Supporting Information

Intrinsically fluorescent gold nanoclusters stabilized within a copper storage protein that follow Irving-Williams trend in metal ion sensing

Dhanashree Selvan,† Pallavi Prasad,† Skyler Crane,† Abubkr Abuhagr,‡ Richard Covington,† Kateryna Artyushkova,‡ Guda Ramakrishna,§ Saumen Chakraborty†,*

†Department of Chemistry and Biochemistry, University of Mississippi, University, MS 38677, United States
‡Department of Chemistry, Western Michigan University, Kalamazoo, MI 49008, United States
§Department of Chemical and Biological Engineering, Center for Micro-Engineered Materials (CMEM), University of New Mexico, Albuquerque, NM 87131, United States
DNA sequence of Csp1 with N-terminal Strep-Tag:

TGGAGCCACCCGCAGTTCCGAGAAGGGTGGCAGCGTGAAGAACCCGCACGCGGGCCACAAAA
TGAGCCACGGTGCGAAGTTAACAGCGCTGCTGGACAGCAGCAGCCACTGCCTGGCGGGTTGG
TGAAGATTTGTGCTGCTCAGTGGGGTTGAATGGGCGCGATGAAACGACGCAGCATGCGGTGCT
GCACTAAGGCGACCTATGATCTGTTGGCGGCGTCGCTGGCGGTGCGCTGGCGAAACTGGCGGGTAC
CAACAGGCGTGTTCCCGGCGTTTGCGGAAGGTGGTTTGGGACGTGCTTTGCGCGGGCTGCAAGA
AAGAGTGCGATAAGTTCCCGAGCATCGCGGAGTGCAAAGCGCATGCGGTGAAGCGTCCAGC
GTGCGCGGAGGAAATGCCACAAAAAGTGCCGCGCTAA
Figure S1. X-ray structure of Cu(I)-Csp1 showing the orientation of all coppers along the protein core.
Figure S2. Photographs of the control sample with a variant of Csp1 where all the Cys residues were mutated to non-coordinating Ala and Leu residues. 25 μM of protein was incubated with 150 μM Au³⁺ in the presence of NaOH (4.8% v/v) for 18h at 37°C, under normal light (left); under uv light (right). A lack of luminescence in the absence of thiols indicates that the observed luminescence of the clusters presented in this work is arising from thiol-associated Au at the interior of the protein.
Figure S3. Photographs of AuNC@Csp1 in the presence of 1 to 21 equivalents of Au$^{3+}$ prepared using NaBH$_4$ as reductant, viewed under normal light (top) and uv light (below). Protein concentration was kept constant at 25 μM.
Figure S4. a) UV-vis spectra of AuNC@Csp1 prepared using NaBH₄ in the presence of 1-21 equivalents of Au³⁺. b) Representative photoexcitation and photoemission spectra of the NaBH₄ reduced samples. The excitation spectrum for 1 and 3 equivalent Au³⁺ samples is shown as dotted red, while the corresponding emissions are shown in orange/purple in the 450 nm region. The excitation for the 12 equivalent Au³⁺ samples is in dotted green, and the corresponding emission spectra are shown with maxima ~622 nm. The figure legend represents the equivalents of Au³⁺ with respect to protein.
Figure S5. Photoexcitation and photoemission spectra of selected samples prepared by the endogenous reduction method. The figure legend represents the equivalents of Au$^{3+}$ with respect to Csp1.
Figure S6. Photoexcitation and photoemission spectra of 9 equivalent Au sample reduced with 15% NaOH (v/v), along with a photograph showing the orange color under UV light (inset).
Figure S7. MALDI-MS spectrum of AuNC@Csp1_a in CHCA matrix.
Figure S8. Emission spectra of AuNC@Csp1_a containing 30 ppm Au after incubation with 10-fold excess metal ions for 10 minutes excited at 365 nm (a). The pH-dependent emission spectra of AuNC@Csp1_a are shown in (b). Photographs of the corresponding samples under UV light are shown in (c) and (d), respectively.
Figure S9. Emission spectra of AuNC@Csp1_a containing 30 ppm Au with increasing concentrations of Co$^{2+}$, Ni$^{2+}$ and Cu$^{2+}$ excited at 365 nm.
**Figure S10.** Emission spectra and photographs of a representative cluster sample freshly synthesized (red) and after 4 months (green) demonstrating shelf stability of the clusters over time. Insets show the photographs of these samples under UV light.