Electronic Supplementary Material (ESI) for Journal of Materials Chemistry B.
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Supporting Information

Self-assembly of a metallo-peptide into a drug delivery system using a ‘‘switch on’’ displacement strategy

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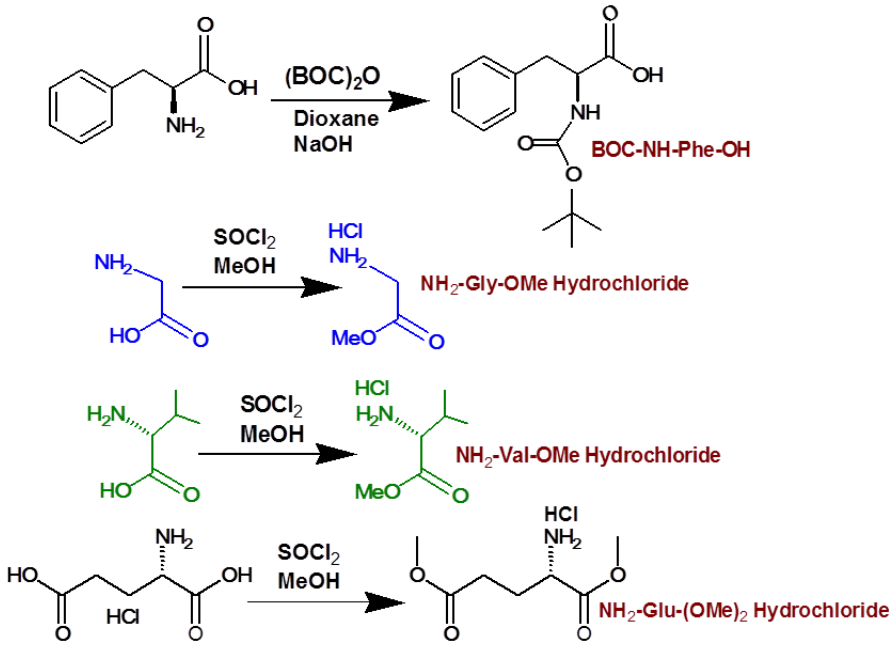
Experimental Section

Peptide synthesis: Peptides were synthesized by conventional solution-phase methods. Peptide coupling was mediated by dicyclohexylcarbodiimide/1-hydroxybenzotriazole (DCC/HOBt). The products were purified by column chromatography using silica gel (100–200 mesh) as the stationary phase and an n-hexane–ethyl acetate mixture as an eluent. The final compounds were fully characterized by Bruker 400 MHz ^{1}H-NMR spectroscopy and mass spectroscopy (Applied Biosystems Voyager-DE Pro MALDI-TOF and Accela Autosampler, Thermo Scientific (CCQ Fleet)).



Scheme S1: Synthetic methodologies adopted for the synthesis of BOC-protected phenylalanine and methyl esetr hydrochloride of glycine, valine, and glutamic acid.

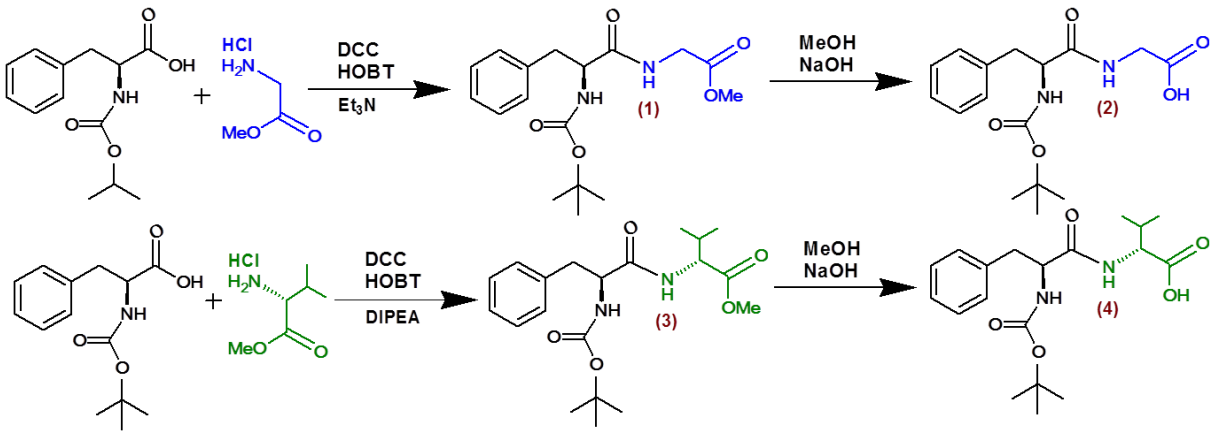
Synthesis of BOC-NH-Phe-OH: A solution of L-phenylalanine (4 g, 24.21 mmol) in a mixture of dioxane (45 mL), water (25 mL), and 1 M NaOH (25 mL) was stirred and cooled

in an ice-water bath. Di-tertbutylpyrocarbonate (5.46 g, 25 mmol) was added and stirred continuosly at room temperature (RT) for 6h. Then, the solution was concentrated using vacuum to about 10–15 mL, cooled in an ice-water bath, covered with a layer of ethyl acetate (about 50 mL), and acidified with a dilute solution of $KHSO_4$ to pH 2–3 (determined by congo red). The aqueous phase was extracted with ethyl acetate and this operation was performed repeatedly. The ethyl acetate extracts were pooled, washed with water, dried over anhydrous Na_2SO_4, and evaporated using vacuum. The pure material was obtained as a waxy solid. Yield 5.27 g (19.88 mmol, 82.14%) (Scheme S1).

Synthesis of NH_2-Gly-OMe Hydrochloride: 3.75g (50 mmol) of L-glycine was dissolved in 75 mL of MeOH and cooled in an ice bath. Then, 10 ml of $SOCl_2$ were added dropwise and stirred for 8h. The excess solvent was evaporated using a rotary vacuum. The dried crystalline solid product obtained was L-glycine methyl ester hydrochloride. Yield 5.10 g (40.8 mmol, 81.6%) (Scheme S1).

Synthesis of NH_2-Val-OMe hydrochloride: 3.515g (30 mmol) of L-valine were dissolved in 60 mL MeOH and cooled in an ice bath. Then, 10 mL of $SOCl_2$ were added dropwise and stirred for 8h. The excess solvent was evaporated under a rotary vacuum. The dried crystalline solid product obtained was L-valine methyl ester hydrochloride. Yield 4.26 g (25.44 mmol, 84.8%) (Scheme S1).

Synthesis of NH_2-Glu-$(OMe)_2$ Hydrochloride: 4.413g (30 mmol) of L-glutamic acid were dissolved in 45 mL MeOH and cooled in an ice bath. Then, 12 mL of $SOCl_2$ were added dropwise and stirred for 8h. The excess solvent was evaporated using a rotary vacuum. The dried crystalline solid product obtained was L-glutamic acid methyl ester hydrochloride. Yield 5.28 g (24.96 mmol, 83.2%) (Scheme S1).



Scheme S2: Synthetic methodologies adopted for the synthesis of compounds 1, 2, 3, and 4.

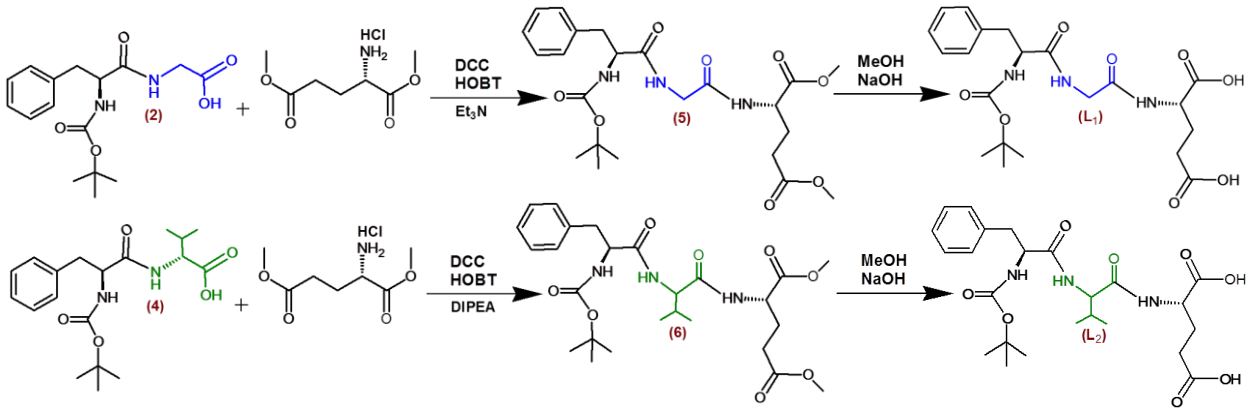
Synthesis of BOC-Phe-Gly-OMe (1): 2.0 g (7.5 mmol) of Boc-Phe-OH were dissolved in 40 mL dry DCM in an ice-water bath. NH_2-Gly-OMe.HCl 1.255 g (10.0 mmol) and Et_3N 2 ml, 15 mmol) were then added to the reaction mixture, followed immediately by the addition of 1.856 g (9.0 mmol) dicyclohexylcarbodiimide (DCC) and 1.216 g (9.0 mmol) of HOBt. The reaction mixture was allowed to warm-up to RT and was stirred for 48 h. DCM was evaporated and the residue was dissolved in ethyl acetate (45 mL). The dicyclohexylurea (DCU) was filtered off. The organic layer was washed with 2M HCl (3 X 50 mL), brine (2 X 50 mL), 1 M sodium carbonate (3 X 50 mL), and brine (2 X 50 mL), and finally dried over anhydrous sodium sulfate. It was then evaporated using vacuum to yield Boc-Phe-Gly-OMe as a white solid. The product was purified by silica gel (100–200 mesh) using n-hexane–ethyl acetate (3 : 1) as eluent. Yield: 1.87 g (5.58 mmol, 74.48%). ^{1}H NMR ($CDCl_3$, 400 MHz, δ_{ppm}): 7.31-7.28 (m, 2H, ArH of Phe), 7.23-7.20 (m, 3H, ArH of Phe), 6.52 (b, 1H, NH), 5.05 (b, 1H, NH), 4.42-4.41 (m, 1H, C_αH, Phe) 4.07-3.91 (dd, 2H, -CH_2-Gly), 3.73 (s, 3H, OMe), 3.14-3.05 (m, 2H, C_βH, Phe) 1.39 (s, 9H, Boc). ESI-MS(m/z): [M]=336.38 (calculated); 336.43 (observed), $[M+Na+H]^+$=360.38 (calculated); 360.23 (observed); $[M+K+H]^+$=376.38 (calculated); 376.42 (observed), $[M+2Na]^+$=382.38 (calculated); 381.31 (observed) (Scheme S2).

Synthesis of BOC-NH-Phe-Gly-OH (2): To 3.5 g (10.4 mmol) of Boc-Phe-Gly-OMe, 40 mL MeOH and 2M 15 mL NaOH were added and the progress of saponification was monitored by thin layer chromatography (TLC). The reaction mixture was stirred. After10h, the methanol was removed under vacuum; the residue was dissolved in 50 mL of water, and washed with diethyl ether (2 X 50 mL). Then, the pH of the aqueous layer was adjusted to 2 using 1M HCl and extracted with ethyl acetate (3 X 50 mL). The extracts were pooled, dried over anhydrous sodium sulfate, and evaporated under vacuum to obtain the compound as a waxy solid. Yield: 3.217 g (9.98 mmol, 96%). ^{1}H NMR (DMSO-d_6, 400 MHz, δ_{ppm}): 12.56 (s, 1H, COOH), 8.23 (t, 1H, NH),7.27-7.16 (m, 5H, ArH of Phe), 6.89 (d, 1H, NH), 4.23-4.17 (m, 1H, C_αH, Phe) 3.86-3.72 (m, 2H, -CH_2-Gly), 3.03-2.69 (m, C_βH, Phe) 1.28 (s, 9H, Boc). ESI-MS(m/z): $[M+Na+H]^+$=346.35 (calculated); 346.42 (observed); $[M+K+H]^+$ = 362.35 (calculated); 362.37 (observed) (Scheme S2).

Synthesis of BOC-Phe-Val-OMe (3): 2.0 g (7.5 mmol) of Boc-Phe-OH were dissolved in 40 mL dry DCM in an ice-water bath. NH_2-Val-OMe.Hcl 1.67 g (10.0 mmol) and N-methyl diisopropylethylamine (DIPEA) 2.56 ml (15 mmol) were then added to the reaction mixture, followed immediately by the addition of 1.856 g (9.0 mmol) dicyclohexylcarbodiimide (DCC) and 1.216 g (9.0 mmol) of HOBt. The reaction mixture was allowed to warm-up to RT and was stirred for 48 h. DCM was evaporated and the residue was dissolved in ethyl acetate (45 mL). The dicyclohexylurea (DCU) was filtered off. The organic layer was washed with 2M HCl (3 X 50 mL), brine (2 X 50 mL), 1 M sodium carbonate (3 X 50 mL), and brine (2 X 50 mL) and dried over anhydrous sodium sulfate. It was then evaporated under vacuum to yield Boc-Phe-Val-OMe as a white solid. The product was purified by silica gel (100–200 mesh) using n-hexane–ethyl acetate (3 : 1) as eluent. Yield: 2.11 g (5.37 mmol, 71.67%). ^{1}H NMR ($CDCl_3$, 400 MHz, δ_{ppm}): 7.31-7.27 (m, 2H, ArH of Phe), 7.26-7.20 (m, 3H, ArH of Phe), 6.34 (J =8.72 Hz, d, 1H, NH), 5.01 (b, 1H, NH), 4.47-4.44 (m, 1H, C_αH, Phe), 4.35-4.33 (m, 1H, Val), 3.69 (s, 3H, OMe), 3.08-3.05 (m, 2H, C_βH, Phe), 2.14-2.05 (m, 1H, Val) 1.41 (s, 9H,

Boc) 0.87-0.83 (dd, 6H, -$(CH_3)_2$). ESI-MS(m/z): $[M+2H]^+$=380.21 (calculated); 380.35 (observed) (Scheme S2).

Synthesis of BOC-NH-Phe-Val-OH (4): To 4 g (10.56 mmol) of Boc-Phe-Val-OMe, 40 mL MeOH and 2M 15 mL NaOH were added and the progress of saponification was monitored by thin layer chromatography (TLC). The reaction mixture was stirred. After 10h, the methanol was removed using vacuum; the residue was dissolved in 50 mL of water, and washed with diethyl ether (2 X 50 mL). Then, the pH of the aqueous layer was adjusted to 2 using 1M HCl and extracted with ethyl acetate (3 X 50 mL). The extracts were pooled, dried over anhydrous sodium sulfate, and evaporated under vacuum to obtain the compound as a white solid. Yield: 3.74 g (10.26 mmol, 97.23%). ^{1}H NMR ($CDCl_3$, 400 MHz, δ_{ppm}): 7.29-7.26 (m, 2H, ArH of Phe), 7.22-7.19 (m, 3H, ArH of Phe), 6.64 (d, *J* =8.3 Hz, d, 1H, NH), 5.22 (b, 1H, NH), 4.51-4.47 (m, 1H, C_αH, Phe), 4.43 (d, *J* =5.12 Hz, 1H, Val), 3.06 (b, 2H, C_βH, Phe), 2.23-2.15 (m, 1H, Val) 1.39 (s, 9H, Boc) 0.92-0.88 (dd, 6H, -$(CH_3)_2$). ESI-MS(m/z): $[M+K+H]^+$ = 404.16 (calculated); 404.72 (observed) (Scheme S2).



Scheme S3: Synthetic methodologies adapted for the synthesis of compounds 5, 6, $\mathbf{L_1}$, and $\mathbf{L_2}$.

Synthesis of BOC-NH-Phe- Gly-Glu-$(OMe)_2$ (5): 2.0 g (6.191 mmol) of Boc-NH-Phe-Gly-OH **(2)** were dissolved in 30 ml dry DCM in an ice-water bath. NH2-Glu-$(OMe)_2$

Hydrochloride 1.703g (8.048 mmol) and N-methyl diisopropylethylamine (DIPEA) 2.06 ml, (12.072 mmol) were then added to the reaction mixture, followed immediately by the addition of 1.532 g (7.429 mmol) dicyclohexylcarbodiimide (DCC), and 1.003 g (7.429 mmol) of HOBt. The reaction mixture was allowed to warm-up to RT and stirred for 48 h. DCM was evaporated and the residue was dissolved in ethyl acetate (60 mL) and the dicyclohexylurea (DCU) was filtered off. The organic layer was washed with 2M HCl (3 X 50 mL), brine (2 X 50 mL), 1 M sodium carbonate (3 X 50 mL) brine (2 X 50 mL), dried over anhydrous sodium sulfate, and finally evaporated under vacuum to yield **BOC-NH-Phe-Gly-Glu-(OMe)$_2$ (5)** as a white solid. The product was purified by silica gel (100–200 mesh) using n-hexane–ethyl acetate (3: 1) as eluent. Yield: 2.085 g (4.35 mmol, 70.35%). ^{1}H NMR ($CDCl_3$, 400 MHz, δ_{ppm}): 7.31-7.27 (m, 2H, ArH of Phe), 7.25-7.19 (m, 3H, ArH of Phe), 6.81 (b, 1H, NH), 5.18 (d, *J* =7.6 Hz, 1H, NH), 4.60-4.54 (m, 1H, C_αH, Phe), 4.39-4.34 (m, 2H), 4.12-4.06 (dd, 1H, Glu), 3.74 (s, 3H, -OMe), 3.67 (s, 3H, -OMe), 3.07-2.99 (m, 2H, C_βH, Phe), 2.43-2.39 (m, 2H, Glu), 2.24-2.16 (m, 2H, Glu), 1.37 (s, 9H, Boc). ESI-MS(m/z): $[M+Na+H]^+$=503.224 (calculated); 503.40 (observed); $[M+K+H]^+$ = 519.198 (calculated); 519.36 (observed) (Scheme S3).

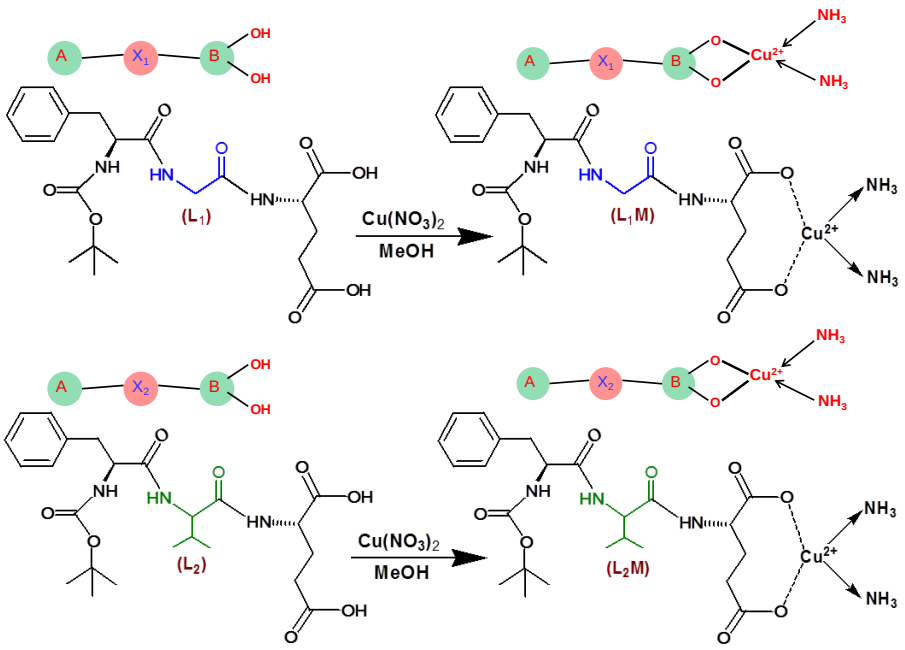
Synthesis of BOC-NH-Phe-Gly-Glu-(OH)$_2$ (L$_1$): To 2 g (4.17 mmol) of Boc-NH-Phe-Gly-Glu-(OMe)$_2$, 40 mL MeOH and 2M 10 mL NaOH were added and the progress of saponification was monitored by thin layer chromatography (TLC). The reaction mixture was stirred. After 10h, the methanol was removed using vacuum; the residue was dissolved in 50 mL of water, and washed with diethyl ether (2 X 50 mL). Then, the pH of the aqueous layer was adjusted to 2 using 1M HCl and extracted with ethyl acetate (3 X 50 mL). The extracts were pooled, dried over anhydrous sodium sulfate, and evaporated under vacuum to obtain the compound as a waxy like solid. Yield: 1.780 g (3.943 mmol, 94.56%). Elemental analysis calcd (%) for L$_1$ ($C_{21}H_{29}N_3O_8$): C 55.87, H 6.47, N 9.31, O 28.35; found: C 55.17, H 6.41, N

9.16. ^{1}H NMR (DMSO-d_6, 400 MHz, δ_{ppm}): 8.23-8.19 (m, 1H, NH), 8.02 (d, *J*= 7.52 Hz, 1H, NH),7.30-7.25 (m, 5H, ArH of Phe), 6.99 (d, *J*= 8.36 Hz, 1H, NH), 4.26-4.21 (m, 1H, C_αH, Phe), 4.19-4.14 (m, 1H, Glu), 3.80-3.73 (m, 2H, Gly), 3.03-2.99 (m, 2H, C_βH, Phe), 2.75-2.68 (m, 2H, Glu), 2.29-2.25 (m, 2H, Glu), 1.28 (s, 9H, Boc). ESI-MS(m/z): $[M]^+$=451.19 (calculated); 450.25 (observed) (Scheme S3).

Synthesis of BOC-NH-Phe-Val-Glu-(OMe)$_2$ (6): 3.0 g (8.241 mmol) of Boc-NH-Phe-Val-OH **(4)** were dissolved in 30 ml dry DCM in an ice-water bath. NH_2-Glu-(OMe)2 Hydrochloride 2.266 g (10.71 mmol) and N-methyl diisopropylethylamine (DIPEA) 2.74 ml, (16.06 mmol) were then added to the reaction mixture, followed immediately by the addition of 2.04 g (9.89 mmol) dicyclohexylcarbodiimide (DCC) and 1.336 g (9.89 mmol) of HOBt. The reaction mixture was allowed to warm-up to RT and stirred for 48 h. DCM was evaporated and the residue was dissolved in ethyl acetate (60 mL) and the dicyclohexylurea (DCU) was filtered off. The organic layer was washed with 2M HCl (3 X 50 mL), brine (2 X 50 mL), 1 M sodium carbonate (3 X 50 mL) brine (2 X 50 mL), dried over anhydrous sodium sulfate, and finally evaporated under vacuum to yield **BOC-NH-Phe-Val-Glu-(OMe)$_2$ (5)** as a white solid. The product was purified by silica gel (100–200 mesh) using n-hexane–ethyl acetate (: 1) as eluent. Yield: 2.97 g (5.71 mmol, 69.32%). ^{1}H NMR ($CDCl_3$, 400 MHz, δ_{ppm}): 7.31-7.27 (m, 2H, ArH of Phe), 7.25-7.20 (m, 3H, ArH of Phe), 7.10 (d, *J*= 7.6 Hz, 1H, NH), 6.80 (d, *J*= 8.4 Hz, 1H, NH), 5.19 (d, *J* =7.72 Hz, 1H, NH), 4.58-4.53 (m, 1H, C_αH, Phe), 4.43-4.41 (m, 1H), 3.74 (s, 3H, -OMe), 3.67 (s, 3H, -OMe), 3.14-3.03 (m, 2H, C_βH Phe) 2.46-2.33 (m, 2H), 2.25-2.18 (m, 2H), 2.06-1.99 (m, 1H), 1.39 (s, 9H, Boc), 0.93-0.88 (dd, 6H, -$(CH_3)_2$). ESI-MS(m/z): $[M+Na+H]^+$=545.26224 (calculated); 545.73 (observed); $[M+K+H]^+$ = 561.23 (calculated); 561.72 (observed) (Scheme S3).

Synthesis of BOC-NH-Phe-Val-Glu-(OH)$_2$ (L$_2$): To 2 g (3.83 mmol) of Boc-NH-Phe-Val-Glu-$(OMe)_2$, 40 mL MeOH, and 2M 10 mL NaOH were added and the progress of

saponification was monitored by thin layer chromatography (TLC). The reaction mixture was stirred. After 10h, the methanol was removed under vacuum; the residue was dissolved in 50 mL of water, and washed with diethyl ether (2 X 50 mL). Then, the pH of the aqueous layer was adjusted to 2 using 1M HCl and extracted with ethyl acetate (3 X 50 mL). The extracts were pooled, dried over anhydrous sodium sulfate, and evaporated under vacuum to obtain the compound as a powder-like solid. Yield: 1.74 g (3.52 mmol, 91.89%). Elemental analysis calcd (%) for L_2 ($C_{24}H_{35}N_3O_8$): C 58.40, H 7.15, N 8.51, O 25.93; found: C 57.57, H 7.08, N 8.37. ^{1}H NMR (DMSO-d_6, 400 MHz, δ_{ppm}): 12.56 (s, 2H, COOH), 8.27 (J = 7.28 Hz, d, 1H, NH), 7.73 (J = 8.96 Hz, d, 1H, NH), 7.26-7.17 (m, 5H, ArH of Phe), 7.03 (J = 8.64 Hz, d, 1H, NH), 4.27-4.22 (m, 1H, C_αH, Phe), 4.19-4.15 (m, 1H), 2.98-2.93 (m, 2H, C_βH, Phe), 2.76-2.70 (m, 1H), 2.31-2.26(m, 2H, Glu), 1.99-1.95 (m, 2H, Glu), 1.83-1.74 (m, 1H, Val), 1.29 (s, 9H, Boc), 0.89-0.84 (dd, 6H, -(CH_3)$_2$). ESI-MS(m/z): $[M+H]^{++}$= 494.24 (calculated); 494.08 (observed) (Scheme S3).



Scheme S4: Synthetic methodologies adapted for the synthesis of the metallo-peptides **L_1M** and **L_2M**.

Synthesis of L_1M/L_2M: The metal-peptide complexes (**L_1M** and **L_2M**) were prepared by dissolving peptides **L_1** (180 mg, 0.40 mmol) or **L_2** (1.97 mg, 0.40 mmol) in a 60/40 (v/v) ethanol/water mixture. The pH of the solution was adjusted to pH 8 with diluted aqueous ammonia. In a typical experiment, 0.5 mmol, 138.5 mg copper(II) nitrate dissolved in 0.5 mL of triple distilled water were added to the peptide solution at RT with stirring. After the copper salt addition, the pH was readjusted to 8. The copper peptide complexes (**L_1M** and **L_2M**) immediately precipitated. To complete the reaction, the reaction mixture was further reacted for 6 hours at RT. Then, the solids were centrifuged, washed with ethanol, and dried under vacuum overnight. All precipitates had a 1:1 metal/peptide stoichiometry confimed by mass spectrometry. Yields were above 95%. L_1M, L_2M corresponding to the dibasic form of **L_1** plus 1 Cu^{2+} plus 2 NH_3 plus 2 H_2O: Elemental analysis calcd (%) for L_1M ($C_{21}H_{37}CuN_5O_{10}$): C 43.26, H 6.40, Cu 10.90, N 12.01, O 27.44; found: C 44.09, H 6.02, N 10.97. Elemental analysis calcd (%) for L_2M ($C_{24}H_{43}CuN_5O_{10}$): C 46.11, H 6.93, Cu 10.16, N 11.20, O 25.59; found: C 46.97, H 6.52, N 10.22. ESI-MS (m/z) for **L_1M**: [**L_1-Cu^{2+}**+2H]$^+$= 514.13 (calculated); 514.58 (observed) (Scheme S4). ESI-MS(m/z) for **L_2M**: [**L_2-Cu^{2+}**+H]$^+$= 557.18 (calculated); 557.87 (observed) (Scheme S4).

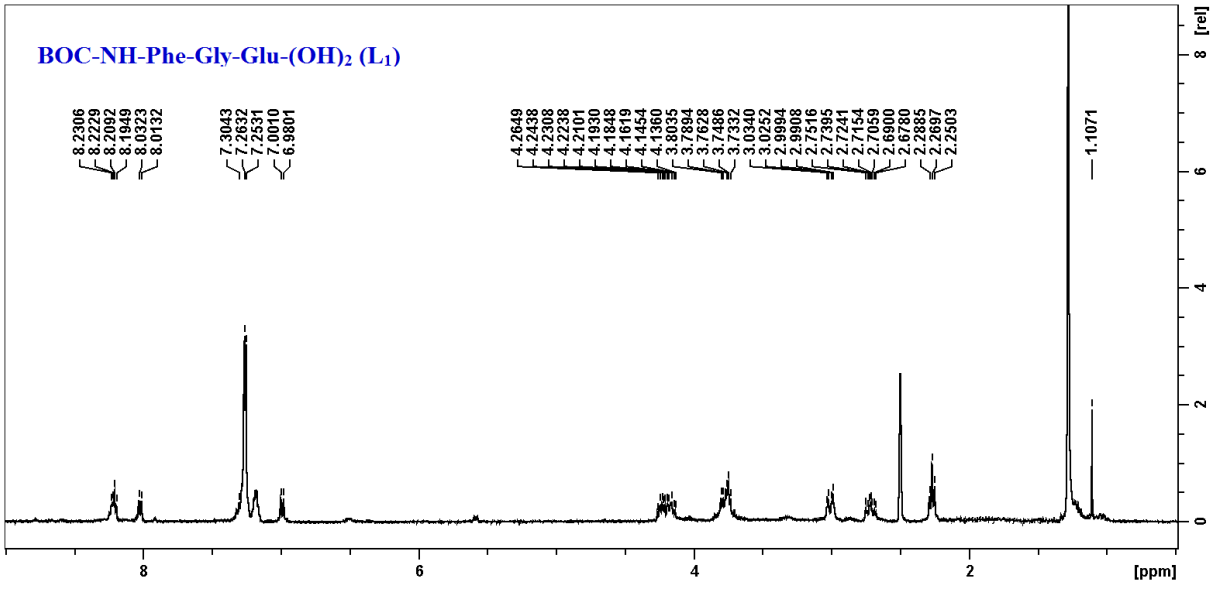


Figure S1. ^{1}H NMR (DMSO(d^{6}), 400 MHz, δppm) of Boc-NH-Phe-Gly-Glu-(OH)$_2$ (**L$_1$**).

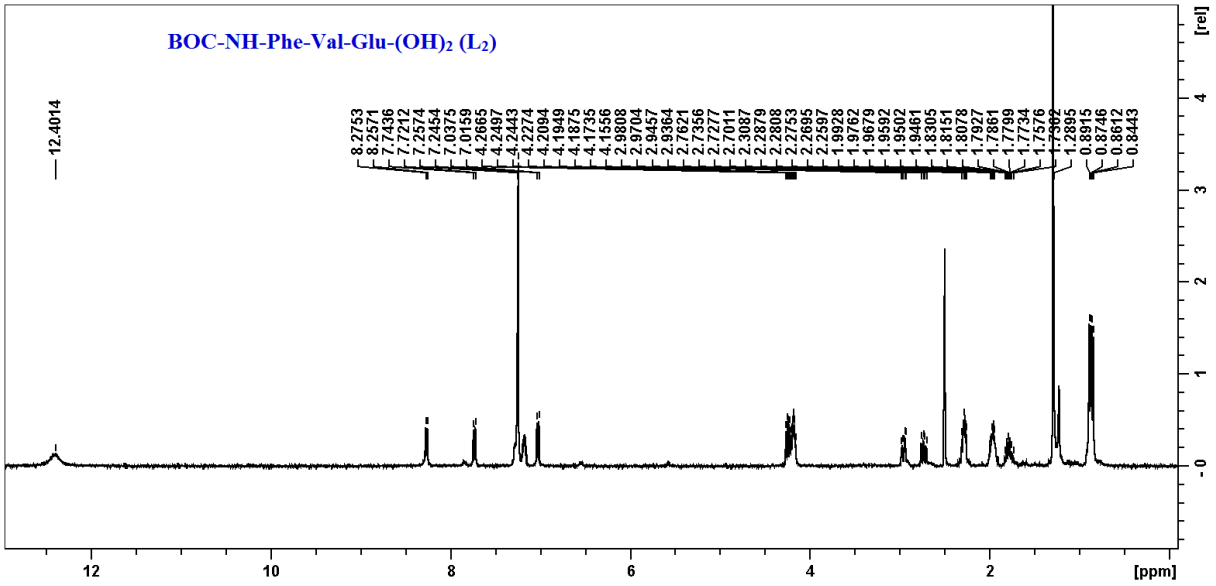


Figure S2. ^{1}H NMR (CDCl$_3$, 400 MHz, δppm) of Boc-NH-Phe-Val-Glu-(OH)$_2$ (**L$_2$**).

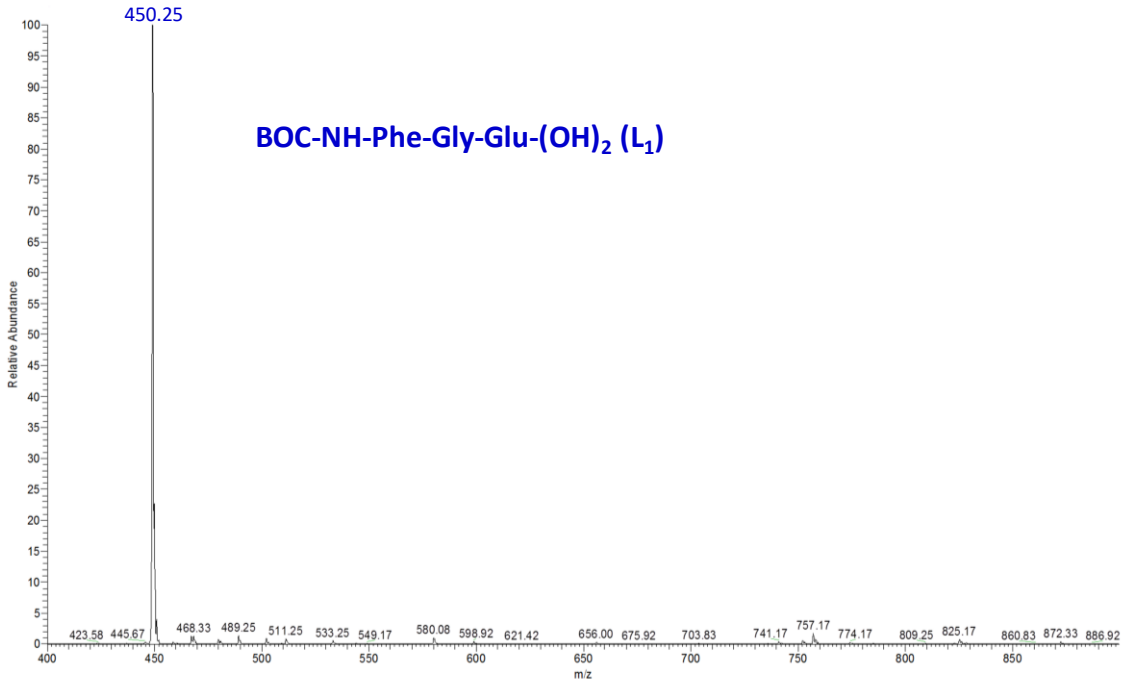


Figure S3. ESI Mass spectra of Boc-NH-Phe-Gly-Glu-(OH)$_2$ (**L$_1$**).

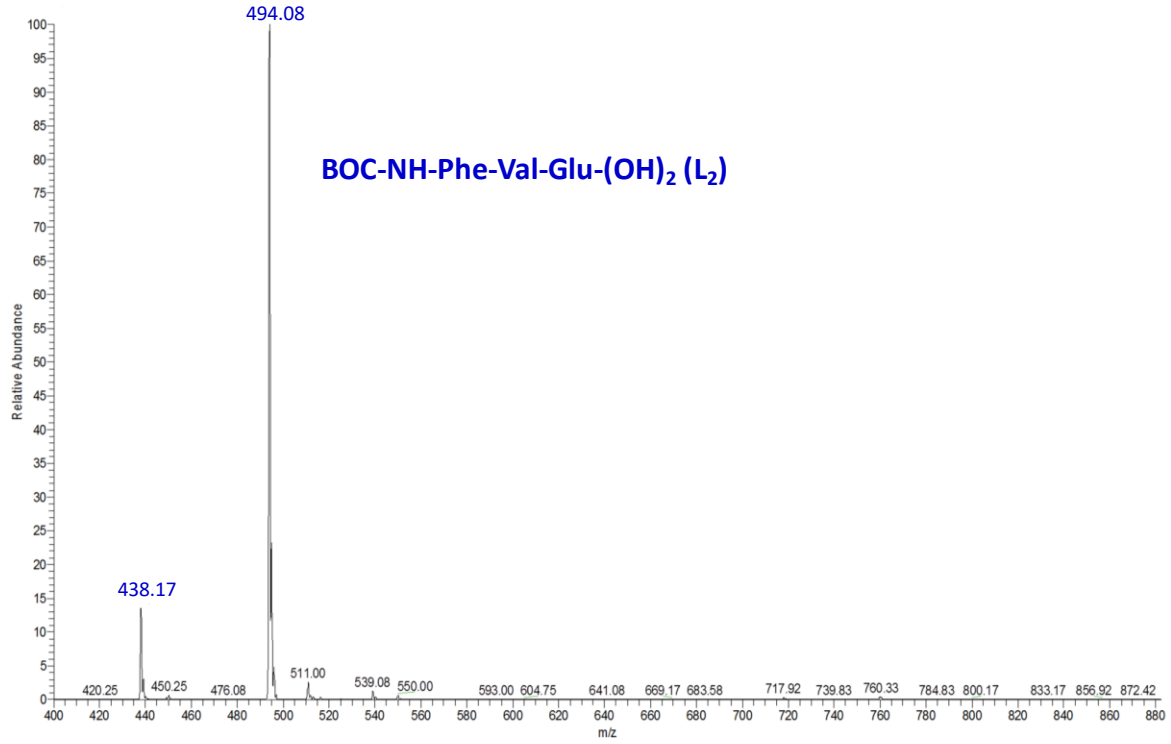


Figure S4. ESI Mass spectra of Boc-NH-Phe-Val-Glu-$(OH)_2$ (**L_2**).

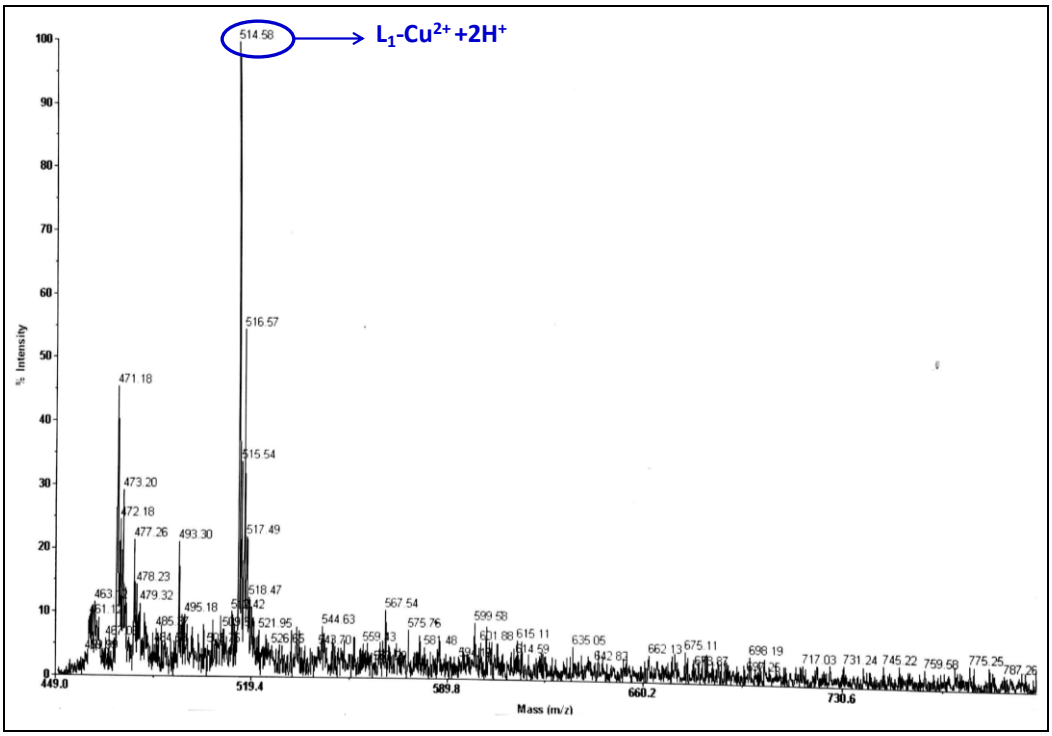


Figure S5. ESI Mass spectra of **L_1M**.

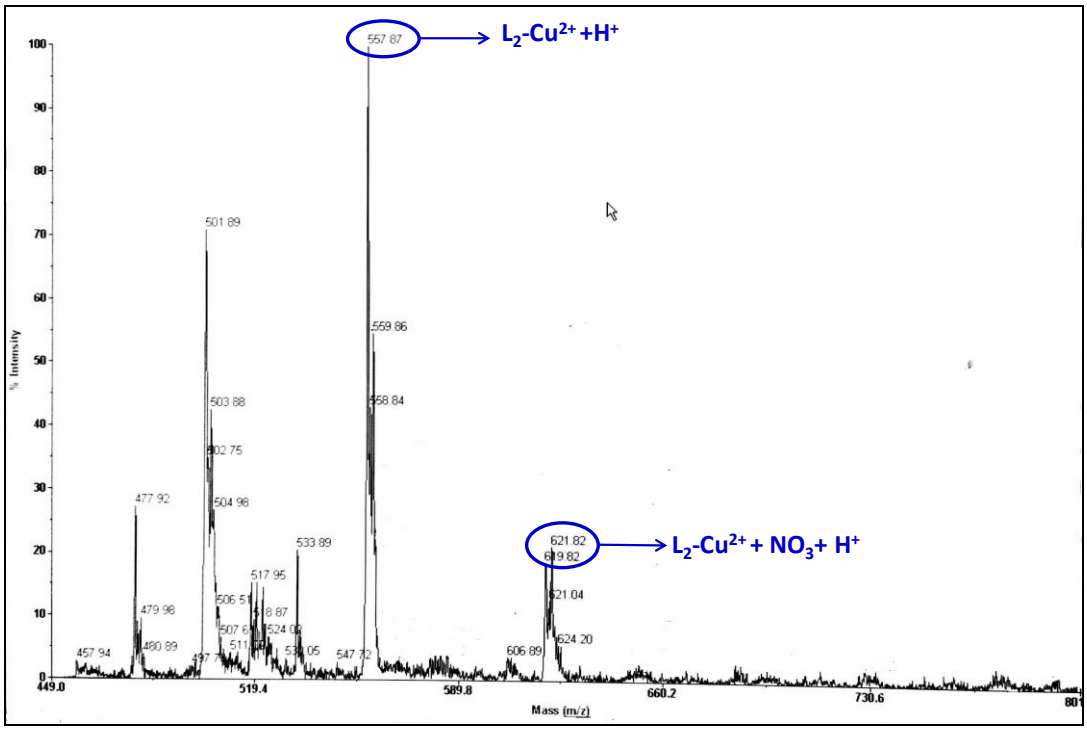


Figure S6. ESI Mass spectra of **L_2M**.

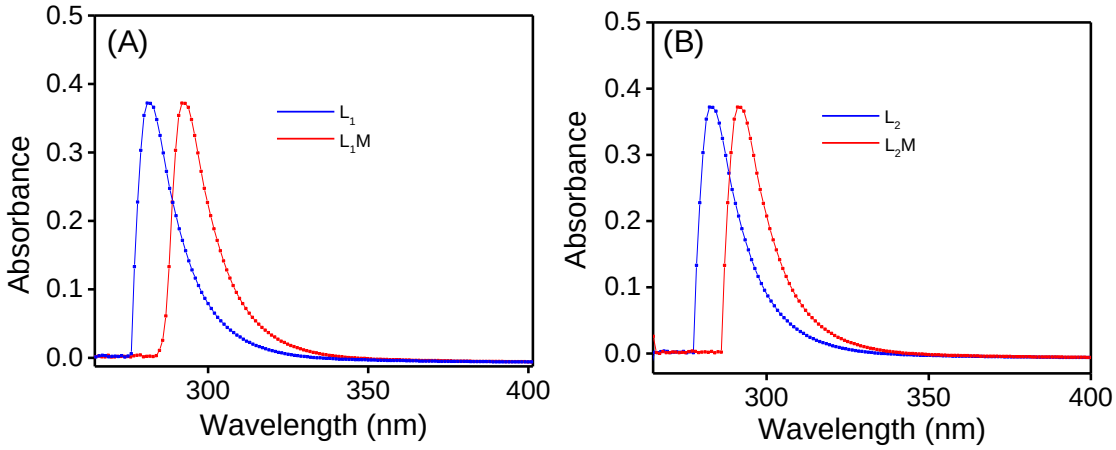


Figure S7. UV-Vis absorbance spectra of **L_1, L_2** (blue line) and **L_1M, L_2M** (red line)

Difference in hydrophobicity between glycine (Gly) and valine (Val) in a different hydrophobicity scale: Table S1

Amino Acid	Kyte-Dolytel Hydrophobicity	hhHydrophobicity	Eisenberg and Weiss
Glycine	-0.4	0.74	0.16
Valine	4.2	-0.31	0.54

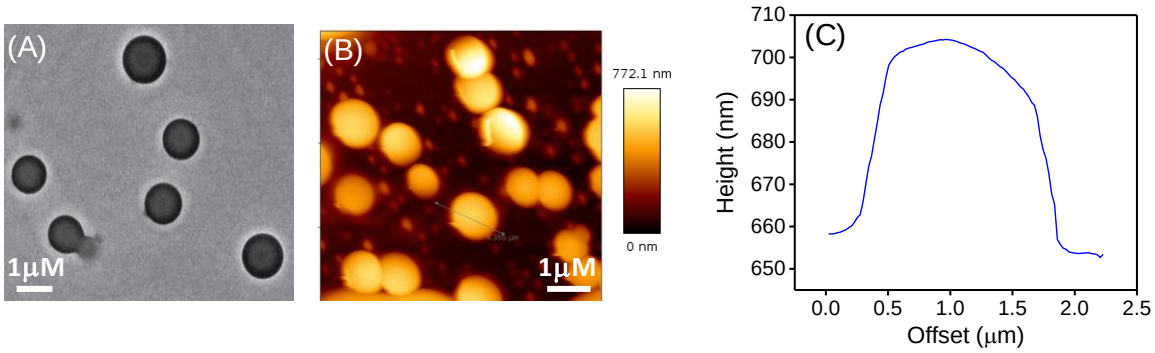


Figure S8. (A) TEM micrograph of self-assembled structures formed by $\mathbf{L_2}$ in water. AFM topographic analysis: (B) Two-dimensional representation and (C) height analysis of the self-assembled structures formed by $\mathbf{L_2}$ in water.

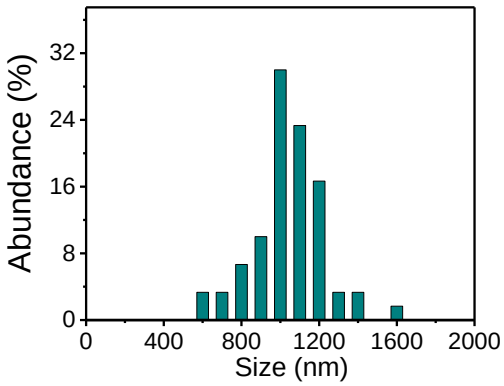


Figure S9. Size distribution obtained from DLS measurements for the spherical particles formed by $\mathbf{L_2}$ in water.

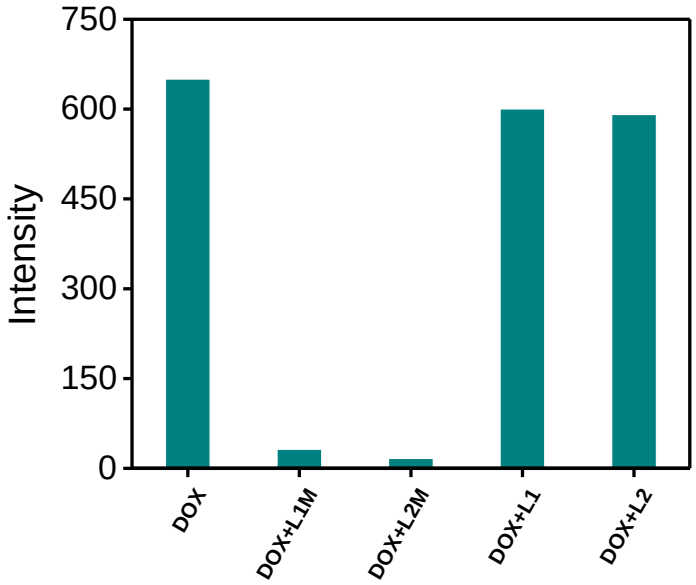


Figure S10. Fluorescence quenching of DOX (10 μM) in the presence of different analytes (λ_{Ext} = 490 nm, λ_{Ext} = 590 nm): Only DOX in tris-HCl buffer (pH =7.2); DOX in the presence of self-assembled **L_1M** (22.03 X 10^{-5}M); DOX in the presence of self-assembled **L_2M** (20.62 X 10^{-5}M); DOX with self-assembled L_1 (33.2 X 10^{-5}M); DOX with self-assembled **L_2** (30.35 X 10^{-5}M).

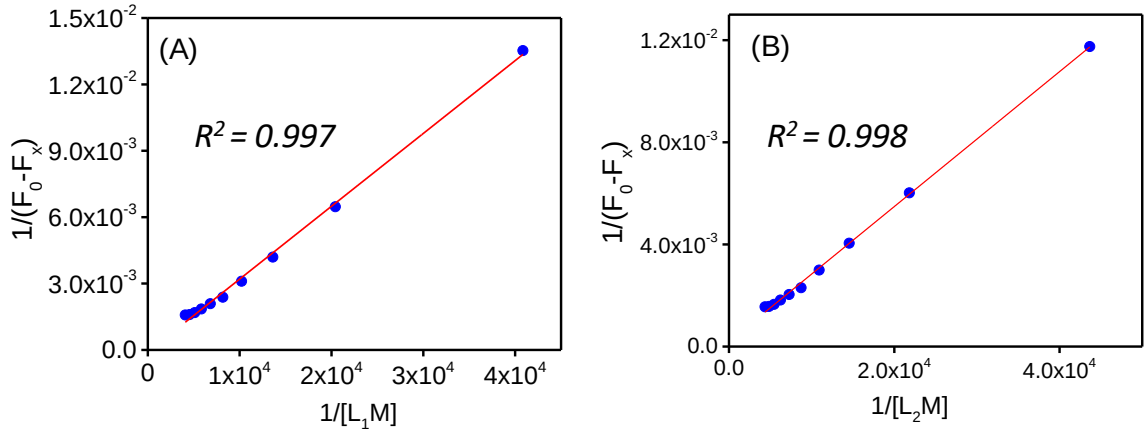


Figure S11. Benesi-Hildebrand plot for evaluating the binding constant and stoichiometry for the formation of the **L_1M**/ **L_2M** -DOX complex. λ_{ext} = 490 nm and λ_{mon} = 590 nm were used for emission studies. The fit of the plot confirms the 1:1 binding stoichiometry.

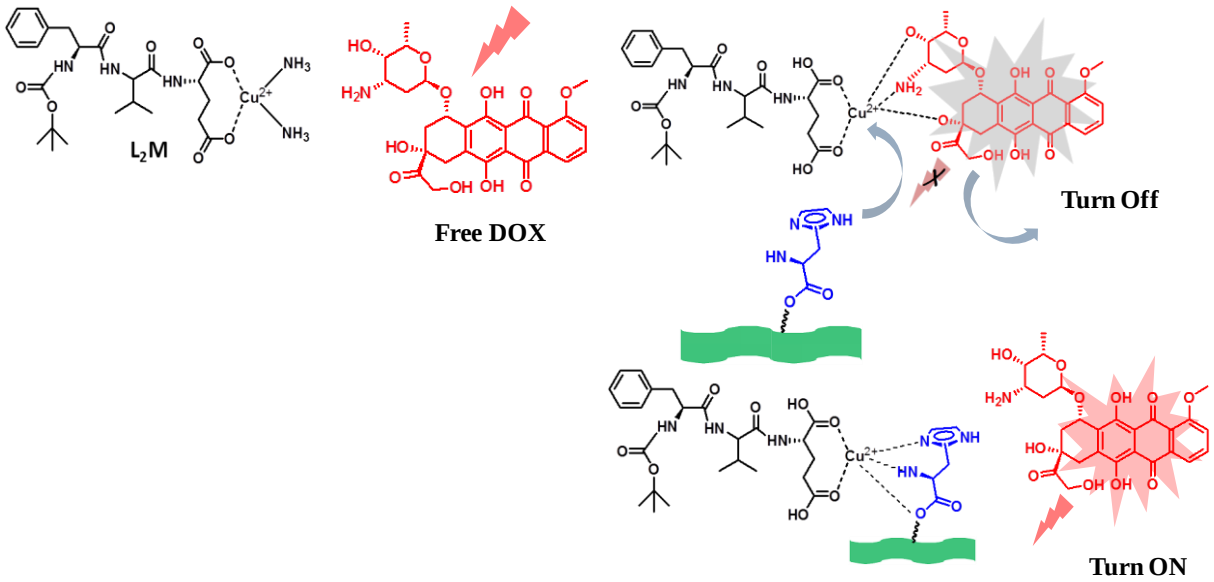


Figure S12. Schematic representation of the drug displacement assay.

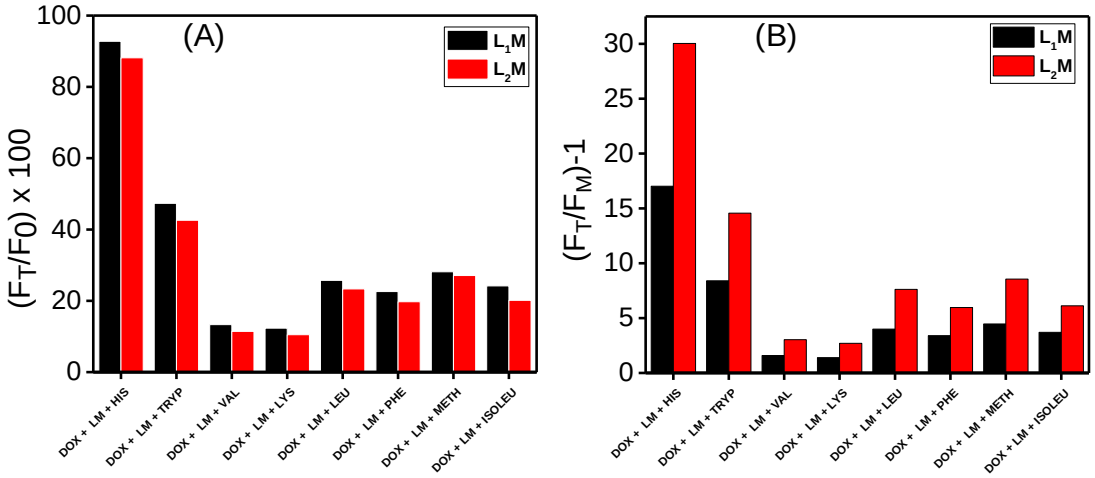


Figure S13. Percentage of fluorescence recovery [(F_T/F_0) X 100] (%) of DOX during the drug displacement process using different essential amino acids. F_0 and F_T are the emission intensities of free DOX and the **L_1M/L_2M**–DOX conjugate in the presence of different essential amino acids at 590 nm, λ_{Ext} = 490 nm; (B) Fluorescence intensity changes (F_T/F_M − 1) of the **L_1M/L_2M**–DOX conjugate in the presence of different essential amino acids; F_M and F_T are the emission intensities of **LM**–DOX in the absence and presence of different essential amino acids. λ_{Ext} = 490 nm, and λ_{Mon} = 590 nm.

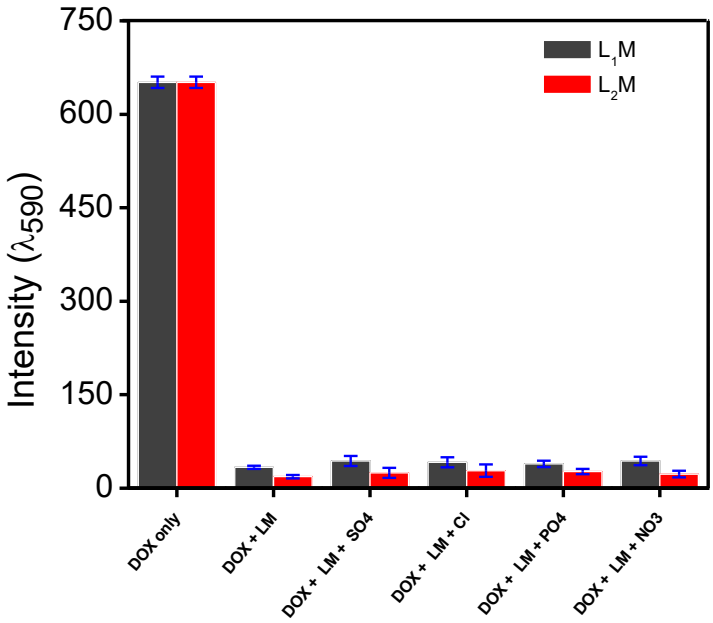


Figure S14. The emission quenching of DOX by metallo-peptides (L_1M and L_2M) in the presence and absence of several interfering anions. λ_{Ext} = 490 nm, and λ_{Mon} = 590 nm.

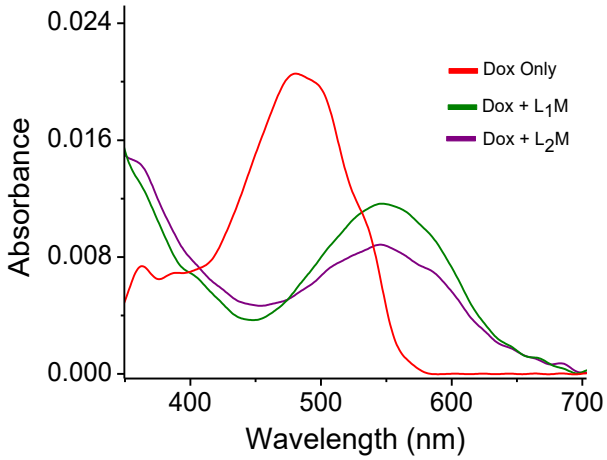


Figure S15. UV-Vis absorbance spectra of DOX (10 μM) in absence and presence **L_1M** (22.03 X 10^{-5}M) and **L_2M** (20.61 X 10^{-5}M)

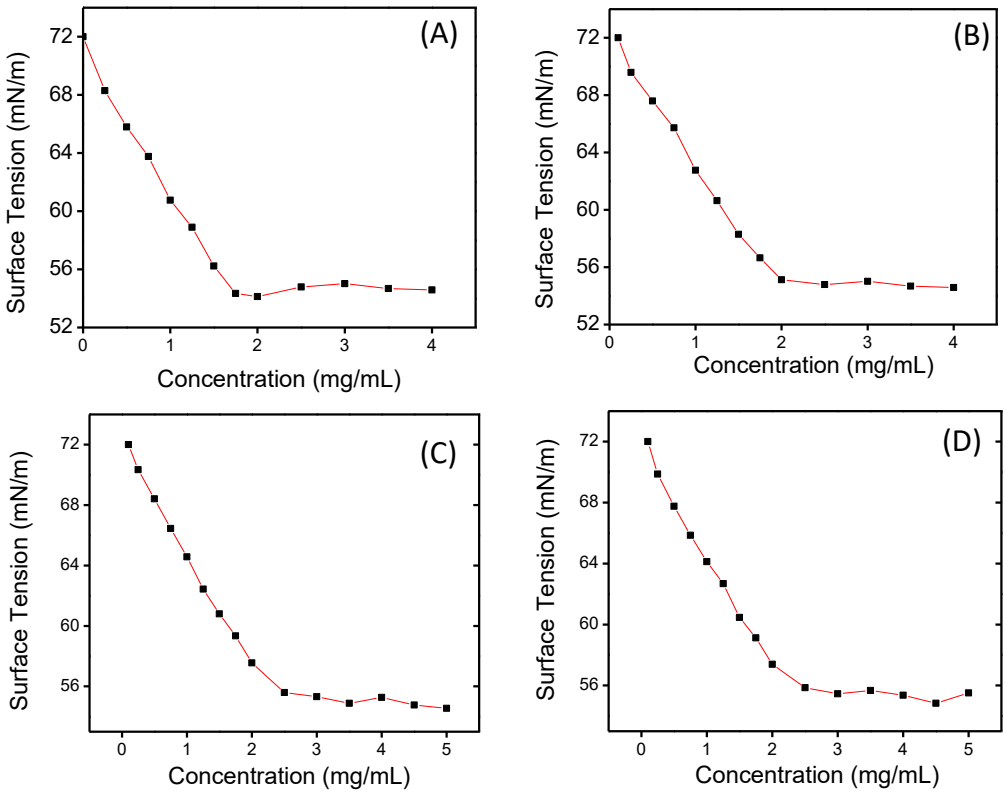


Figure S16. Determination of critical aggregation concentrations (Cac) for L_1 and L_2 and their corresponding metal conjugates (**L_1M** and **L_2M**). Relationship between the surface tension and concentration of (A) **L_1**, (B) **L_2**, (C) **L_1M** and (D) **L_2M** in water solutions.

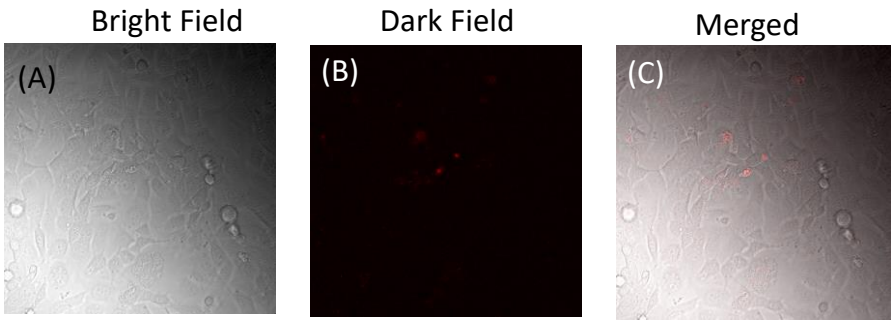


Figure S17. (A) Bright-field transmission image of N2aM cells incubated with Only DOX; (B) Dark field fluorescence image of N2aM cells incubated with Only DOX (from the red channel 590 ± 10 nm); (C) Merging of bright and dark-field transmission images of N2aM cells incubated with only DOX .

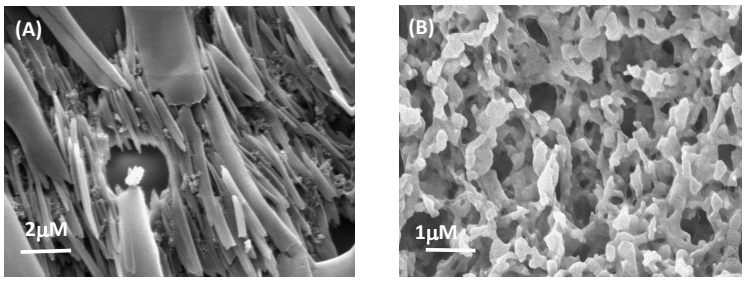


Figure S18. HR-SEM micrographs of the self-assembled structures formed by (A) **L_1M** (B), **L_2M** in blood serum (pH maintained at 7.2; physiological pH).

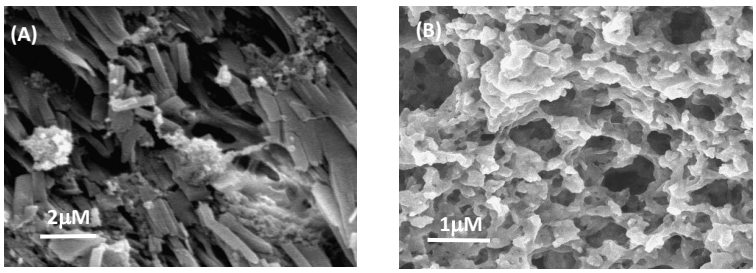


Figure S19 HR-SEM micrographs of the self-assembled structures formed by (A) **L_1M** (B), **L_2M** in presence of DOX in aqueous medium).

MTT assay for assessing the cytotoxicity of the metallo-peptides L_1M and L_2M towards N2aM cells: The *in-vitro* cytotoxicity of **L_1M** and **L_2M** to murine neuroblastoma cells (N2a M) was determined by the conventional MTT (3-(4, 5-Dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide, a yellow tetrazole) assay. N2a M cells in their exponential growth phase were trypsinized and seeded in 96-well flat-bottom culture plates at a density of 3 x 10^3 cells per well in 100 μL DMEM complete medium (Biological Industries, Beit Haemek, Israel). The cells were allowed to adhere and grow for 24h at 37ºC in a 5% CO_2 incubator and then the medium was replaced with 100 μL fresh incomplete medium containing various concentrations of **L_1M** and **L_2M** (0 to 30 μM). Next, the assay was performed in quadruplet for each concentration. Cells were then incubated for 12h, after which the culture medium was removed and 100 μL of 1 mg/mL MTT reagent in PBS was added to each well. Thereafter, it was incubated for 4h; during this period active mitochondria of viable cells reduce MTT to purple formazan. Unreduced MTT was then discarded and DMSO (100 μL) was added into each well to dissolve the formazan precipitate, which was then measured spectrophotometrically using a microplate reader (Biorad, USA) at 570 nm. The cytotoxic effect of each treatment was expressed as the percentage of cell viability relative to the untreated control cells. The following formula was used to calculate the viability of cell growth. Cell viability (%) = (means of absorbance value of treated group/ means of Absorbance value of untreated control) X 100.