

Supporting Information for

Promoting Self-Assembly of Collagen-Related Peptides into Various Higher-Order Structures by Metal-Histidine Coordination

Wei Hsu, Yi-Lun Chen, and Jia-Cherng Horng*

Department of Chemistry, National Tsing Hua University, Hsinchu, Taiwan 30013,
R.O.C.

Frontier Research Center on Fundamental and Applied Sciences of Matters, National
Tsing Hua University, Hsinchu, Taiwan 30013, R.O.C.

Table of Contents

Estimation of T_m values.....	S2
Table S1. Peptides and their sequences used in this study.....	S2
Table S2. Absorption of peptide solutions for chelation competition experiments at 313 nm.....	S2
Figure S1. Thermal unfolding curves monitored by CD for HG(PPG) ₇ GH.....	S3
Figure S2. Analytical HPLC chromatograms for X9 and PHG	S4
Figure S3. Thermal unfolding curves monitored by CD for X9 and PHG	S5
Figure S4. TEM images for the assemblies of X9 and PHG after incubating with Co ²⁺ or Ni ²⁺	S6
Figure S5. SEM images for the aggregates of X9 after incubating with various [Cu ²⁺].....	S7
Figure S6. SEM images for the aggregates of X9 after incubating with various [Zn ²⁺].....	S8
Figure S7. SEM images for the aggregates of PHG after incubating with various [Cu ²⁺].....	S9
Figure S8. SEM images for the aggregates of PHG after incubating with various [Zn ²⁺].....	S10
Figure S9. SEM images for the aggregates of X9 after incubating with Cu ²⁺ or Zn ²⁺ (molar ratio of metal to peptide 1:1).....	S11
Figure S10. DLS analysis of chelation competition experiments for X9 and PHG	S12

Estimation of T_m values

All the T_m values were obtained from the first derivative of the ellipticity versus temperature curve ($d[\theta]/dT$). The temperature where the $d[\theta]/dT$ value is a minimum was determined to be T_m . For most samples, we could determine their T_m values from the minimal $d[\theta]/dT$ values and the deviation was ± 2 °C. For **X9**/ Zn^{2+} solution, the thermal transition was slightly broad because of aggregation, two distinct minimal $d[\theta]/dT$ values were observed, and the deviation of the determined T_m values was ± 3 °C. For **PHG**/ Cu^{2+} and **PHG**/ Zn^{2+} solutions, the thermal transitions were relatively broad due to the appearance of extensive aggregates, but minimal $d[\theta]/dT$ values could still be found and used to estimate their T_m values. The deviation of the estimated T_m values for these two samples was ± 3 °C. The corresponding thermal unfolding curves and T_m values are shown in Figures S1 and S3, and Table 1.

Table S1. Peptides and their sequences used in this study

Peptide	Sequences ^a
HG(PPG) ₇ GH	Ac-His-Gly-(Pro-Pro-Gly) ₇ -Gly-His-NH ₂
HG(PPG) ₉ GH (X9)	Ac-His-Gly-(Pro-Pro-Gly) ₉ -Gly-His-NH ₂
HG(PPG) ₄ (PHG)(PPG) ₄ GH (PHG)	Ac-His-Gly-(Pro-Pro-Gly) ₄ -(Pro-His-Gly)-(Pro-Pro-Gly) ₄ -Gly-His-NH ₂

^aAc indicates an acetylated N-terminus and NH₂ indicates an amidated C-terminus.

Table S2. Absorption of peptide solutions for chelation competition experiments at 313 nm^a

	HG(PPG) ₉ GH (X9)	HG(PPG) ₄ PHG(PPG) ₄ GH (PHG)
Peptide (400 μ M) only	-0.0171	0.0103
Zn^{2+} (160 μ M) added	0.8433	1.3575
EDTA (1.6 mM) added	0.0213	-0.0062

^aAll measurements were conducted by a UV-Vis spectrometer, and in pH 7.4 20 mM phosphate buffer at ambient temperature.

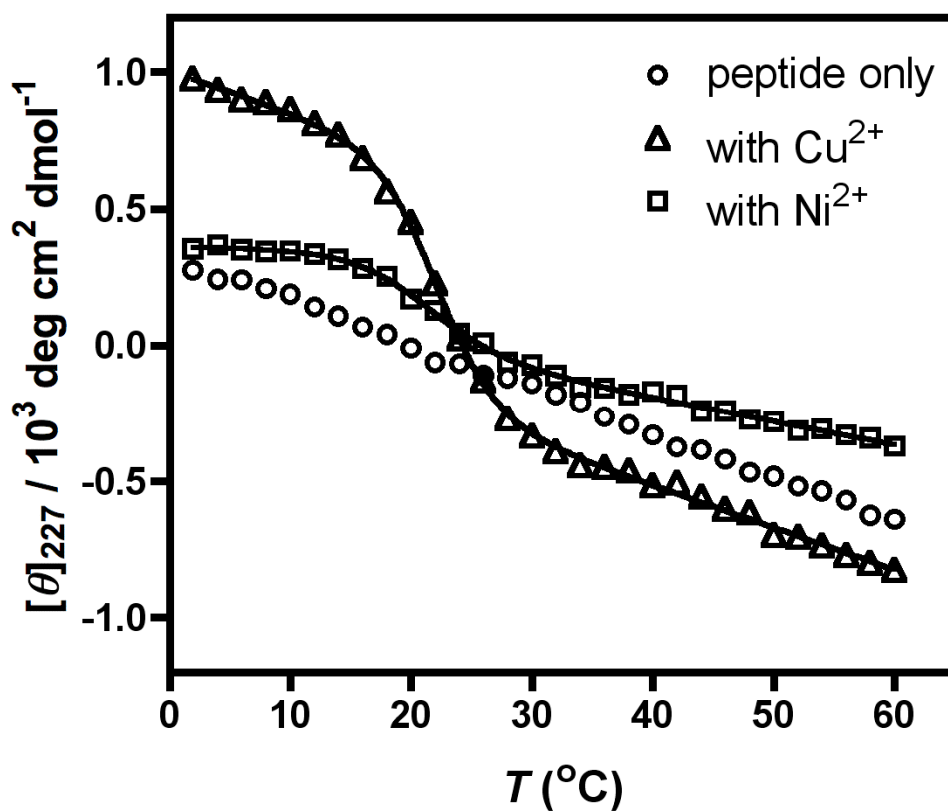


Figure S1. Thermal unfolding curves monitored by CD for HG(PPG)₇GH in the presence of Cu²⁺ and Ni²⁺ ions at pH 8.0. All measurements were performed in 20 mM sodium phosphate buffer. The concentrations of peptide and metal ions were 230 μM and 1 mM respectively. Instead of pH 7.4, pH 8.0 was used for this peptide since it gave a better thermal transition curve.

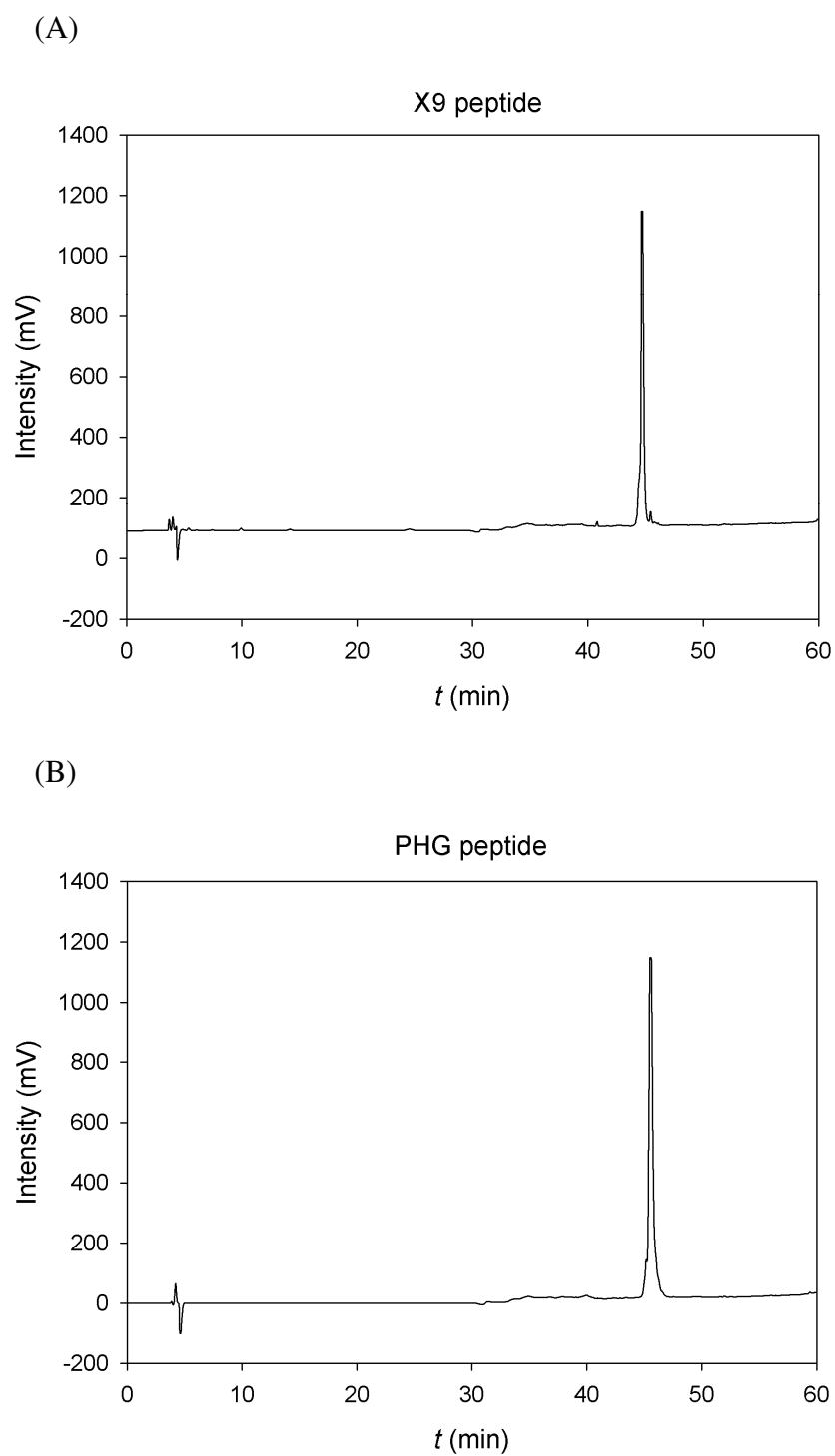


Figure S2. Analytical HPLC chromatograms for (A) **X9** (retention time: 44.7 min) and (B) **PHG** (retention time: 45.6 min). A gradient of 0 to 60% acetonitrile (0.1% TFA by v/v) and a flow rate of 1.0 mL/min were used for the analysis.

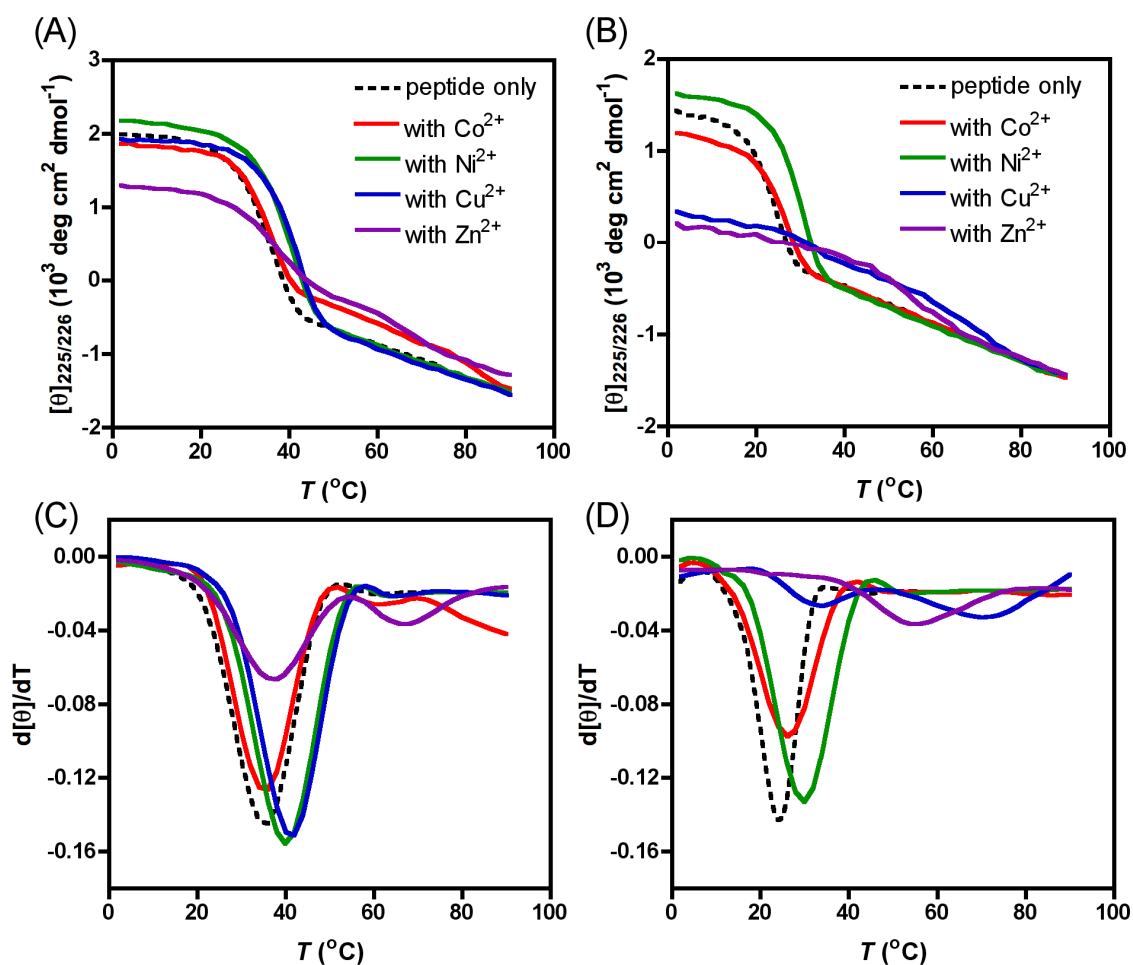


Figure S3. Thermal unfolding curves monitored by CD for (A) **X9** and (B) **PHG** in the presence of different metal ions at pH 7.4. The first derivatives of the thermal unfolding curves versus temperature ($d[\theta]/dT$) are shown in (C) **X9** and (D) **PHG**. All measurements were performed in 20 mM sodium phosphate buffer. The concentrations of peptide and metal ions were 400 μM and 160 μM respectively. The signals for the samples of **PHG**/ Zn^{2+} and **PHG**/ Cu^{2+} were much smaller than the others due to the precipitation in solution.

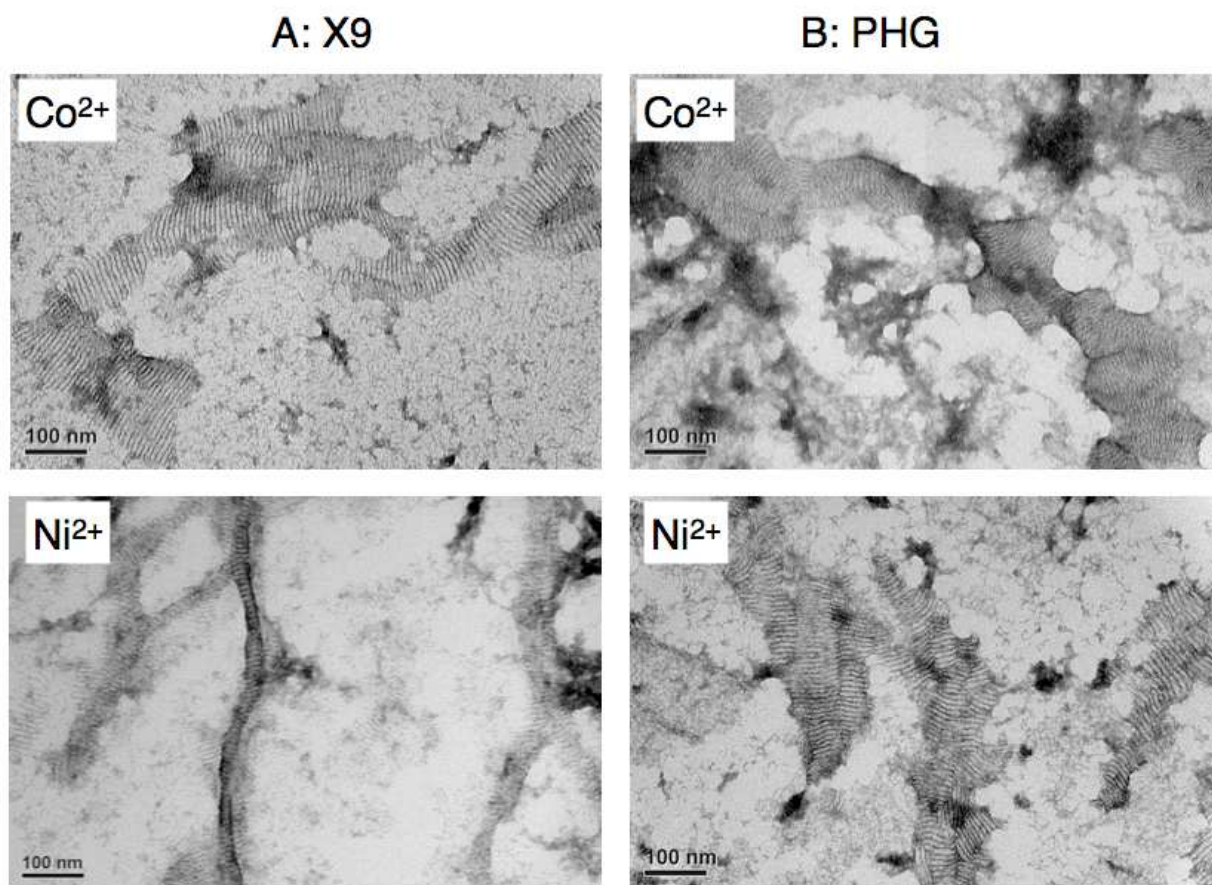


Figure S4. TEM images for the assemblies of (A) **X9** and (B) **PHG** after incubating with Co^{2+} or Ni^{2+} . Before taking TEM images, 400 μM peptides were incubated with 160 μM metal ions (molar ratio of peptide to metal 5:2) in pH 7.4 20 mM phosphate buffer and at 4 $^{\circ}\text{C}$ for more than 2 d. The scale bar is 100 nm.

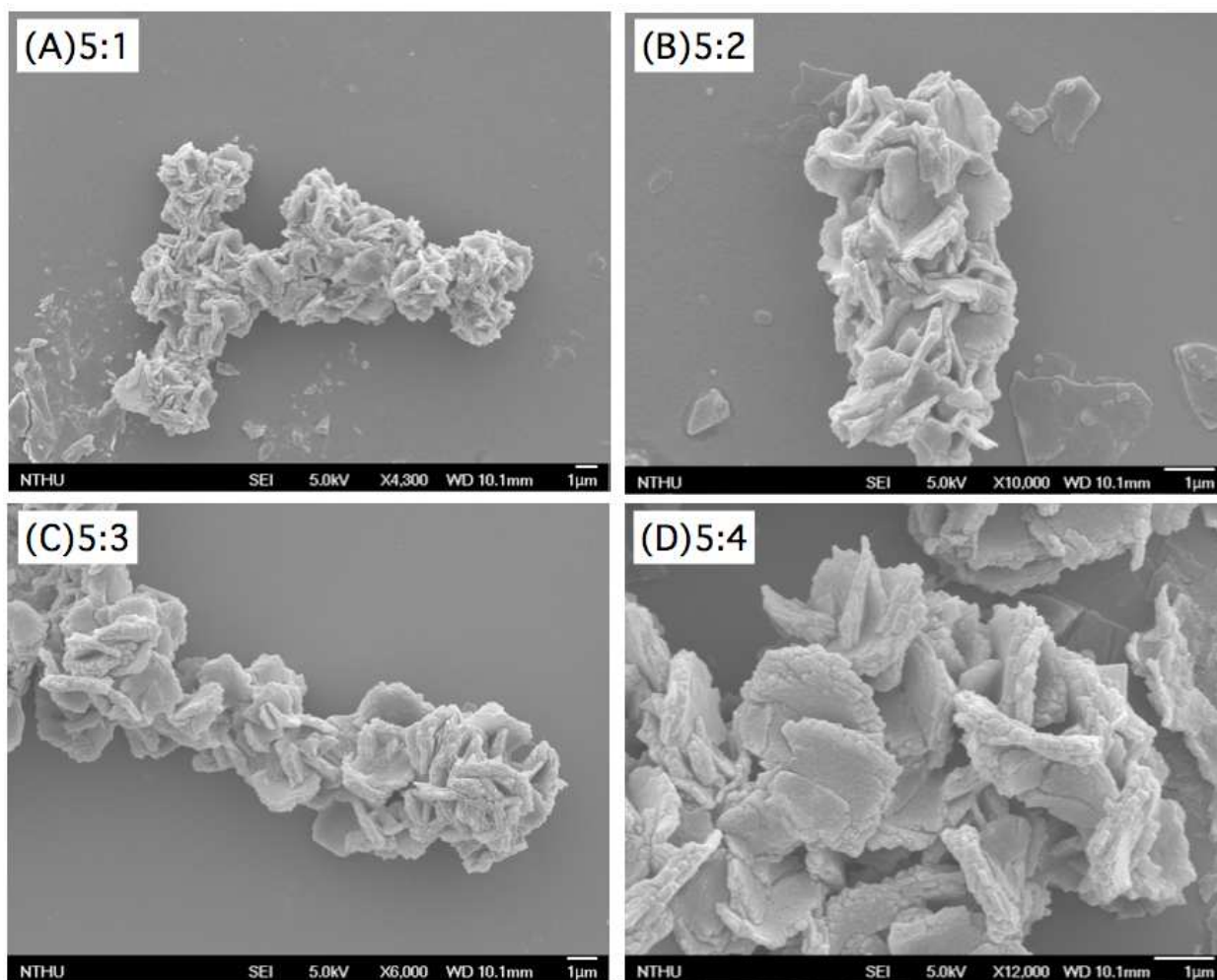


Figure S5. SEM images for the aggregates of 400 μM X9 after incubating with (A) 80 μM , (B) 160 μM , (C) 240 μM , and (D) 320 μM Cu^{2+} (the molar ratio of peptide to Cu^{2+} ranges from 5:1 to 5:4). Before taking SEM images, the samples were incubated in pH 7.4 20 mM phosphate buffer and at 4 $^{\circ}\text{C}$ for more than 4 d. The scale bar is 1 μm .

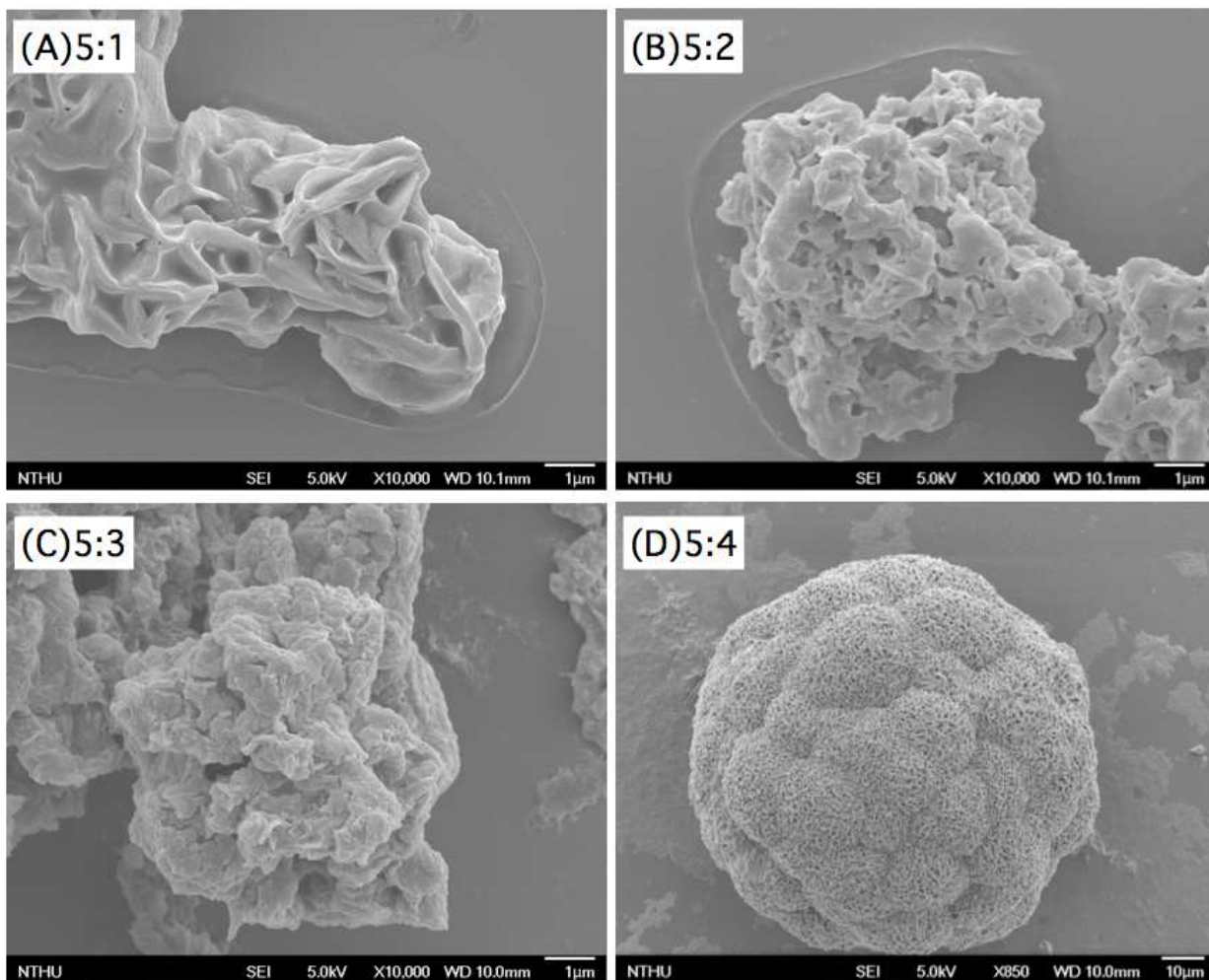


Figure S6. SEM images for the aggregates of 400 μM X9 after incubating with (A) 80 μM , (B) 160 μM , (C) 240 μM , and (D) 320 μM Zn^{2+} (the molar ratio of peptide to Zn^{2+} ranges from 5:1 to 5:4). Before taking SEM images, the samples were incubated in pH 7.4 20 mM phosphate buffer and at 4 $^{\circ}\text{C}$ for more than 4 d. The scale bar is 1 μm for panels (A), (B), and (C), and 10 μm for panel (D).

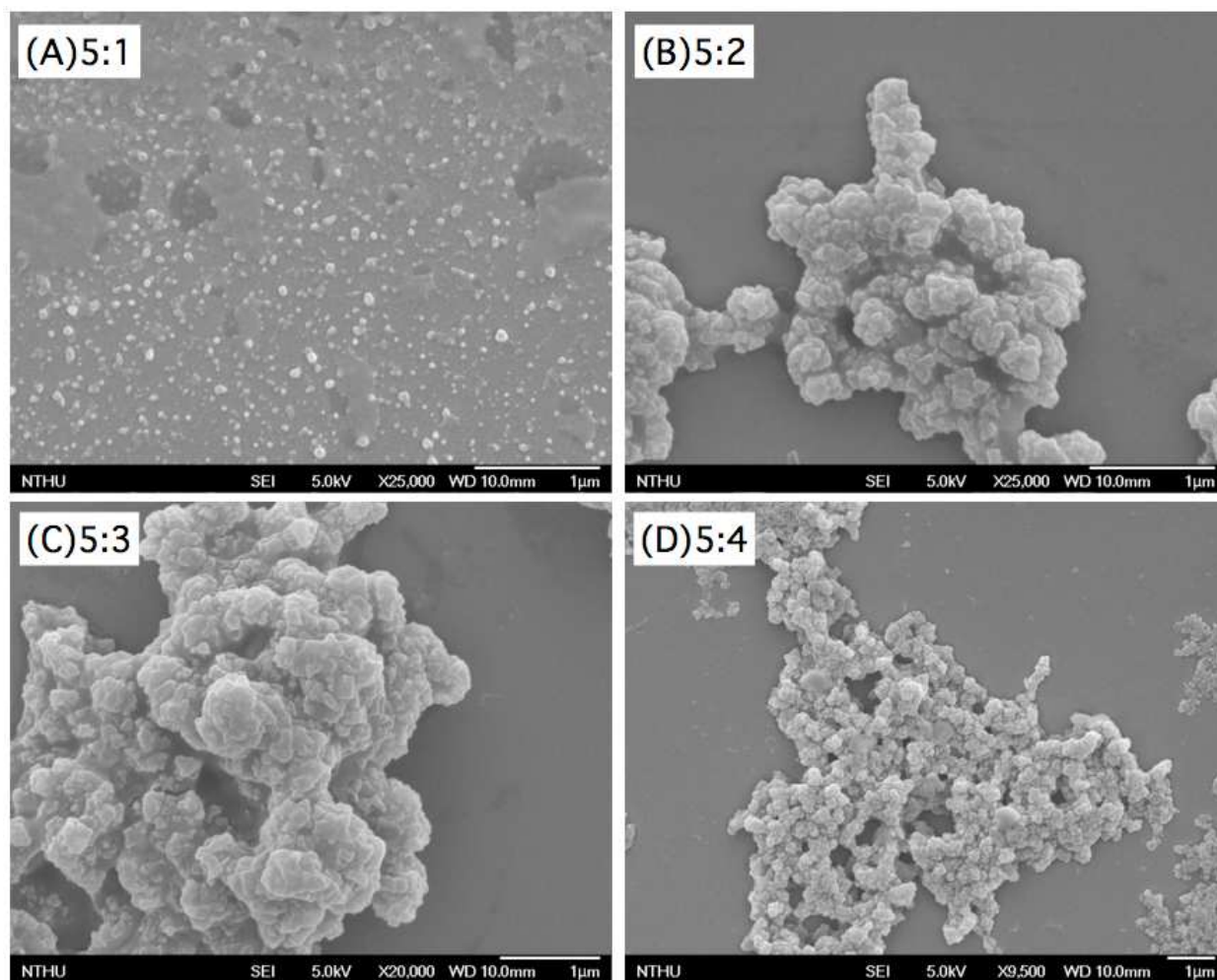


Figure S7. SEM images for the aggregates of 400 μM PHG after incubating with (A) 80 μM , (B) 160 μM , (C) 240 μM , and (D) 320 μM Cu^{2+} (the molar ratio of peptide to Cu^{2+} ranges from 5:1 to 5:4). Before taking SEM images, the samples were incubated in pH 7.4 20 mM phosphate buffer and at 4 $^{\circ}\text{C}$ for more than 4 d. The scale bar is 1 μm .

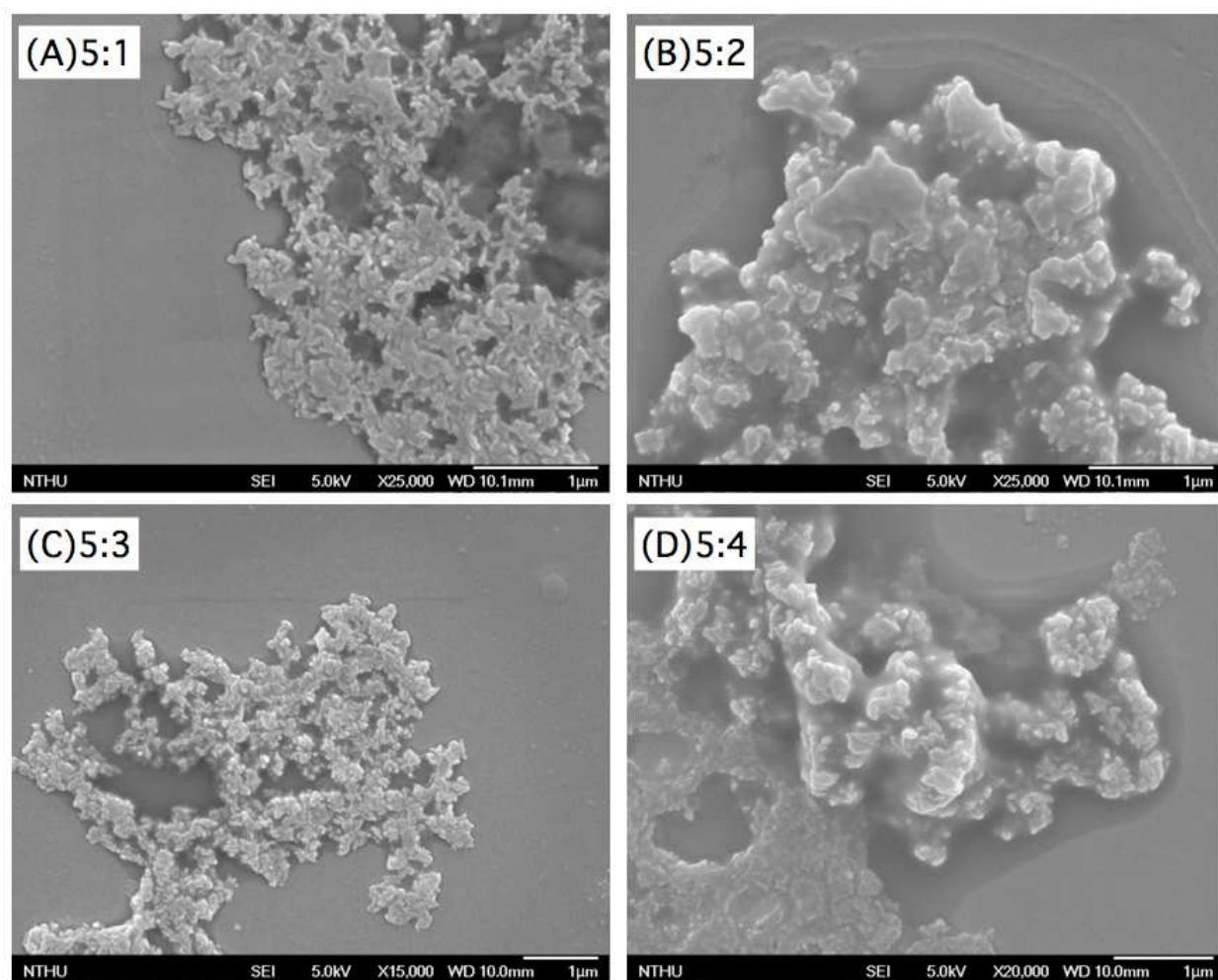


Figure S8. SEM images for the aggregates of 400 μM **PHG** after incubating with (A) 80 μM , (B) 160 μM , (C) 240 μM , and (D) 320 μM Zn^{2+} (the molar ratio of peptide to Zn^{2+} ranges from 5:1 to 5:4). Before taking SEM images, the samples were incubated in pH 7.4 20 mM phosphate buffer and at 4 $^{\circ}\text{C}$ for more than 4 d. The scale bar is 1 μm .

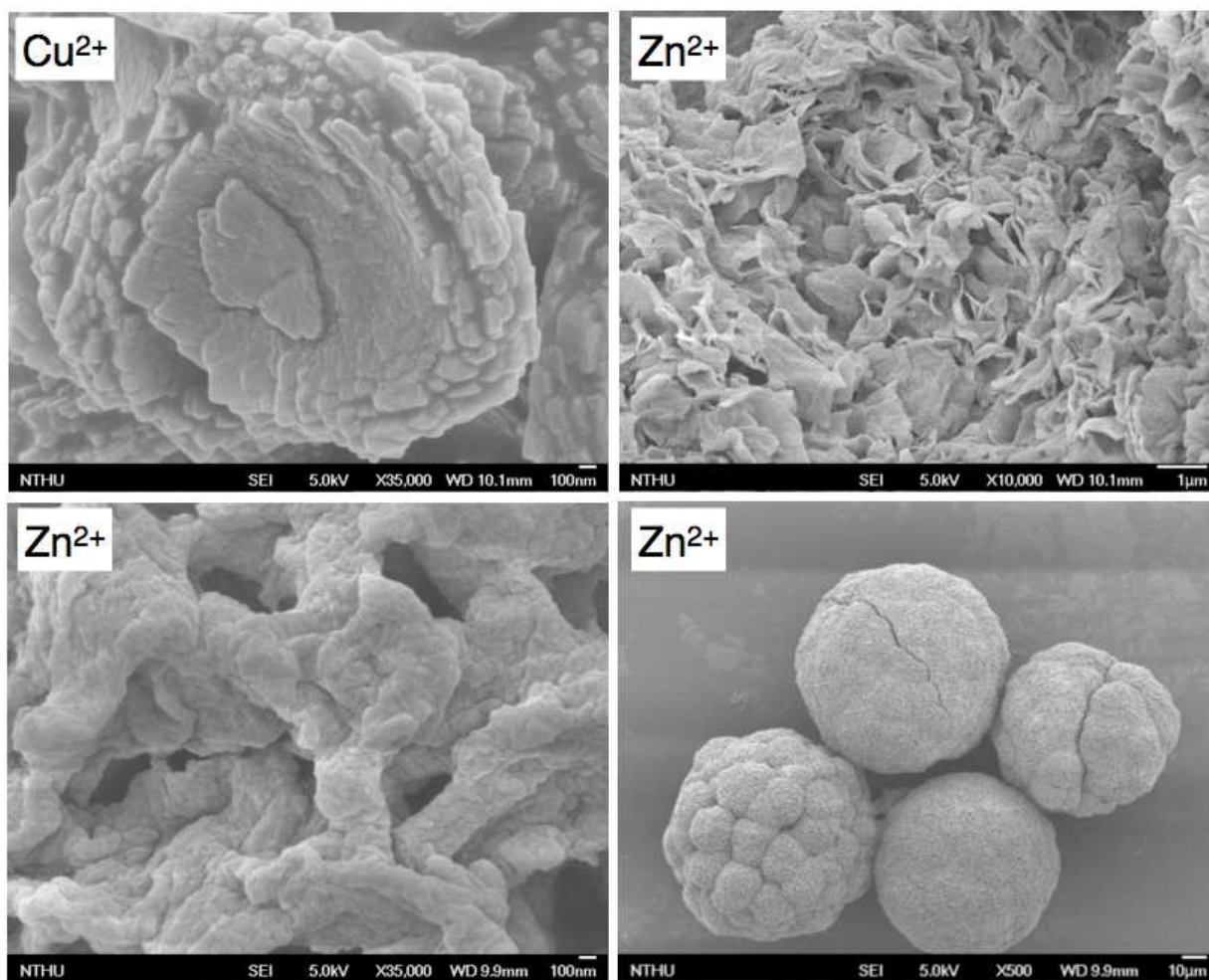


Figure S9. SEM images for the aggregates of 400 μM X9 after incubating with 400 μM Cu^{2+} or Zn^{2+} (molar ratio of peptide to metal 1:1). Before taking SEM images, the samples were incubated in pH 7.4 20 mM phosphate buffer and at 4 °C for more than 4 d. The scale bar is 100 nm in the left panels, 1 μm in the top right panel, and 10 μm in the bottom right panel.

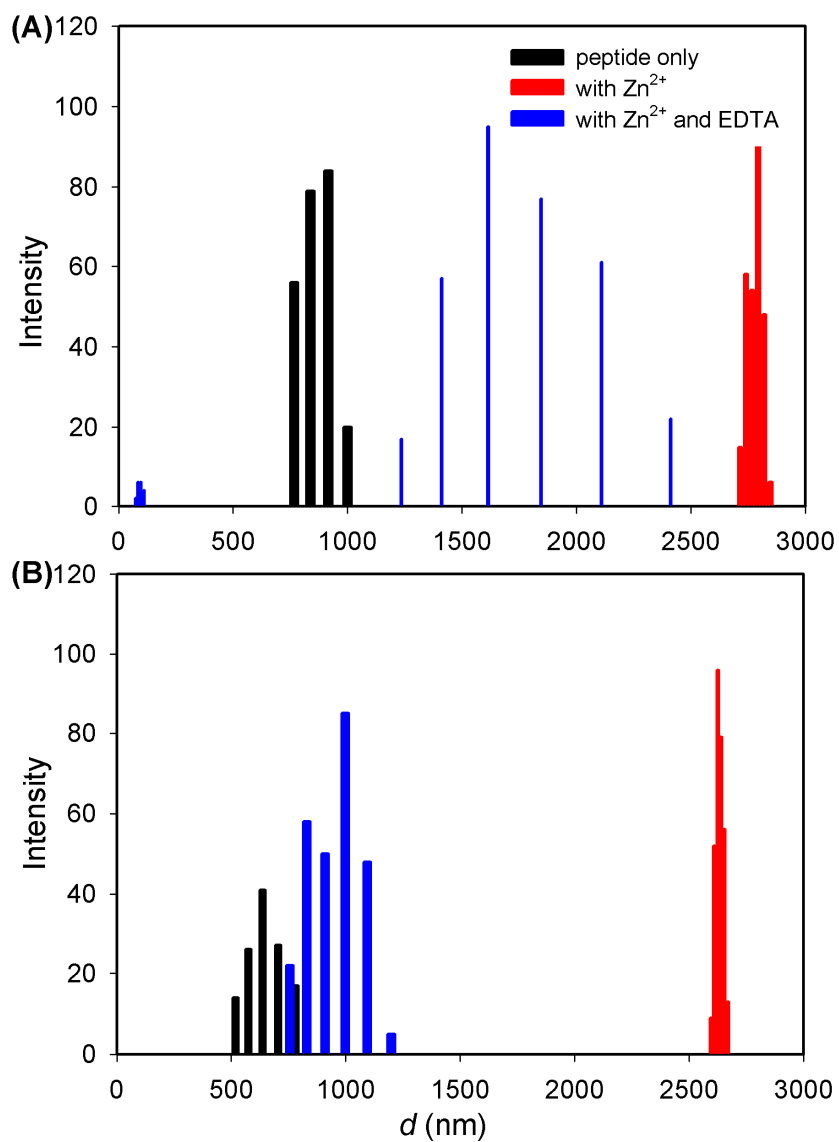


Figure S10. DLS analysis of chelation competition experiments for (A) **X9** and (B) **PHG**.

Hydrodynamic diameters (d) were measured using 400 μ M peptide and 160 μ M Zn^{2+} in pH 7.4 20 mM phosphate buffer followed the addition of 1.6 mM EDTA.