

# **Cu(I) Luminescence from the Tetranuclear Cu<sub>4</sub>S<sub>4</sub> Cofactor of a Synthetic 4-Helix Bundle**

Olesya A. Kharenko<sup>†</sup>, David C. Kennedy<sup>§</sup>, Borries Demeler<sup>‡</sup>, Michael J. Maroney<sup>§</sup>,  
and Michael Y. Ogawa<sup>†</sup>

<sup>†</sup>*Department of Chemistry and Center for Photochemical Sciences, Bowling Green State University, Bowling Green, OH 43403*; <sup>§</sup>*Department of Chemistry, University Massachusetts, Amherst, MA 01003*; <sup>‡</sup>*Center for Analytical Ultracentrifugation of Macromolecular Assemblies, University of Texas Health Science Center, San Antonio, TX 78229*

The binding of Cu(I) to the random coil peptide C16C19-GGY produces a self-organized, metal-bridged 4-helix bundle which displays an intense room-temperature luminescence at 600 nm. The C16C19-GGY peptide has the sequence Ac-K(IEALEGK)<sub>2</sub>(CEACEGK)-(IEALEGK)GGY-amide which is similar to those previously shown to exist as two-stranded coiled-coils. However, in this peptide the Cys-X-X-Cys metal binding motif was introduced into positions 16-19 of the sequence in order to place metal-binding cysteine residues at the “a” and “d” positions of the third heptad repeat. The addition of [Cu(CH<sub>3</sub>CN)<sub>4</sub>]<sup>+</sup> to samples of C16C19-GGY results in an intense ( $\phi = 0.053$ ) ambient temperature luminescence which is centered at 600 nm and is stable upon standing overnight under ambient conditions. The luminescence has an excitation spectrum with a maximum at 275 nm and can be quenched by the addition of ferricyanide, oxygen, or urea to the solution. Emission, CD, and UV titrations indicate a 1:1 metal:peptide stoichiometry.

Cu(I) binding is also accompanied by significant changes to the far UV circular dichroism spectrum of the peptide to indicate that C16C19-GGY undergoes a metal-induced folding to a helical conformation when it binds Cu(I). Analytical ultracentrifugation shows that the metal peptide exists as a tetramer (MW  $\approx$  12 kDa). Thus, four Cu atoms are bound to 4-helix bundle. The Cu K-edge XANES spectrum shows the presence of three-coordinate Cu(I) centers in Cu-C16C19-GGY, and EXAFS analysis is consistent with the formation of a Cu(I) cluster containing a Cu<sub>4</sub>S<sub>4</sub> ring in which each Cu atom is bridged by the side chains of two cysteine residues and has a terminal N/O ligand. These results were not anticipated from the original design and illustrate how the structures of metalloproteins may be controlled by the coordination chemistry of their inorganic cofactors.

This work was supported by NIH grants GM61171 (MYO) and GM69696 (MJM).