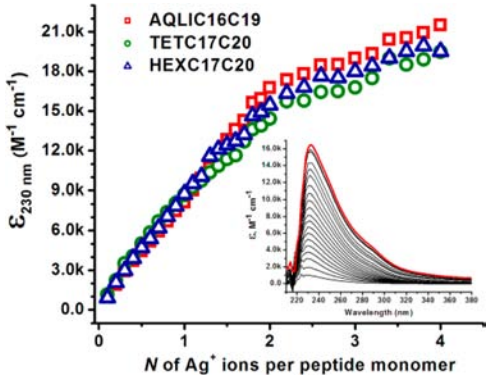

Figure 2. Plot of the extinction coefficient at 232 nm as a function of the molar equivalents Ag^+ added into AQLIC16C19 (red squares), TETC17C20 (green circles), and HEXC17C20 (blue triangles) in 50 mM MES/100 mM NaCl at pH 5.5 and 25 °C. Inset: UV difference of the Ag^+ to AQLIC16C19 additions.

The degree of metal loading in these peptides was further studied by ESI-MS in which peptide solutions were treated with excess $AgNO_3$ and purified by SEC to remove any unbound metal ion. The ESI-MS spectra of AQLIC16C19, TETC17C20, and HEXC17C20 all show a set of multiple peaks corresponding to the apopeptide monomer, the peptide monomer with one Ag^+ ion, and the peptide monomer with two Ag^+ ions (see the SI). These results are consistent with those of the UV−vis titration plots described above by showing a maximum degree of metal loading of two Ag^+ per peptide monomer. It is noted that, whereas the peak corresponding to the M + Ag_2 species is the dominant one for HEXC17C20, the major peaks in the spectra for TETC17C20 and AQLIC16C19 correspond to the relevant M + Ag_1 species. This might be due to the occurrence of a higher degree of fragmentation of these smaller peptides.

The desired Ag^0 NCs were prepared by treating the Ag^+-containing peptides with $NaBH_4$, as described in the SI. The formation of Ag NCs was monitored by observing the growth in the emission intensity at ca. 450 nm, and the reaction was seen to be complete within 24 h. Solutions stored in the dark at room temperature remained emissive for at least 3 months, at which point they retained ca. 85% of their original emission intensity. The reduced clusters are characterized by the appearance of the new absorption features at ca. 350−400 nm accompanied by a low quantum yield fluorescence between 420 and 475 nm (Figure 3).

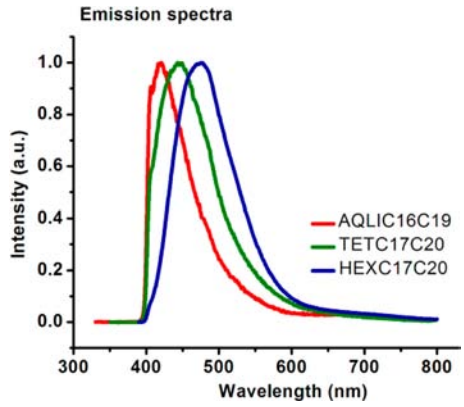

Figure 3. Photoluminescence spectra of the Ag NC adduct of AQLIC16C19, TETC17C20, and HEXC17C20 recorded in 50 mM MES buffer (pH 5.5)/100 mM NaCl.

The excitation spectrum of this emission has a maximum at ca. 380 nm. The emission lifetimes of the three Ag-peptide NCs were fit to biexponential decays (see the SI), for which the shorter-lived components having lifetimes of 3−6 ns comprised ca. 90% of the emitting species (Table 1).

Table 1. Emission Properties of the Ag^0 NCs[a]

	ϕ (%)	τ_1 (ns)	A_1 (%)	τ_2 (ns)	A_2 (%)
AQLIC16C19	0.25	17.4 ± 3.6	12	5.8 ± 2.9	88
TETC17C20	1	17.0 ± 0.7	9	2.9 ± 1.2	91
HEXC17C20	4	16.3 ± 2.8	9	3.0 ± 1.0	91

[a] ϕ, A_1, A_2, τ_1, and τ_2 are as defined in the SI.

It is noted that lifetimes of this magnitude are typical for small Ag^0 NCs stabilized by DNA and peptide frameworks.[4,13,14,24] An interesting feature of Figure 3 is that it shows the occurrence of a distinct red shift in emission as the oligomerization state of the peptide increases. Thus, the Ag^0 NC within AQLIC16C19 emits at λ_{em} = 421 nm, the NC within TETC17C20 emits at λ_{em} = 443 nm, and the NC within HEXC17C20 emits at λ_{em} = 475 nm. Assuming that the degree of metal loading in the various metallopeptides remains unchanged by treatment with $NaBH_4$, these results show that the emission energies of the peptide-bond NCs decrease with increasing cluster size. It is of further interest

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to note that the jellium free-electron model predicts that the emission energy of metal NCs should follow eq 1

$$E_{Em} = E_{Fermi}/N^{1/3} \quad (1)$$

where E_{Fermi} = 5.5 eV is the Fermi energy of bulk Ag and N is the number of atoms in the cluster.[25] Figure 4 plots the emission

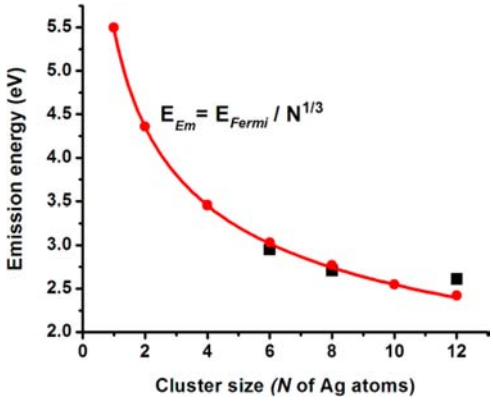

Figure 4. Correlation of the emission energy as a function of the number of incorporated metal atoms. The squares are the experimental values, and the circles are the values calculated from eq 1.

energies calculated according to eq 1 with no adjustable parameters for clusters having up to 12 Ag atoms superimposed on the values observed for the 4-, 8-, and 12-atom NCs in AQLIC16C19, TETC17C20, and HEXC17C20, respectively. As shown, the observed values are in very close agreement with those predicted by theory. It is acknowledged, however, that further work is needed to unequivocally verify the number of Ag atoms present in each of the emissive NCs. Nevertheless, it is of interest to note that both the oxidized and reduced peptides show an induced CD signal between 240 and 300 nm, a region of the spectrum where the clusters absorb (see the SI). These results suggest that both the Ag^+ and Ag^0 clusters exist within similar chiral environments before and after treatment with $NaBH_4$.

In summary, evidence is presented to show that the size-controlled preparation of the emissive Ag^0 NCs can be achieved through the use of appropriately designed metal-binding coiled coils. The Ag NCs prepared in this way are photoluminescent, have narrow emission profiles with nanosecond lifetimes, and are highly stable even in the presence of the high salt concentrations.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

Methods and procedures, ESI-MS spectra, SEC-MALS chromatograms and CD spectra of the apopeptides, CD spectra of holopeptides, UV−vis spectra, emission spectra, CD spectra of the emissive Ag^0 peptides, and fluorescence lifetime decays. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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