Supplemental Information

Transporter protein and drug-conjugated gold nanoparticles capable of bypassing the blood-brain barrier

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1. Nanoconjugate chemical composition calculations

Calculations of nanoconjugate drug and protein loading amounts based on TGA and TEM results are shown below.

The weight of one molar AuNP (W) with a diameter (D = 4 nm based on the TEM measurements) was estimated using the following formula using the bulk density of gold (ρ = 19.3 g/cm^3):

$$W = \rho \times \frac{1}{6}\pi D^3 \times 6.022\times10^{23} = 19.3\frac{g}{cm^3} \times \frac{1}{6}\pi\left(4\times10^{-7}cm\right)^3 \times 6.022\times10^{23} = 3.89\times10^5\, g/mol\,.$$

The molecular weight of MSA ($C_4H_6O_4S$) and pro-THP are 150 g/mol and 210 g/mol, respectively. The weight loss, 100:6.6 = AuNP:MSA, corresponds to 171 MSA molecules per AuNP as calculated below. As a result, each MSA-AuNP has 342 maximum reactive sites.

$$\text{No. of MSA molecules} = \frac{6.6}{150} \times \frac{3.89 \times 10^5}{100} = 171.16 .$$

The area of MSA coverage on AuNP (A) is calculated below:

$$A = \frac{\pi D^2}{\text{No. of MSA molecules}} = \frac{\pi (4nm)^2}{171.16} = 0.294 nm^2 = 29.4 \text{Å}^2 .$$

The weight loss of 15.0% (AuNP : MSA : pro-THP = 100 : 6.6 : 11.1) shows that 205.6 pro-THP molecules are conjugated to one AuNP as calculated below:

$$\text{No. of pro-THP molecules} = \frac{11.1}{210} \times \frac{3.89 \times 10^5}{100} = 205.6 .$$

This means that 60% of all possible reactive sites provided by MSA are occupied by pro-THP.

The total weight loss for the three-part nanoconjugate is 21.6% (AuNP : MSA : pro-THP : WGA-HRP = 100 : 6.6 : 11.1 : 11.5) indicates that each WGA-HRP is conjugated to 2 AuNPs as calculated below:

$$\text{No. of WGA-HRP molecules} = \frac{11.5}{22367} \times \frac{3.89 \times 10^5}{100} \approx 2 .$$

For pro-DPCPX drug (TGA spectra shown in Supplementary Fig. 4), the weight loss of 12.9% (AuNP : MSA : pro-DPCPX = 100 : 6.6 : 8.2) corresponds to 96 pro-DPCPX molecules conjugated to one MSA-capped AuNP. This means that 28% of MSA reactive sites are occupied by pro-DPCPX as calculated below:

$$\text{No. of pro-DPCPX molecules} = \frac{8.2}{332} \times \frac{3.89 \times 10^5}{100} = 96 .$$

The total weight loss for the three-part nanoconjugate is 21.0% (AuNP : MSA : pro-DPCPX : WGA-HRP = 100 : 6.6 : 8.2 : 11.8) indicates that each WGA-HRP is conjugated to 2 AuNPs as calculated below:

$$\text{No. of WGA-HRP molecules} = \frac{11.8}{22367} \times \frac{3.89 \times 10^5}{100} \approx 2 .$$

2. Synthesis and characterization of DPCPX three-part nanoparticles

The synthesis steps of the three-part DPCPX nanoconjugate are similar to those of THP as illustrated in Fig. 1. DPCPX was converted to its pro-drug form, pro-DPCPX, using the Mannich reaction in order to conjugate DPCPX to MSA-capped AuNP with an ester bond linkage. The ester bond enables the hydrolytic release of DPCPX from its nano-carrier. In a typical reaction, 100 mg DPCPX was added to 1 ml 37% formaldehyde (in water), followed by the addition of 1.5 ml Et_3N with vigorous stirring. After the mixture became homogeneous, 2 ml THF was added. The reaction proceeded for 2 days in quiescent conditions to allow crystallization of pro-DPCPX. The pro-DPCPX crystals were dried and then dissolved in 20 mL DMSO to reach a final concentration of 5 mg/ml.

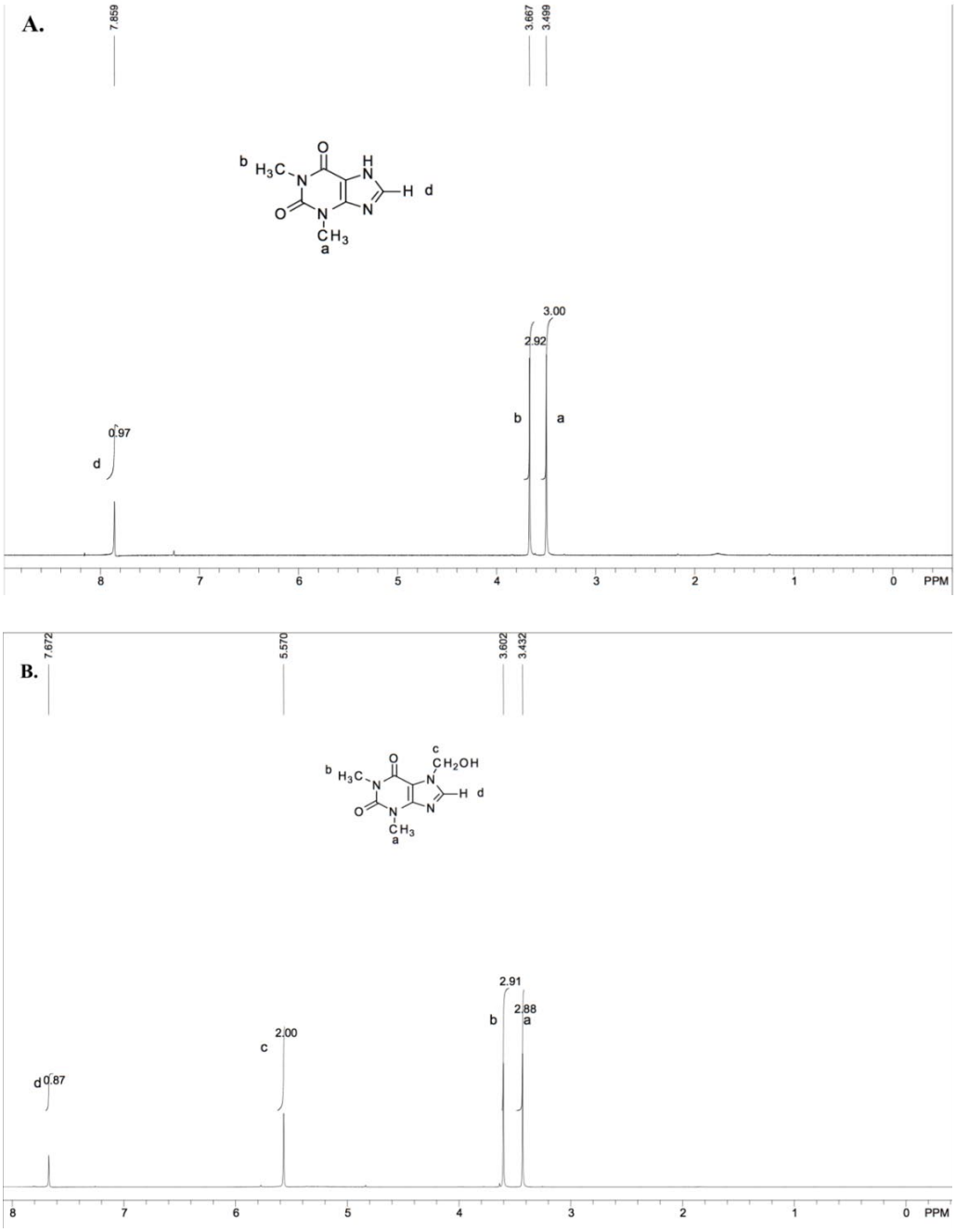
MSA-capped AuNP was concentrated to 5 mg(gold)/ml in 5 ml 0.1 M MES buffer. The pH value was adjusted to 4.7 using 0.1 M MES buffer to favor the ester bond formation. MES was used instead of acetic acid because acetic acid can react with the solvent. One ml of 5 mg/ml pro-DPCPX in DMSO solution was added to 5 ml DMSO containing 4.8 mg EDC and 1.7 ml of DMAP to start the Steglich Reaction. After 40 h reaction, the AuNP-pro-DPCPX product was washed in deionized water and concentrated several times.

In order to conjugate WGA-HRP to the above product through an amide bond to make the final nanoconjugate, the solution of the above product was diluted to 0.1 mg/ml (pro-DPCPX concentration) by deionized water and was maintained at 4°C and pH of 6.6. 5 mg WGA-HRP, 10 mg EDC, and 3 mg NHS were added to the solution. The reaction proceeded for 1 h followed by washing with deionized water. The concentration of the final stock was diluted to 0.5 mg (pro-DPCPX)/ml based on the pro-DPCPX input amount, the actual concentration was 0.14 mg/mL, which was determined by TGA analysis. The stock was diluted 50, 100, 150, and 300 times, corresponding to 2.8, 1.4, 0.93, and 0.46 μg (pro-DPCPX)/ml for the *in vivo* experiments. The nanoconjugate solutions can be stored at 4°C up to one month before *in vivo* experiments.

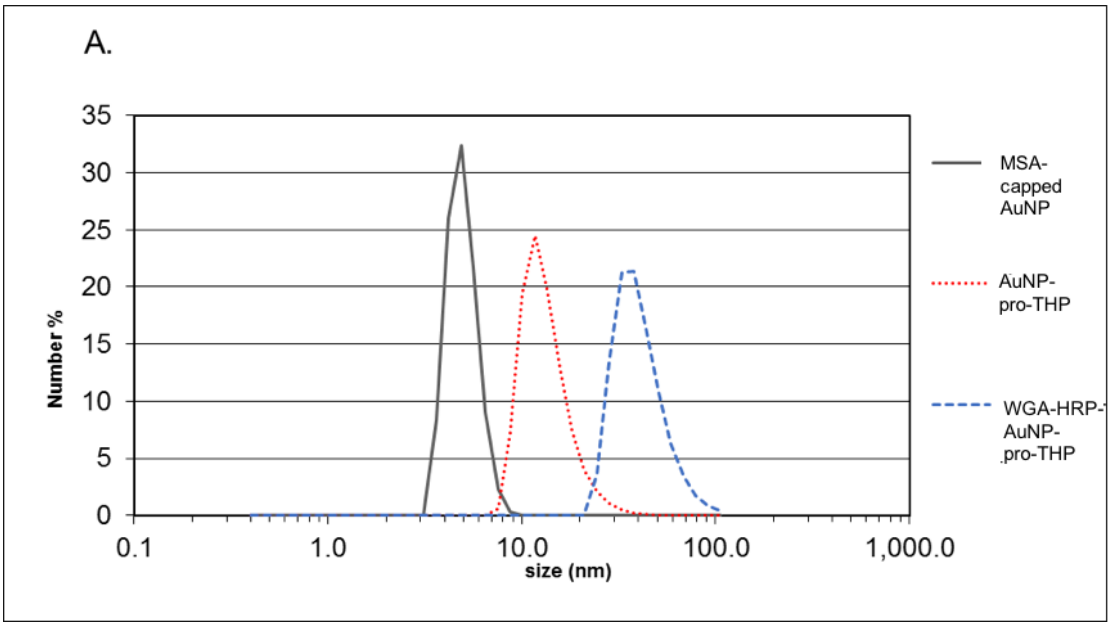
The Zetasizer and TGA results for pro-DPCPX synthesis products are located in SI Fig. 2B and SI Fig. 4, respectively. The hydrodynamic diameters measured by the zetasizer are 13.5 nm (zeta potential = -37 mV) for pro-DPCPX-AuNP and 32.7 nm (zeta potential = -27.4 mV) for WGA-HRP-AuNP-pro-DPCPX. The zetasizer measurements were conducted in PBS buffer (pH = 7.4) at 37°C.

3. Supplementary information figures

SI Figure 1. ^{1}H NMR spectra of A) THP and B) pro-THP. Both THP and pro-THP were dissolved in $CDCl_3$ at room temperature and were analyzed using a Merck 400MHz instrument. ^{1}H NMR δ 5.6 (broad s, 3 CH_2-OH) peak occurred in product confirming the formation of pro-THP.



SI Figure 2. Zetasizer measurements of A) pro-THP nanoconjugates and B) pro-DPCPX nanoconjugates.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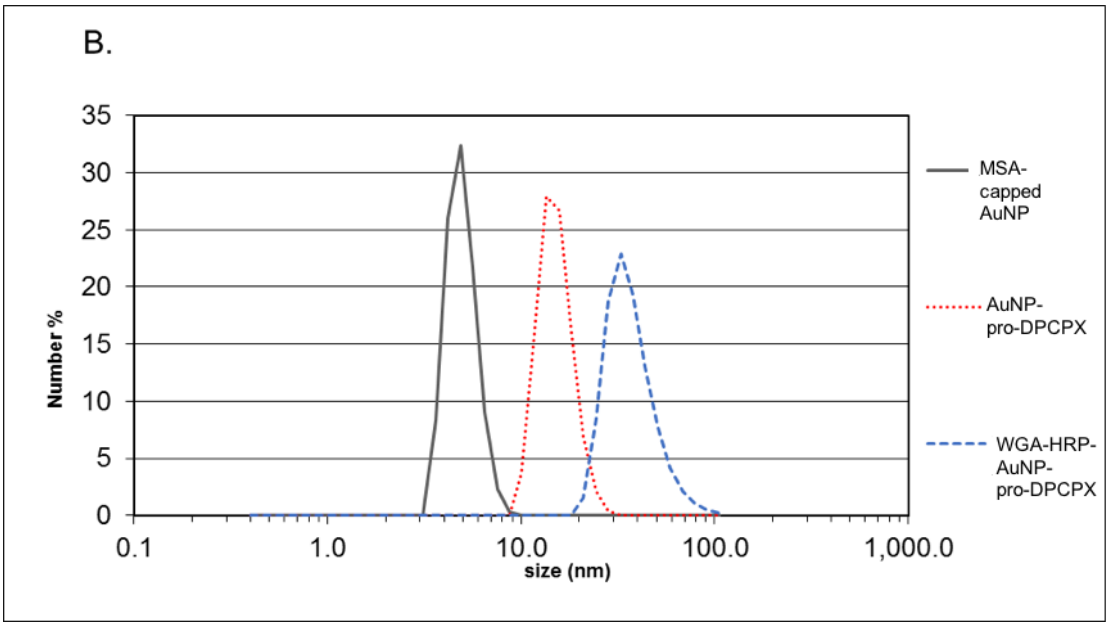
size (nm)
MSA-capped AuNP
AuNP-pro-THP
WGA-HRP-AuNP-pro-THP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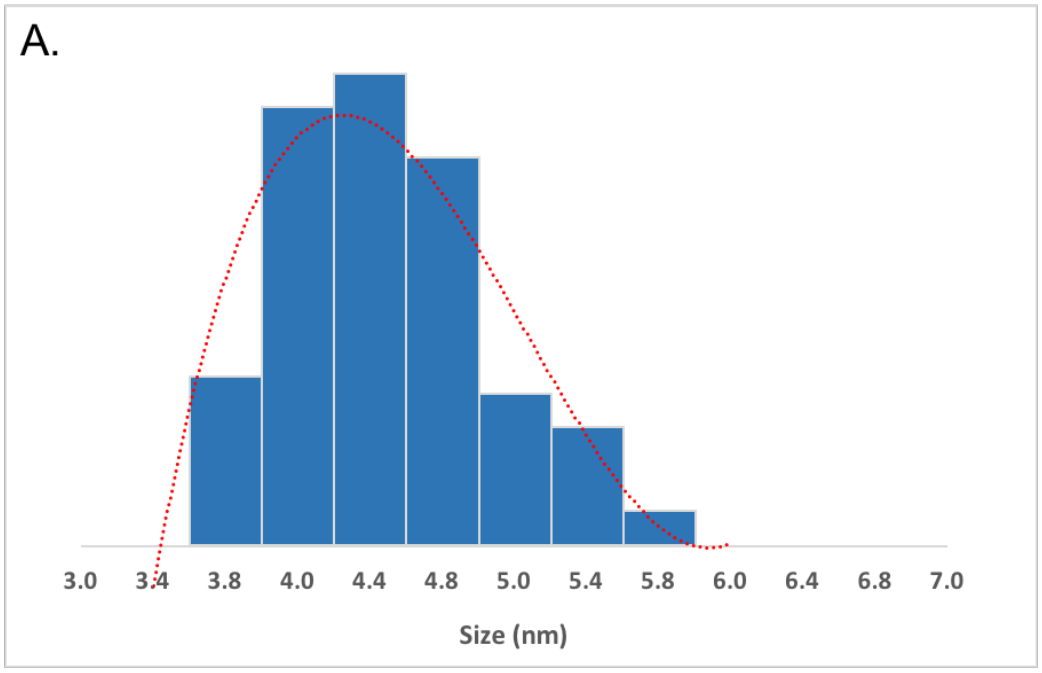
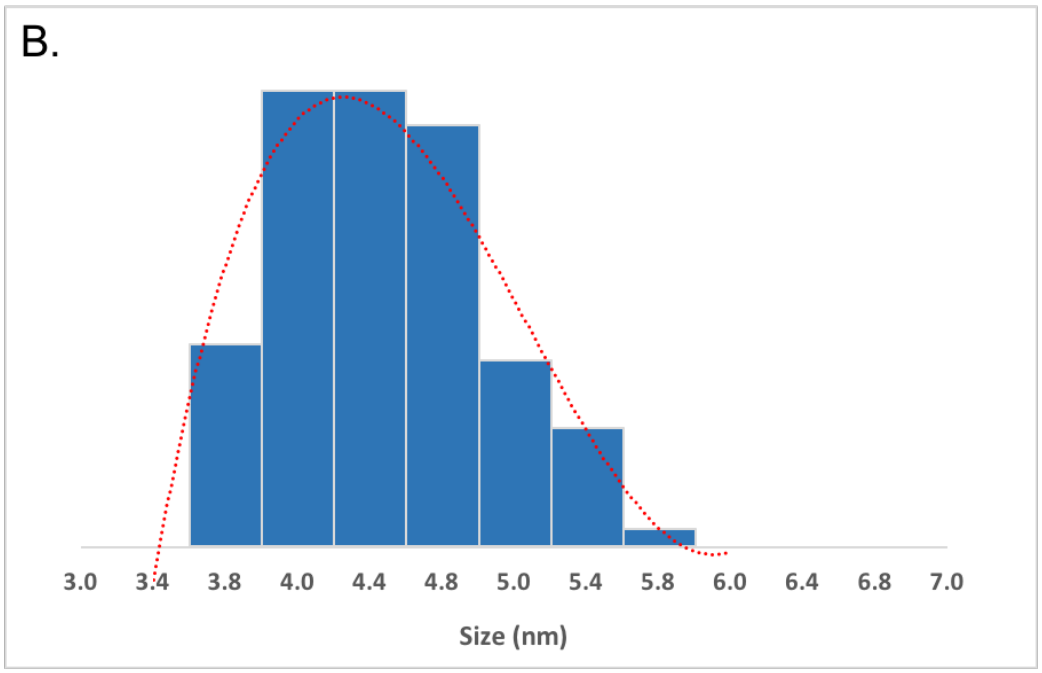
size (nm)
MSA-capped AuNP
AuNP-pro-DPCPX
WGA-HRP-AuNP-pro-DPCPX

SI. Figure 3. TEM size distribution histograms of the AuNP component of the nanoconjugate after each step of synthesis; A) MSA-capped AuNP, B) AuNP-pro-THP, and C) WGA-HRP-AuNP-pro-THP.





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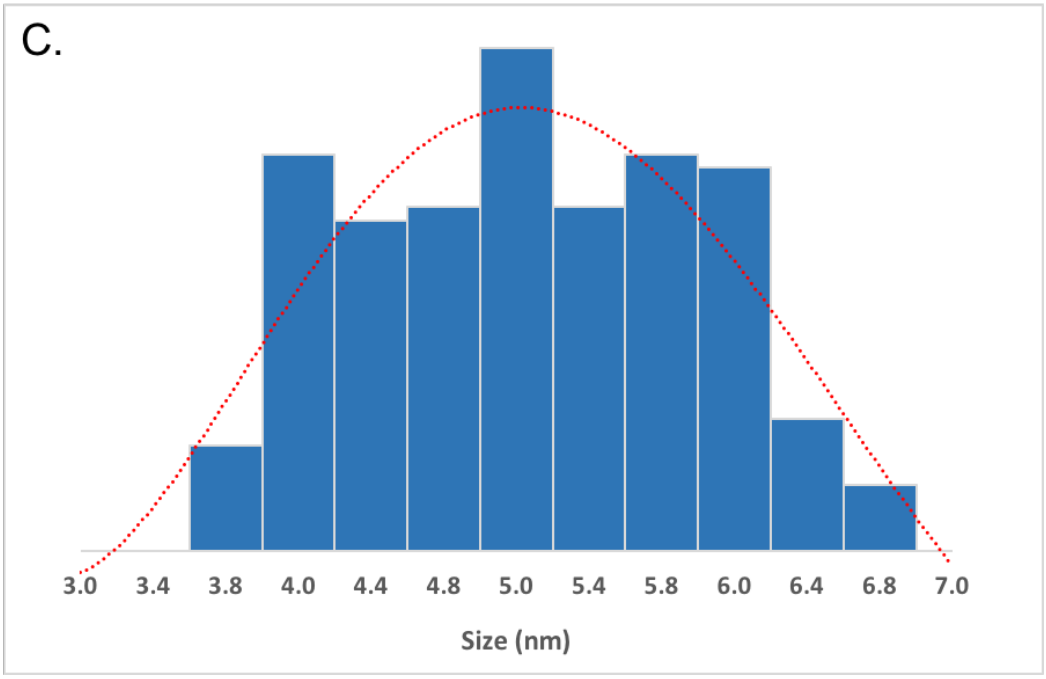
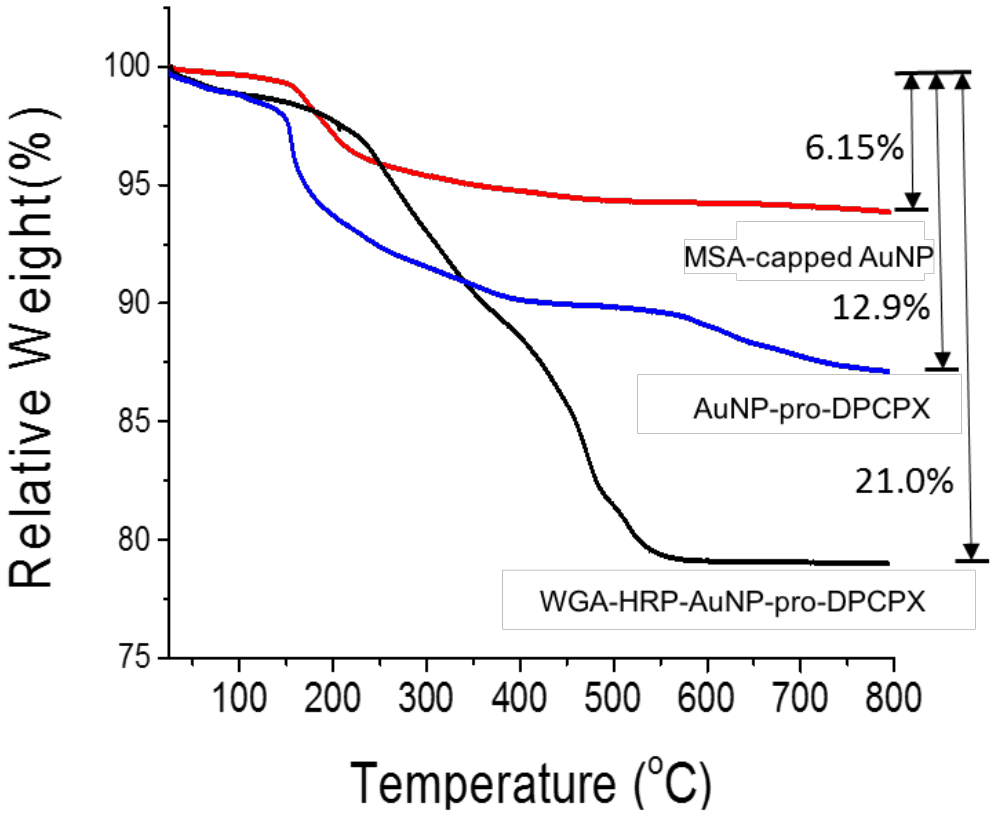


SI. Figure 4. TGA spectra of pro-DPCPX nanoconjugates.



SI. Figure 5. MS spectra of supernatant after 12 h of the AuNP-pro-THP nanoconjugate in A) ACSF or B) HEPES buffer, and C) control pro-THP alone dissolved in HEPES.