Electronic Supplementary Information for Dalton Transactions

Conjugation of Au11 Cluster with Cys-rich Peptides Containing the α-Domain of Metallothionein

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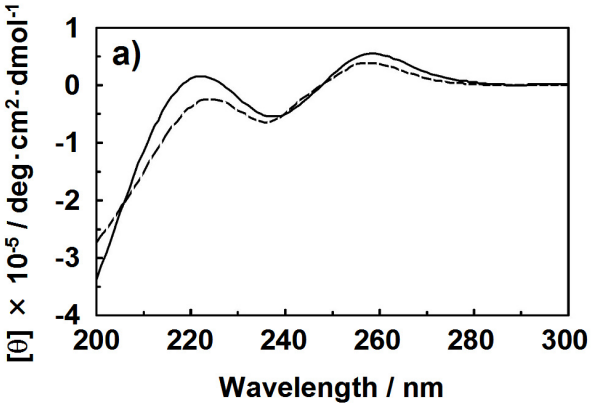
I. Metal ion exchange between CGMTIIα-M^{n+} and Au11 cluster.



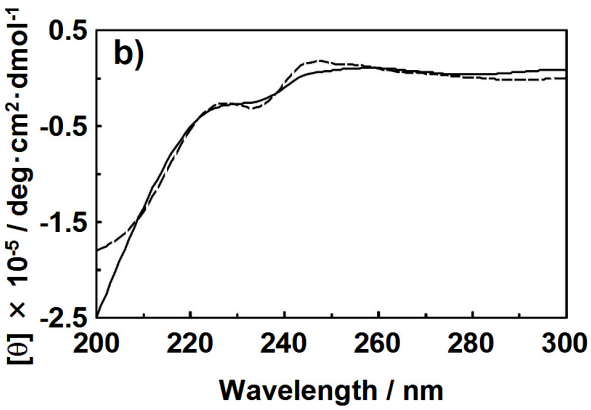


Fig. S1. (a) CD spectral changes observed for the reaction of CGMTIIα and Au11 in the absence and presence of 5 eq. of Cd^{2+}: 25 μM of CGMTIIα-Cd^{2+} (solid line), and 25 μM of CGMTIIα-Cd^{2+} + 25 μM of Au11 (dashed line). (b) CD spectral changes for the reaction of CGMTIIα and Au11 in the absence and presence of 5 eq. of Cu^{+}: 25 μM of CGMTIIα-Cu^{+} (solid line), and 25 μM of CGMTIIα-Cu^{+} + 25 μM of Au11 (dashed line).

As the metal ions make MTIIα withstand O_2 by forming MT clusters (MT-M^{n+}) they were expected to simplify the handling of these systems. Therefore, 1:1 conjugation between CGMTIIα and Au11 was first studied at room temperature in the presence of 5 eq. of M^{n+} (M^{n+} = Zn^{2+}, Cd^{2+}, Co^{2+}, and Cu^{+}). The activities of the peptide/water stock solutions were determined by the Ellman test just before the complexations.

In common with Cd^{2+}-containing mammalian MT,[1] the solution of CGMTIIα and Cd^{2+} shows CD peaks at 220 and 260 nm originating from Cys S-Cd^{2+} LMCT (Fig. S1a, black solid line). The mixing of Au11 to CGMTIIα-Cd^{2+} slightly decreases the two peaks at 220 and 260 nm, but the change in the spectrum is small (Fig. S1a; black dashed line), suggesting that CGMTIIα-Cd^{2+}/Au11 roughly remains the main chain folding of CGMTIIα-Cd^{2+}. Almost all of Cd^{2+} in CGMTIIα-Cd^{2+} will be conserved after the attack of Au11, and probably only a few Cys residues of CGMTIIα contribute to the binding of Au11. CGMTIIα-Zn^{2+} and CGMTIIα-Co^{2+} formed undefined precipitates when treated with Au11 (Tris-HCl buffer at pH ~8), although Zn^{2+} and Co^{2+} do not bind to CGMTIIα at pH ~7 (the CD spectra of apo-CGMTIIα remain unchanged in the presence of these metal ions).

On the contrary, the mixing of Au11 with CGMTIIα-Cu^{+} results in a slight increase in the peak at ~250 nm without precipitation, as well as the disappearance of the weak peak at ~300 nm, but no change at ~220 nm (Fig. S1b). This result suggests that the main chain folding of CGMTIIα-Cu^{+} is almost conserved and Cu^{+} of CGMTIIα-Cu^{+} is only partially exchanged for Au11 as in the case of CGMTIIα-Cd^{2+}. This interpretation is supported by the result of the emission experiments. Similarly to MTII-Cu^{+},[2] CGMTIIα-Cu^{+} shows an emission at 500~700 nm when excited at ~290 nm (Fig. 3 black solid line). The addition of Au11 to this solution reduces this emission by ~40% (Fig. 3, black dashed line) suggesting that Cu^{+} in the original CGMTIIα-Cu^{+} is partially exchanged by Au11. Otherwise, all of the Cu^{+} content is conserved and the emission is quenched by the heavy atom effect of Au11 attached to CGMTIIα-Cu^{+}. Contrarily, the addition of Cu^{+} to the 1:1 mixed solution of CGMTIIα and Au11 only slightly increases the emission from zero (Fig. 3, gray dashed line) to approximately one-third the level of the solution containing CGMTIIα-Cu^{+} and Au11 (Fig. 3, gray solid line). The appearance of this slight emission indicates that the gold cluster (Au_x) trapped in CGMTIIα is only partially exchanged by Cu^{+}; otherwise, Cu^{+} coordinates to the Cys residues that

are not used during the complexation with Au11. As is mentioned in the manuscript, the Ellman test, in which the number of free SH groups was calculated by subtracting the sum of independent absorption of CGMTIIα-Au11 and DTNB from that of CGMTIIα-Au_{11}/DTNB, revealed that 6 of the 12 Cys residues of CGMTIIα are free from the coordination with Au_x in CGMTIIα-Au_x; however, the test for the solution containing CGMTIIα-Au11 and Cu^+ showed no existence of free Cys residues.[3]

This result indicates that all the free-Cys residues of CGMTIIα-Au_x bind to Cu^+. Considering that CGMTIIα-Au_x itself is not emissive, the emission observed in CGMTIIα-Au_x + Cu^+ is attributed to the new coordination between Cys S and Cu^+. If Au_x is cleaved from CGMTIIα-Au_x by the addition of Cu^+, the emission will increase to the level of CGMTIIα-Cu^+. CGMTIIα-Au_x + Cu^+ may contain a small cluster structure of Cu_x(S-Cys)$_y$. It is not clear that the structure of CGMTIIα-Au_x remains unchanged in the presence of Cu^+. Any way, bond between CGMTIIα and Au_x is not cleaved by the addition of Cu^+. In this meaning, CGMTIIα-Au_x is robust. Considering these results and that the two systems CGMTIIα-Cu^+ + Au11 and CGMTIIα-Au_x + Cu^+ contain the same amount of peptides, Au11, and Cu^+, both CGMTIIα-Cu^+ and CGMTIIα-Au_x require large activation energies for the metal ion exchange reactions.

II. Quantification of PPh_3 eliminated from CGMTIIα-Au_{11}

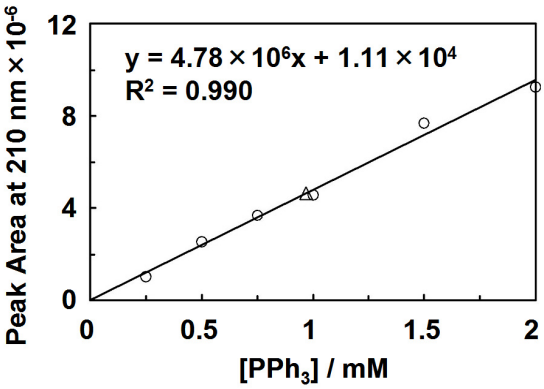


Fig. S2. Calibration curve for the quantification of PPh_3 (HPLC, ODS, 210 nm, 80% MeCN/H_2O, 0.8 ml/min). The peak area of the PPh_3 that eliminated from the reaction mixture of CGMTIIα-Au11, which was prepared by mixing a 10 μl of 700-μM of CGMTIIα aqueous solution and a 20 μl of 350-μM of Au11 MeCN solution, is shown by the black triangle.

III. Complexation of CGMTIIα with Au55.

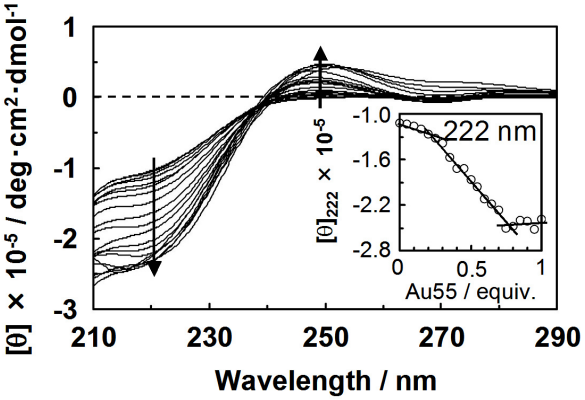


Fig. S3. The results of Au55 titration for CGMTIIα, where a 100-μM solution of Au55 in MeCN was dropwise added to an aqueous solution containing 25 μM of CGMTIIα, monitored with CD spectroscopy. The inset shows the titration curve observed at 222 nm.

The titration curve observed at 222 nm seems to be sigmoidal centered at CGMTIIα:Au55 = 1:0.5, or showing the formation of CGMTIIα-Au55 conjugates with the stoichiometries other than 1:1. However, it should be noted that the mixture of CGMTIIα and Au55 generates a precipitate when the mixing ratio approaches 1:1 in a MeCN/H_2O solution and becomes to yield noisy CD spectra, which disturbs the data collection in the titration region of Au55/CGMTIIα > 0.8. CGMTIIα-Au55 system may also yield a 1:1 conjugate.

Reference

[1] M. J. Stillman, W. Cai, A. J. Zelazowski, *J. Biol. Chem.*, 1987, **262**, 4538-4548.

[2] (a) B. Roschitzki, M. Vašák, *Biochemistry*, 2003, **42**, 9822-9828; (b) A. R. Green, A. Presta, Z. Gasyna, M. J. Stillman, *Inorg. Chem.*, 1994, **33**, 4159-4168; (c) Y. Li, U. Weser, *Inorg. Chem.*, 1992, **31**, 5526-5533; (d) M. J. Stillman, A. J. Zelazowski, J. Szymanska, Z. Gasyna, *Inorganica Chimica Acta*, 1989, **161**, 275-279; (e) Z. Gasyna, A. Zelazowski, A. R. Green, E. Ough, M. J. Stillman, *Inorganica Chimica Acta*, 1988, **153**, 115-118.

[3] Note that the Ellman test is independent of the Cu^+.