Supporting Information

Hormone-Coated NanoContrast Agents Promotes ER+ Breast Cancer CT Detection

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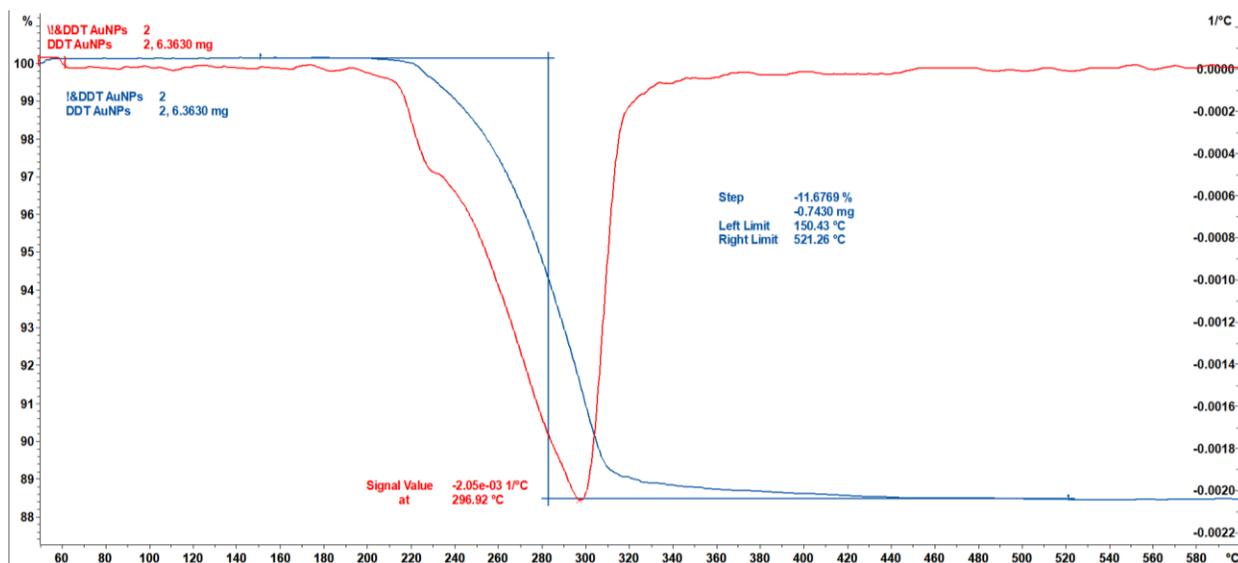
[#] Northeastern University, Department of Pharmaceutical Sciences, Boston, Massachusetts 02115, U.S.A (current address).
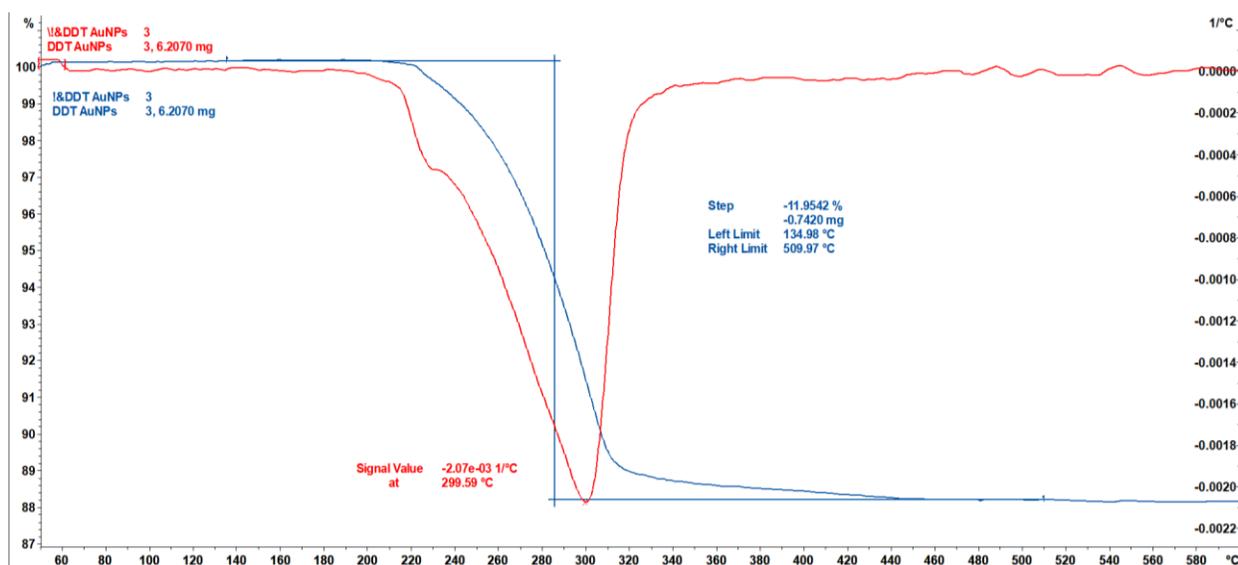
Cumulative **TGA Table** for **C12-S-AuNPs**

Sample	Decomp. T1 (ºC)	Weight Loss 1 (%)	Decomp. T2 (ºC)	Weight Loss 2 (%)
1	307.3	11.4	-	-
2	296.9	11.7	-	-
3	299.6	12.0	-	-
Ave	**301** ± 5	**11.7** ± 0.3	-	-
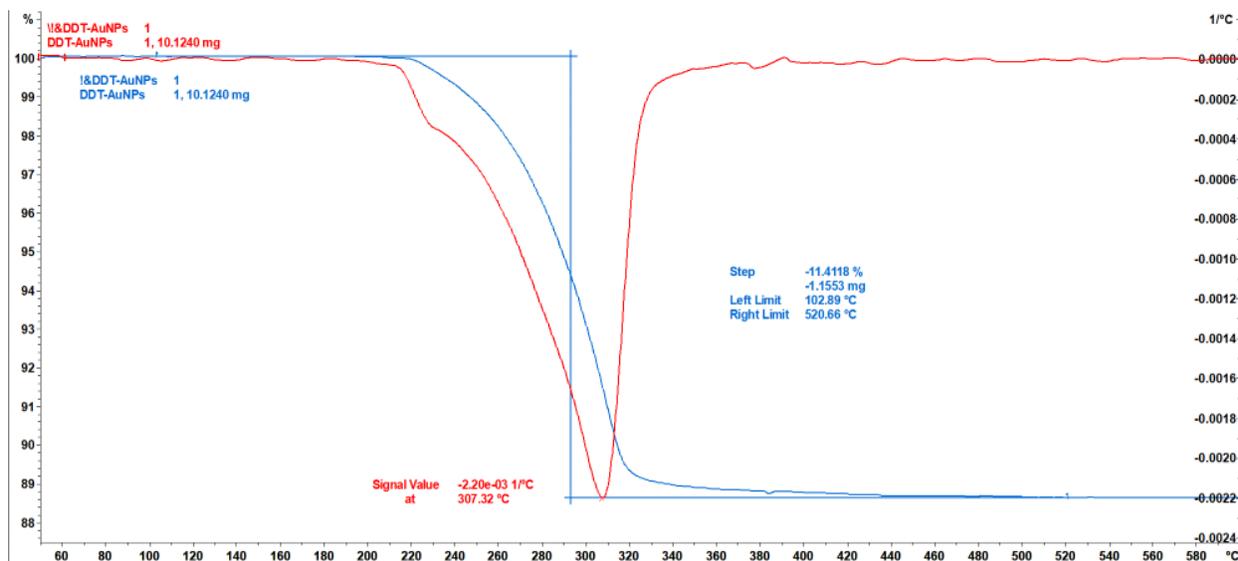
TGA of C12-S-AuNPs #1

TGA of C12-S-AuNPs #2

TGA of C12-S-AuNPs #3
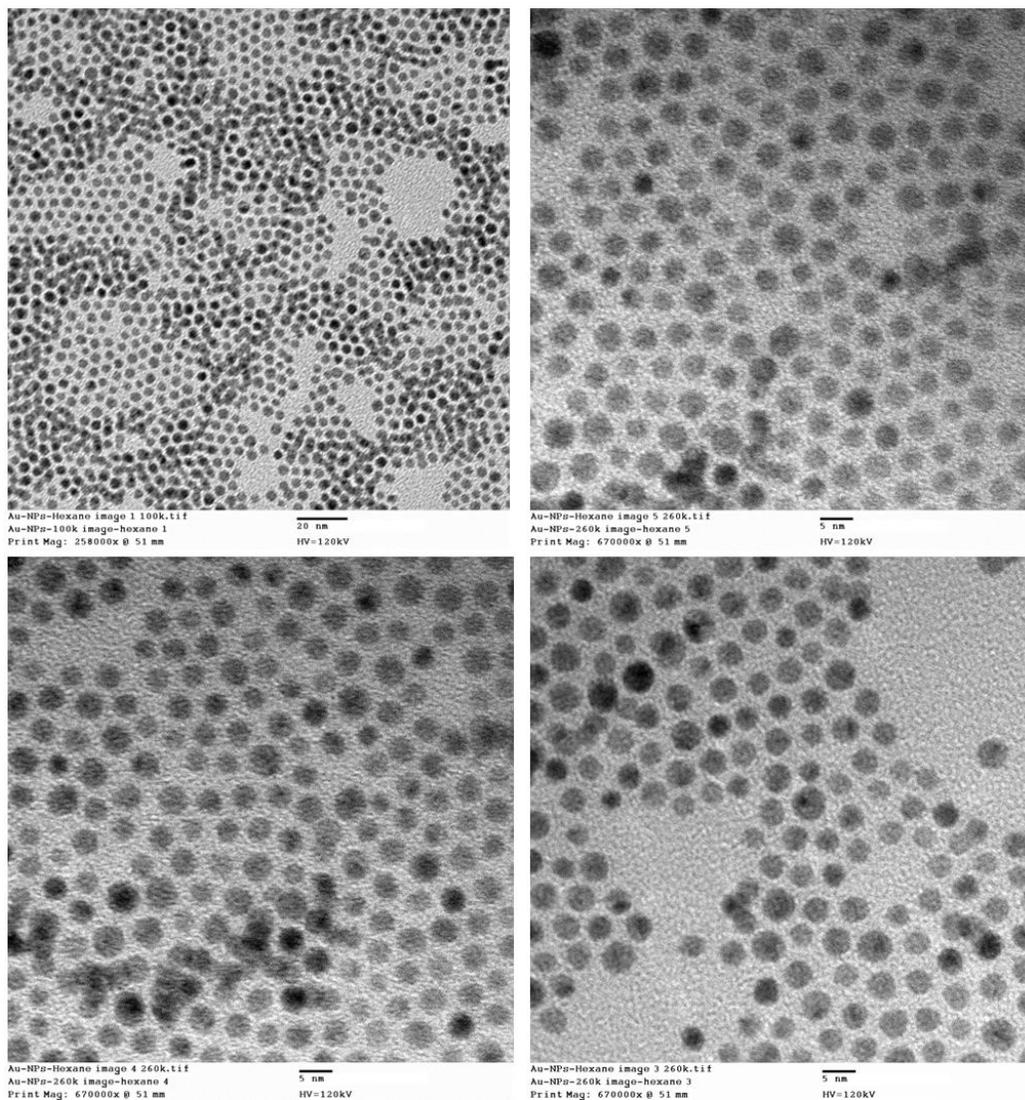
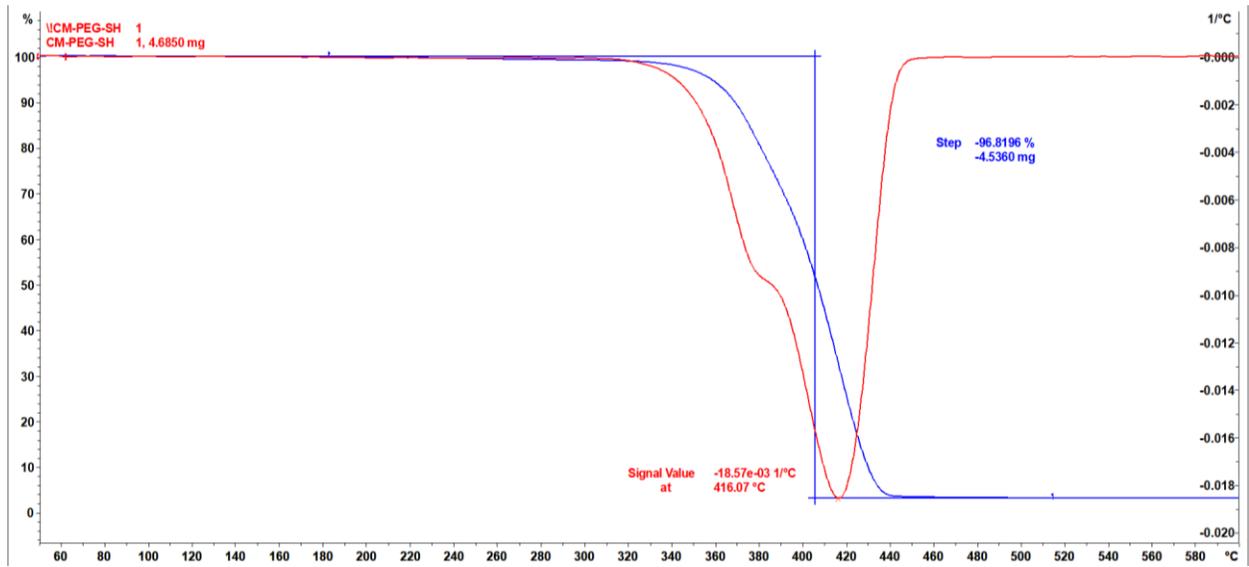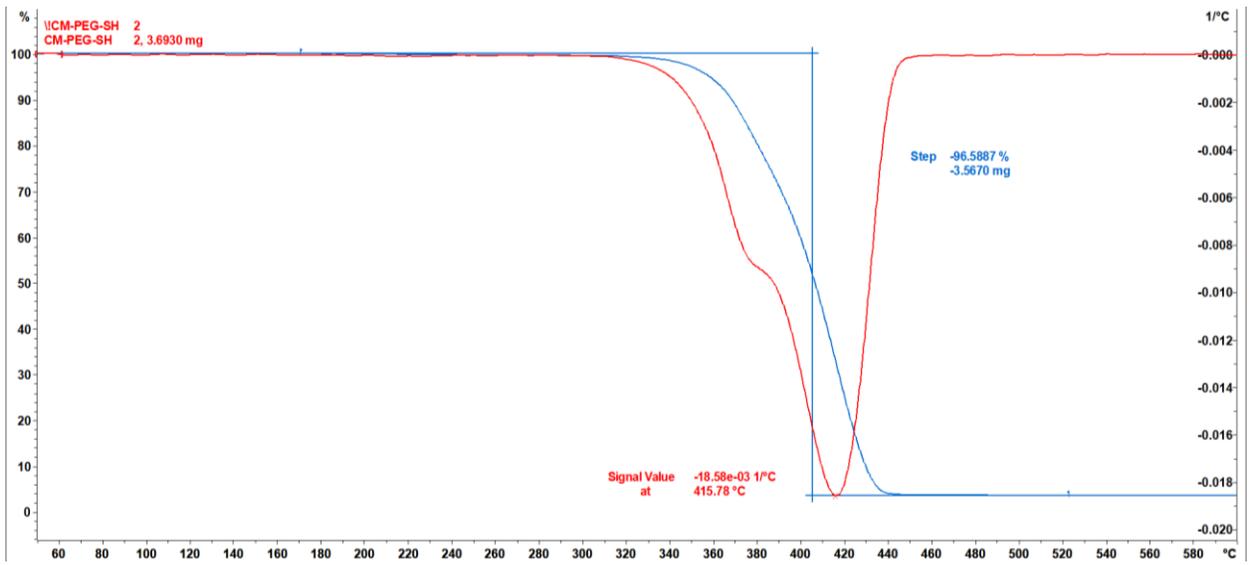**Figure S1**. TGA graphs of C12-S-AuNPs.

Figure S2. TEM of ~3.7 nm **C12-S-AuNPs** at 0.5 mg/mL in hexane.

Cumulative **TGA Table** for **HOOC-PEG-SH**

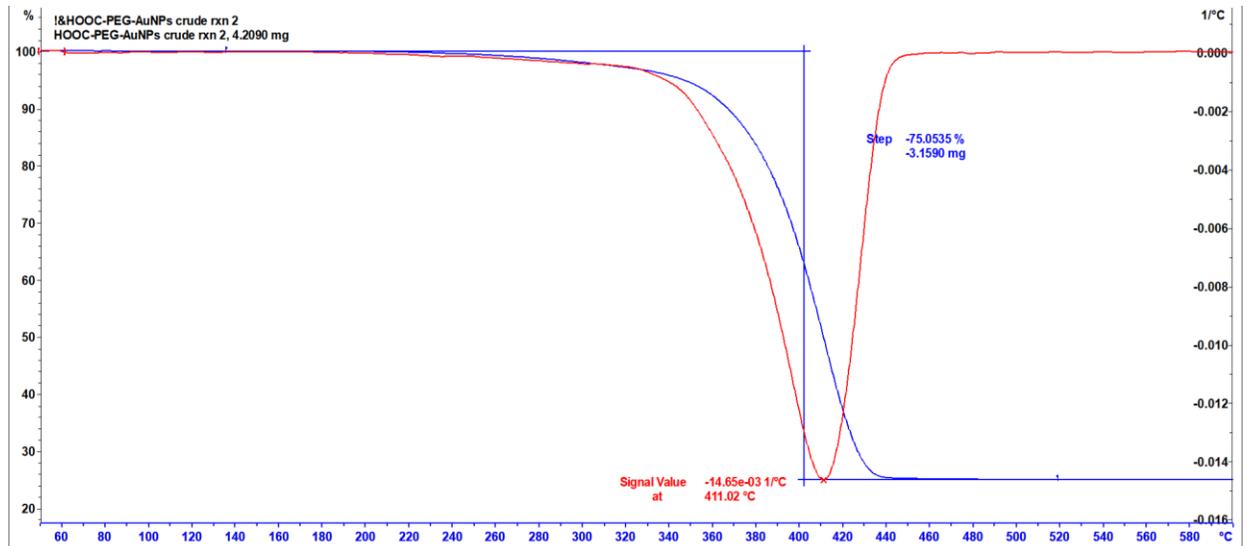| Sample	Decomp. T1 (ºC)	Weight Loss 1 (%)	Decomp. T2 (ºC)	Weight Loss 2 (%)
1	-	-	416.1	96.8
2	-	-	415.8	96.6
3	-	-	415.2	96.4
Ave	-	-	**416** ± 1	**96.6** ± 0.2

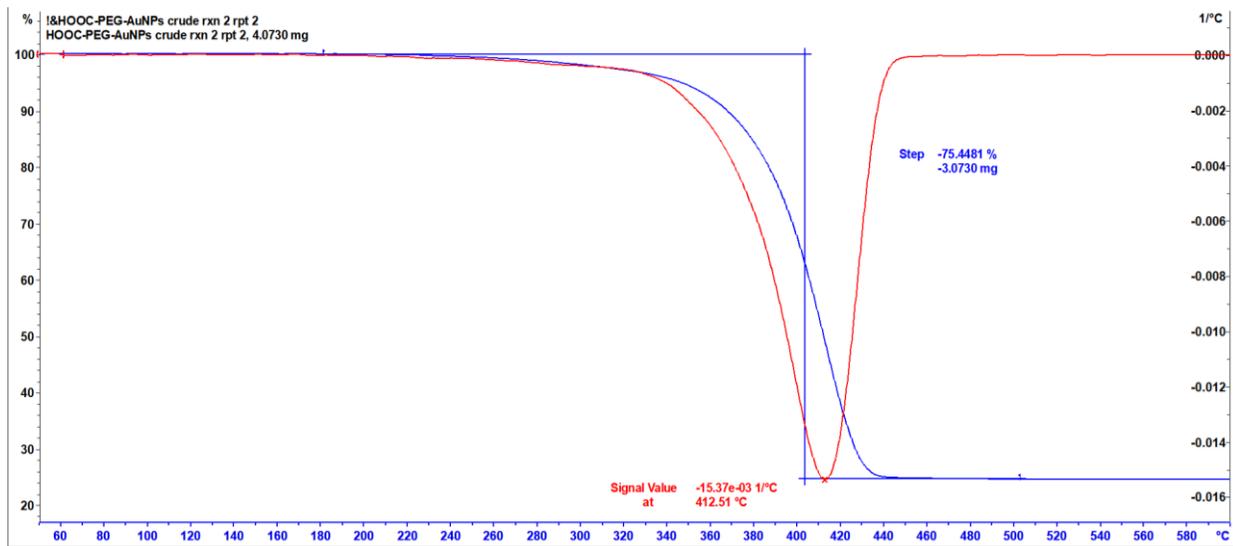TGA of HOOC-PEG-SH #1

TGA of HOOC-PEG-SH #2
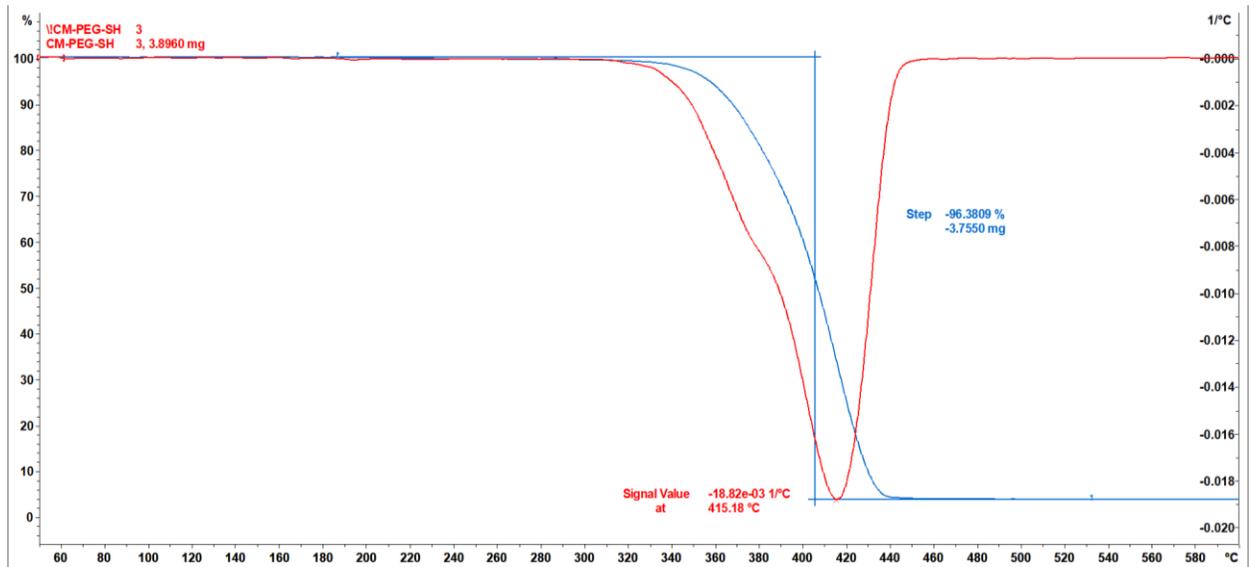
TGA of HOOC-PEG-SH #3

Figure S3. TGA graphs of **HOOC-PEG-SH**.
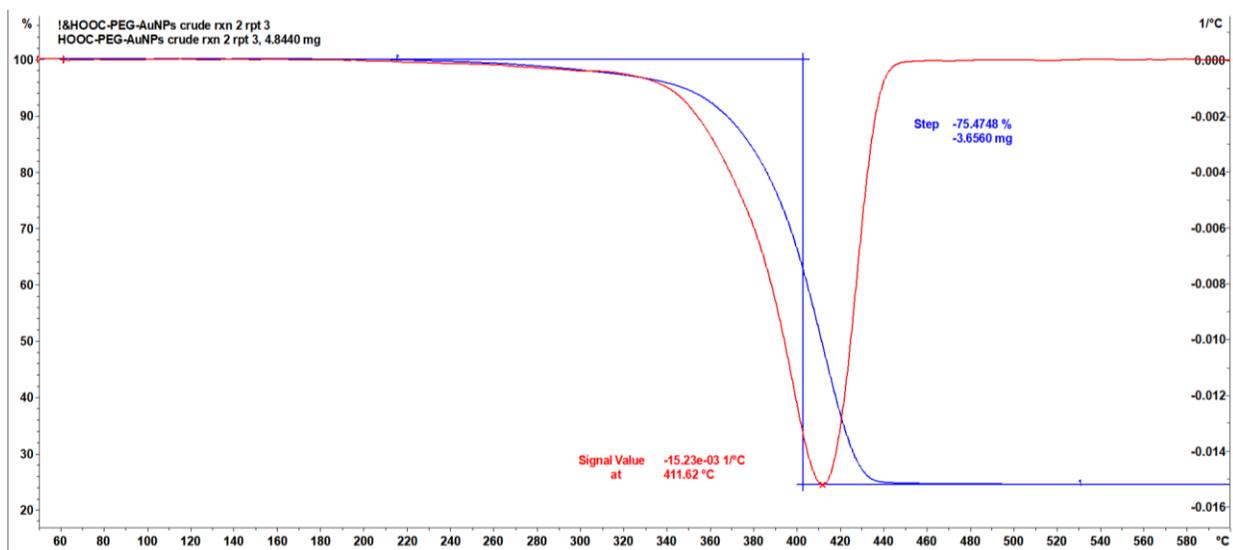
TGA Table of *as prepared* HOOC-PEG-AuNPs

Sample	Decomp. T1 (ºC)	Weight Loss 1 (%)	Decomp. T2 (ºC)	Weight Loss 2 (%)
1	-	-	411.0	75.1
2	-	-	412.6	75.4
3	-	-	411.0	75.5
Ave	-	-	**411** ± 1	**75.3** ± 0.3

TGA of crude HOOC-PEG-S-AuNPs #1
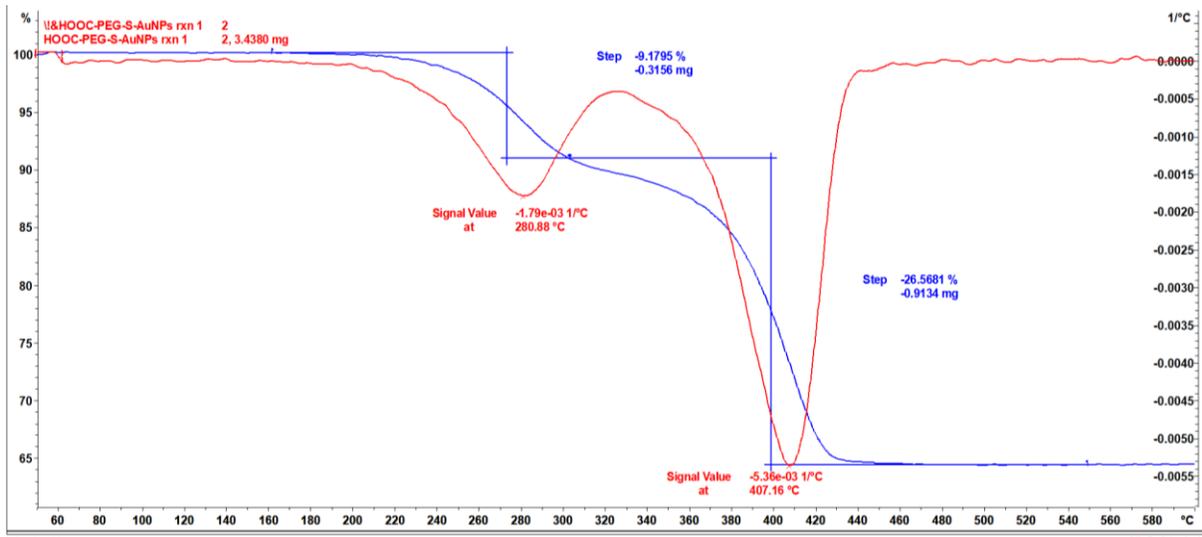
TGA of crude HOOC-PEG-S-AuNPs #2
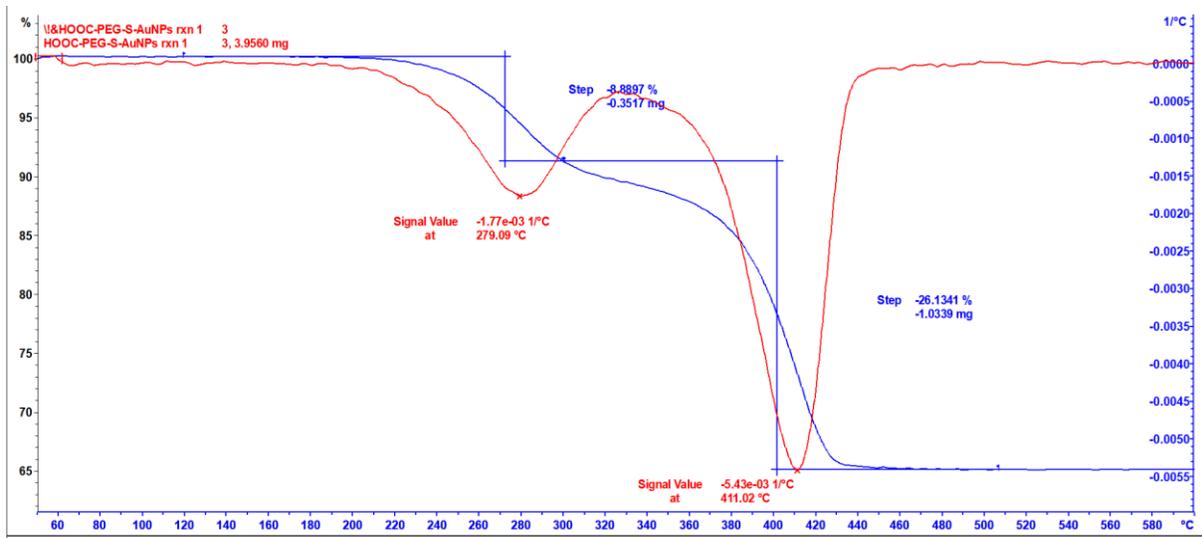

TGA of crude HOOC-PEG-S-AuNPs #3

Figure S4. TGA graphs of **crude HOOC-PEG-S-AuNPs**.
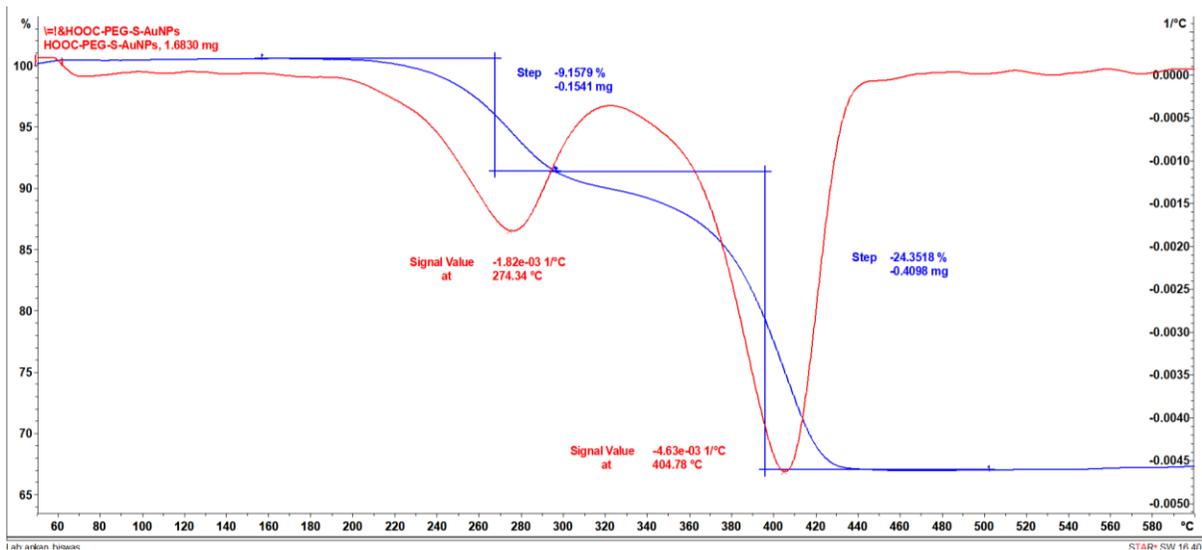
TGA Table of *purified* HOOC-PEG-AuNPs

Sample	Decomp. T1 (ºC)	Weight Loss 1 (%)	Decomp. T2 (ºC)	Weight Loss 2 (%)
1	280.9	9.2	407.2	26.6
2	279.1	8.9	411.0	26.1
3	274.3	9.2	404.8	24.4
Ave	**278.1** ± 3.4	**9.1** ± 0.2	**407.7** ± 3.1	**25.7** ± 1.2

TGA of pure HOOC-PEG-S-AuNPs #1

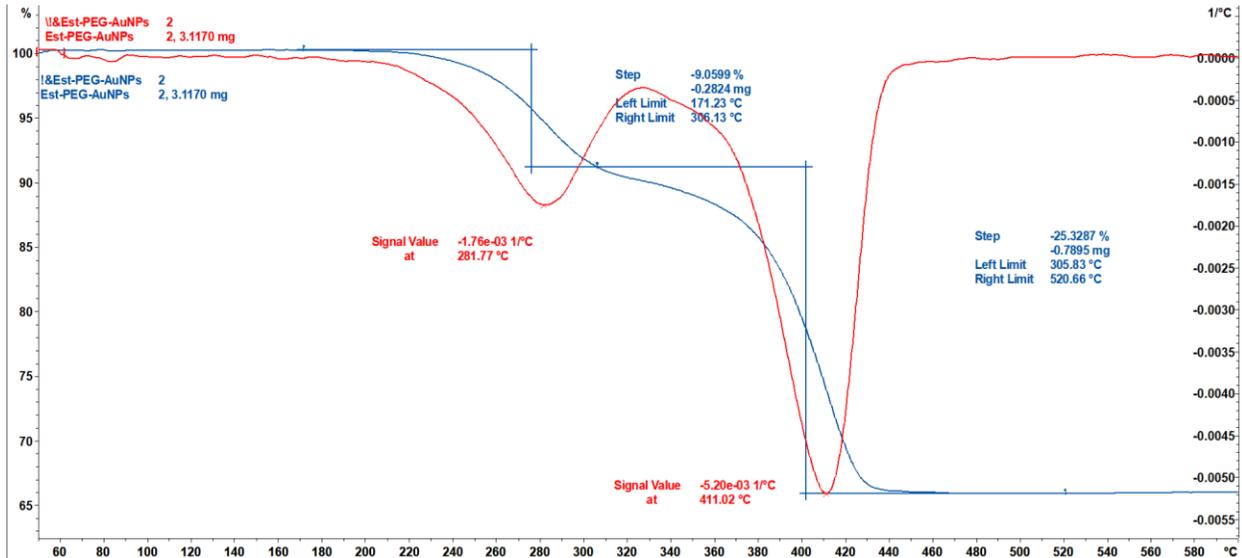TGA of pure HOOC-PEG-S-AuNPs #2

TGA of pure HOOC-PEG-S-AuNPs #3

Figure S5. TGA graphs of **purified HOOC-PEG-S-AuNPs**.
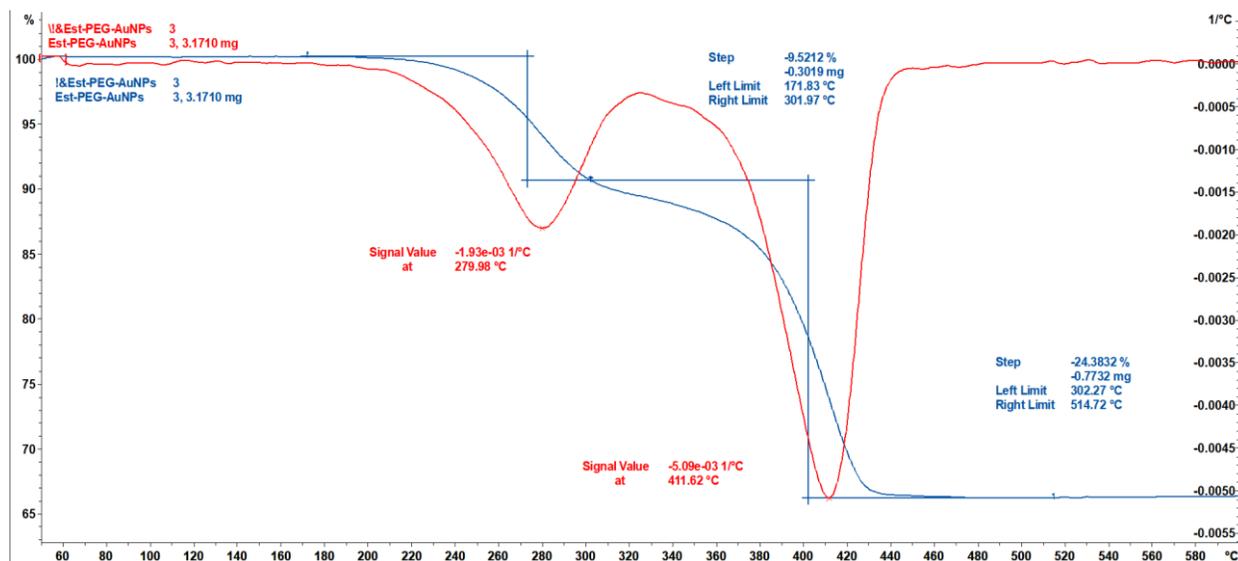
Cumulative **TGA Table** for *E2*-**PEG-AuNPs**

Sample	Decomp. T1 (ºC)	Weight Loss 1 (%)	Decomp. T2 (ºC)	Weight Loss 2 (%)
1	279.7	9.7	410.7	22.6
2	281.8	9.1	411.0	25.3
3	280.0	9.5	411.6	24.4
Ave	**281** ± 1	**9.4** ± 0.3	**411** ± 1	**24.1** ± 1.4
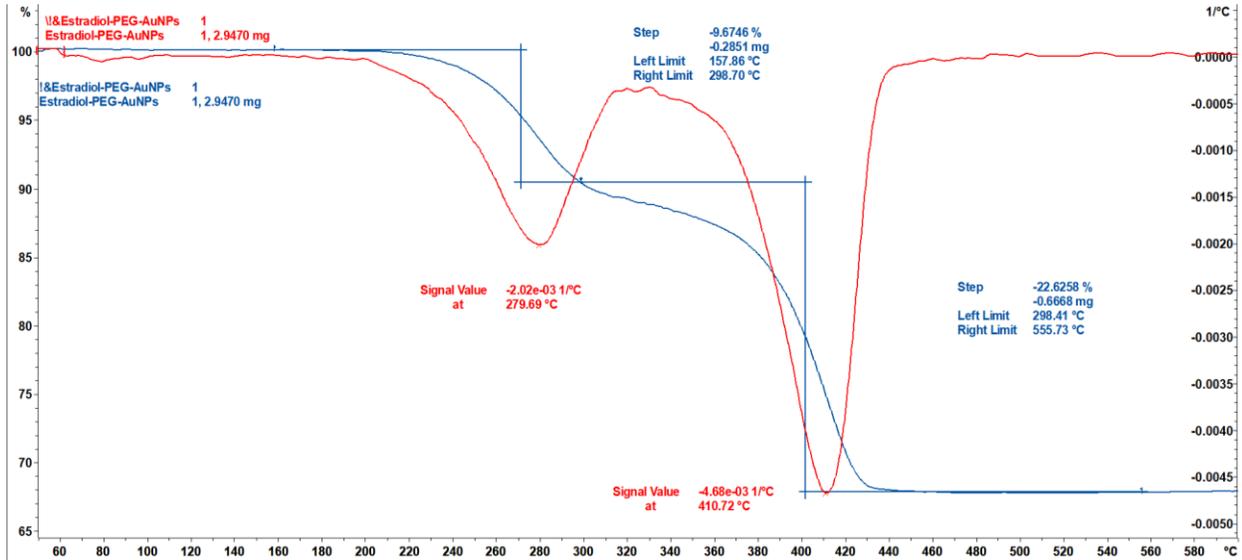
TGA of estradiol-PEG-S-AuNPs #1

TGA of estradiol-PEG-S-AuNPs #2

TGA of estradiol-PEG-S-AuNPs #3

Figure S6. TGA graphs of *E2*-PEG-AuNPs.

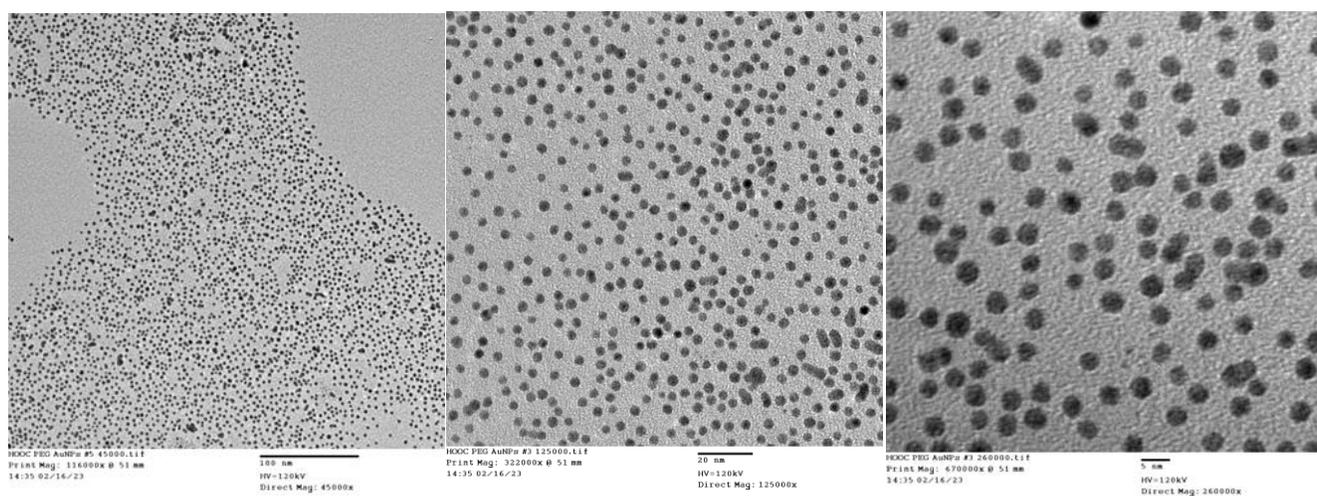**Figure S7.** TLC of *E2*-PEG-AuNPs (left) vs. E2 (right) at 10:1 DCM/MeOH elution.

Figure S8. TEM images of **3.2 ± 0.4 nm** HOOC-PEG-AuNPs at 0.5 mg/mL in water. The examined magnitudes (left to right) were 45000x, 125000x and 260000x.
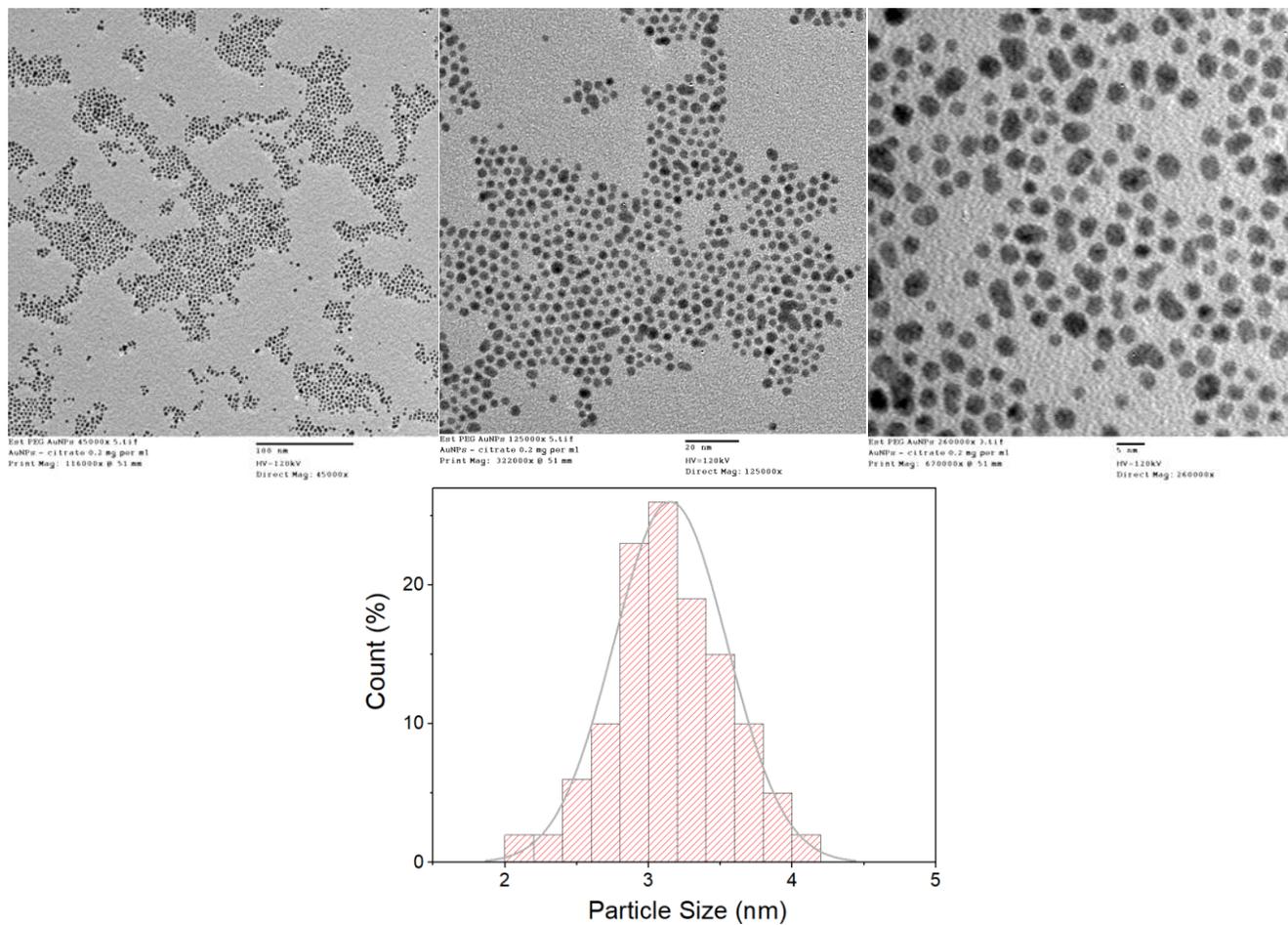
Figure S9. TEM images of **3.9 ± 0.7 nm** *E2*-PEG-AuNPs at 0.5 mg/mL in water at different magnifications; from left to right: 45000x, 125000x and 260000x, along with size distribution.
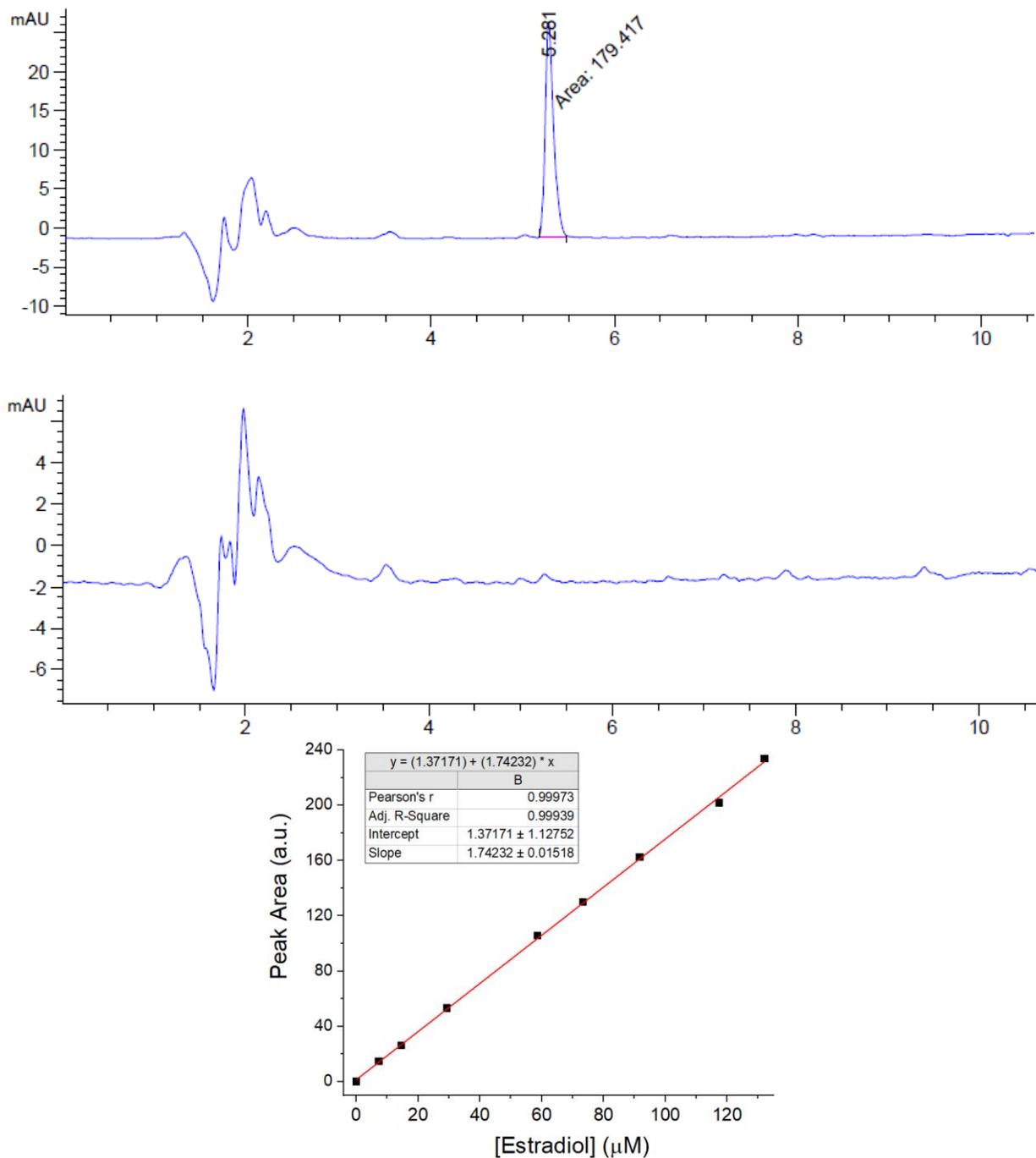
Figure S10. (top) Linear fitting of the *β-estradiol* HPLC peak (retention time at 5.28 mins) between 0-132 μM in DMSO with an equation of y = 1.7423x + 1.3717; (bottom) HPLC spectra of NP-hydrolyzed *β-estradiol* in 1 mL DMSO (4 mg *estradiol*-NPs) vs. control (supernatant residue of PEG-SAuNPs) at the same conditions.
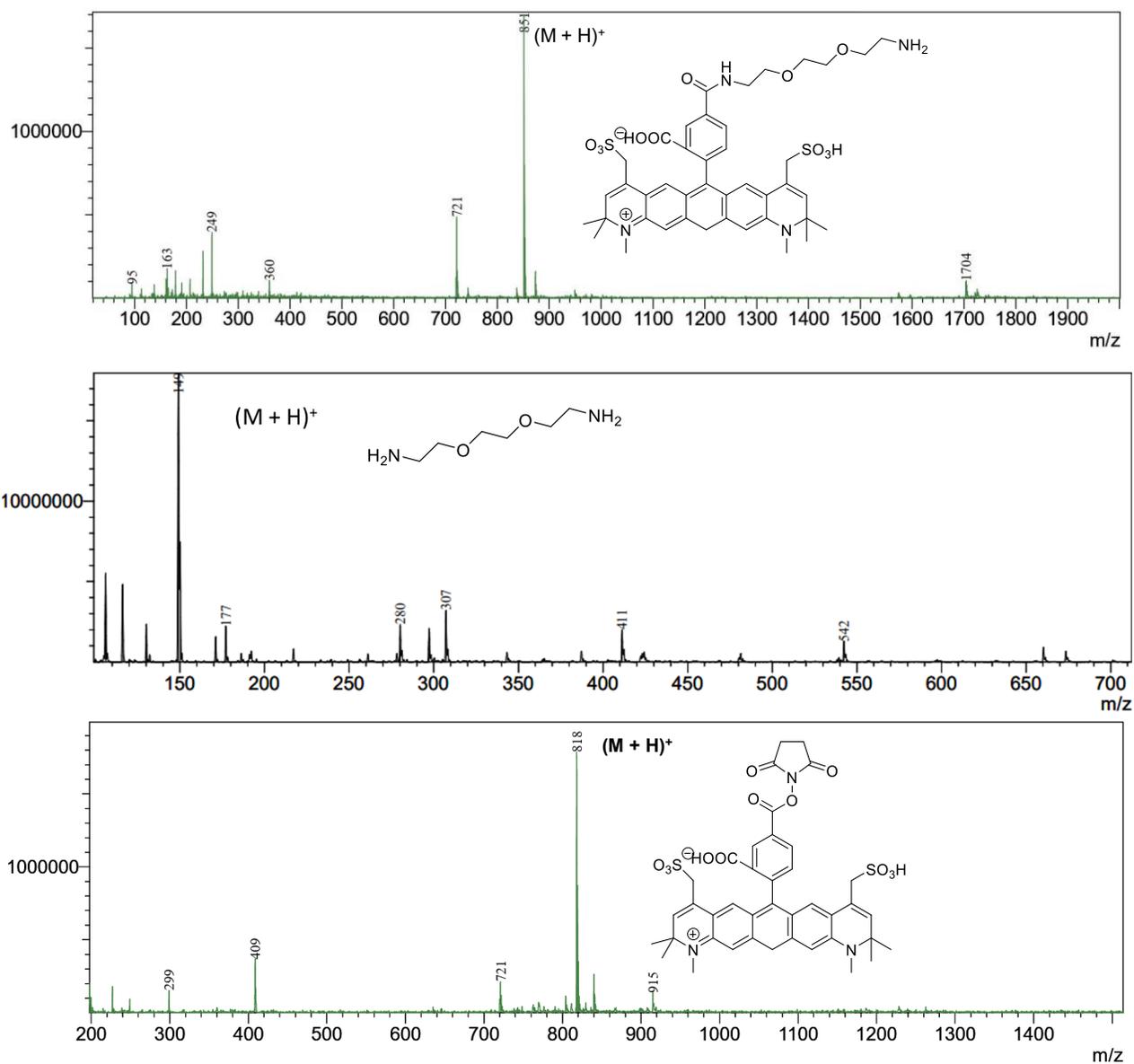
Figure S11. LC-MS spectra of (a) Alexa Fluor 570-EG$_2$-NH$_2$ (MW = 851 g/mol), (b) 1,2-bis(2-aminoethoxy)ethane (MW = 148 g/mol), and (c) Alexa Fluor 594 NHS ester (MW = 818 g/mol).
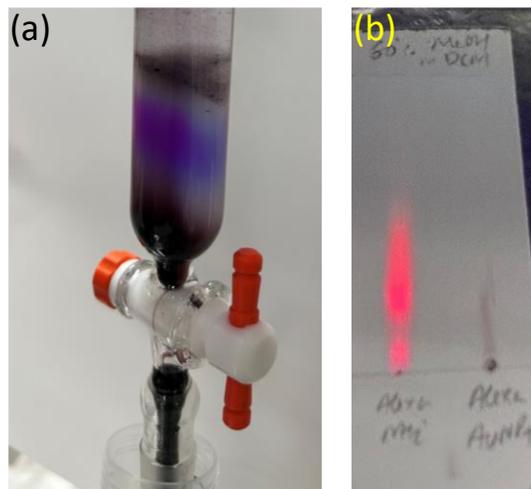
Figure S12. (a) Sephadex purification of (Alexa 594-EG$_2$)/*E2*-PEG-AuNPs (reddish black) from unreacted dye (purple); (b) TLC of Alexa 594 NHS ester (left) and product (right) in DCM/MeOH 1:1.
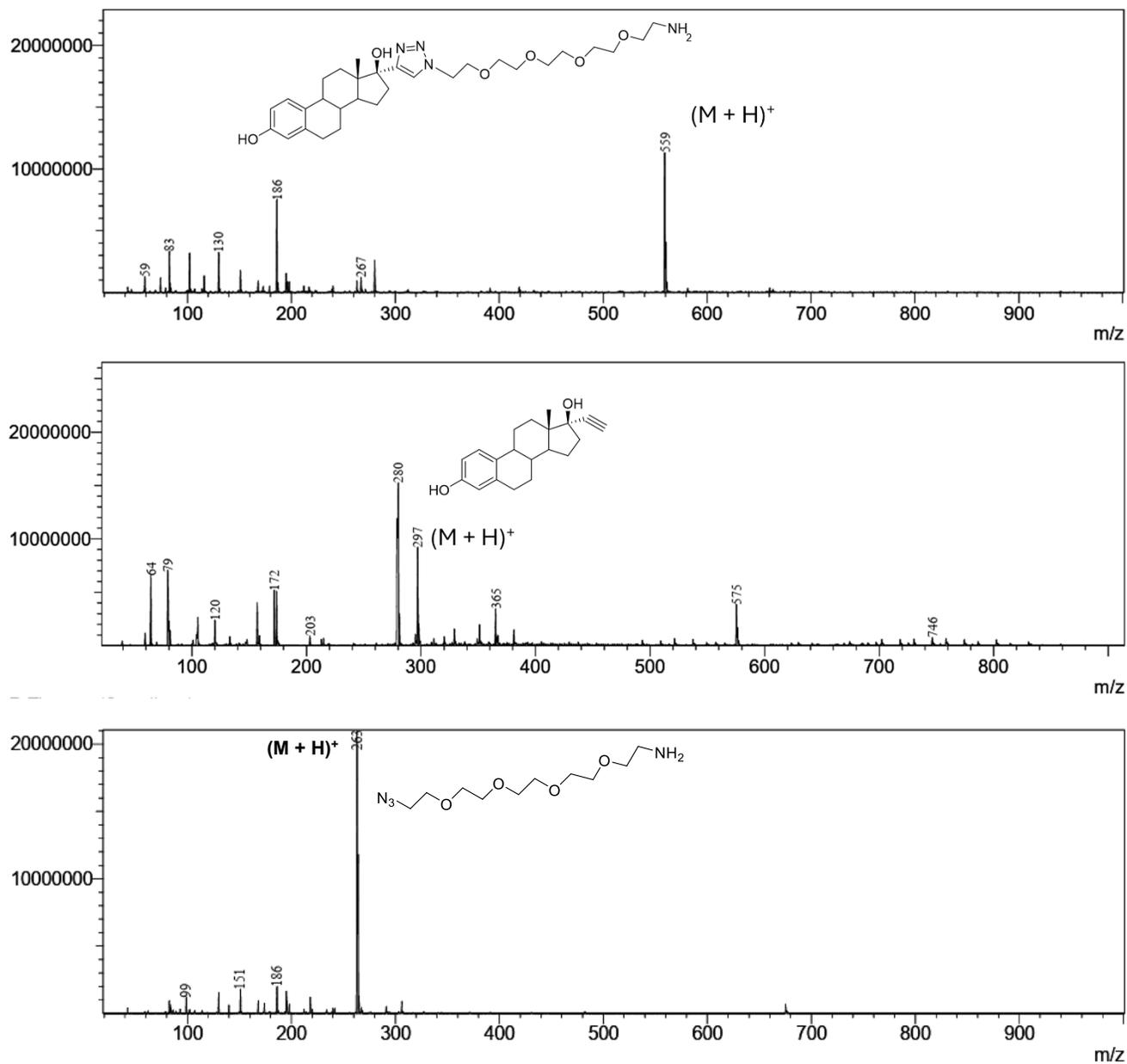
Figure S13. LC-MS spectra of (a) *E2-EG₄-*NH₂ (MW = 558 g/mol; the peak at 186 m/z represents the M/3 +1 ion) and starting materials (b) ethynyl *estradiol* (MW = 296 g/mol) and (c) azido-PEG4-amine (MW = 262 g/mol).
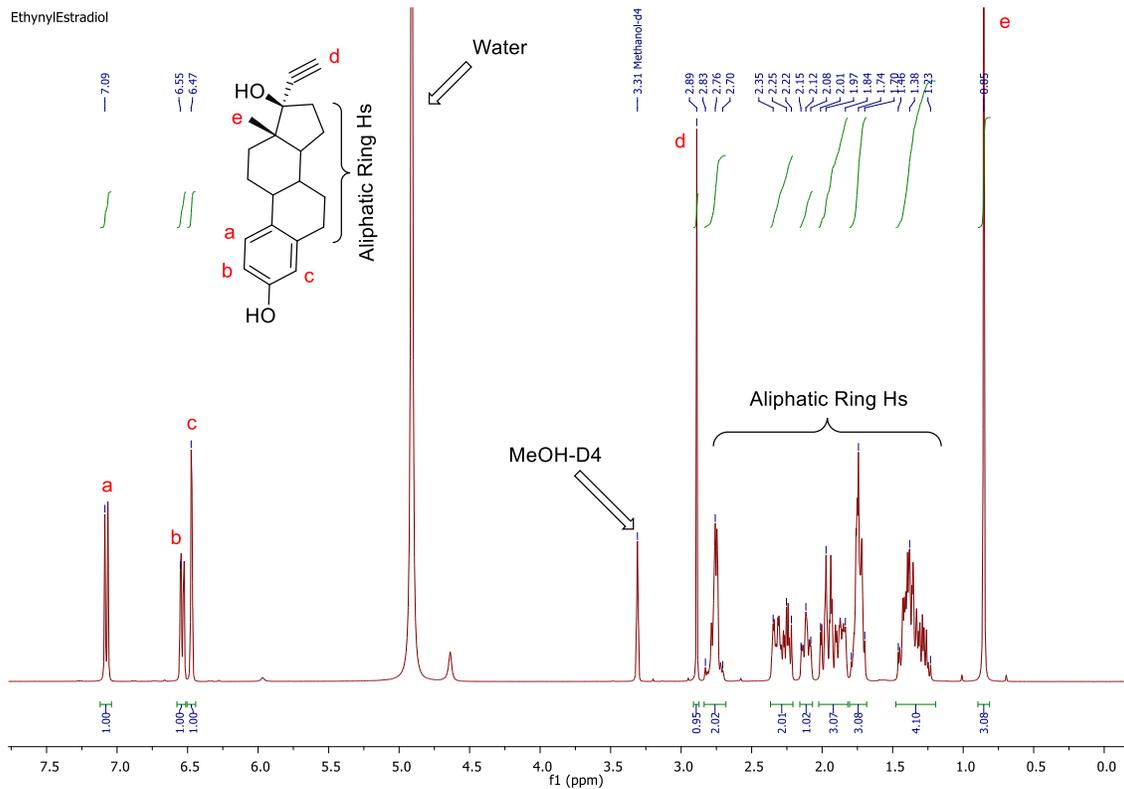
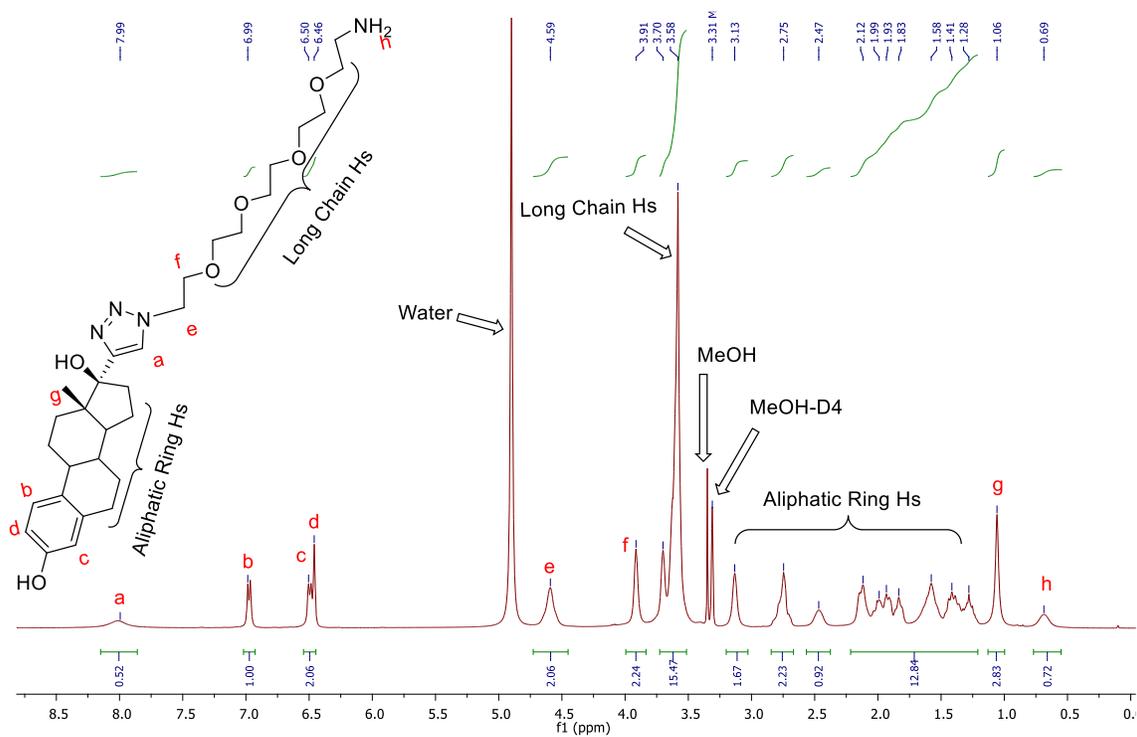**Figure S14**. ^{1}H NMR spectrum of *ethynyl estradiol* in methanol-d4.

Figure S15. ^{1}H NMR spectrum of *E2-EG$_4$*-NH$_2$ in methanol-d4.

PEG4Azide

Figure S16. ^{1}H NMR spectrum of $H_2N\text{-}EG_4\text{-}azide$ in methanol-d4.

Cumulative **TGA Table** for **HOOC-PEG-AuNPs** (purified, new batch)

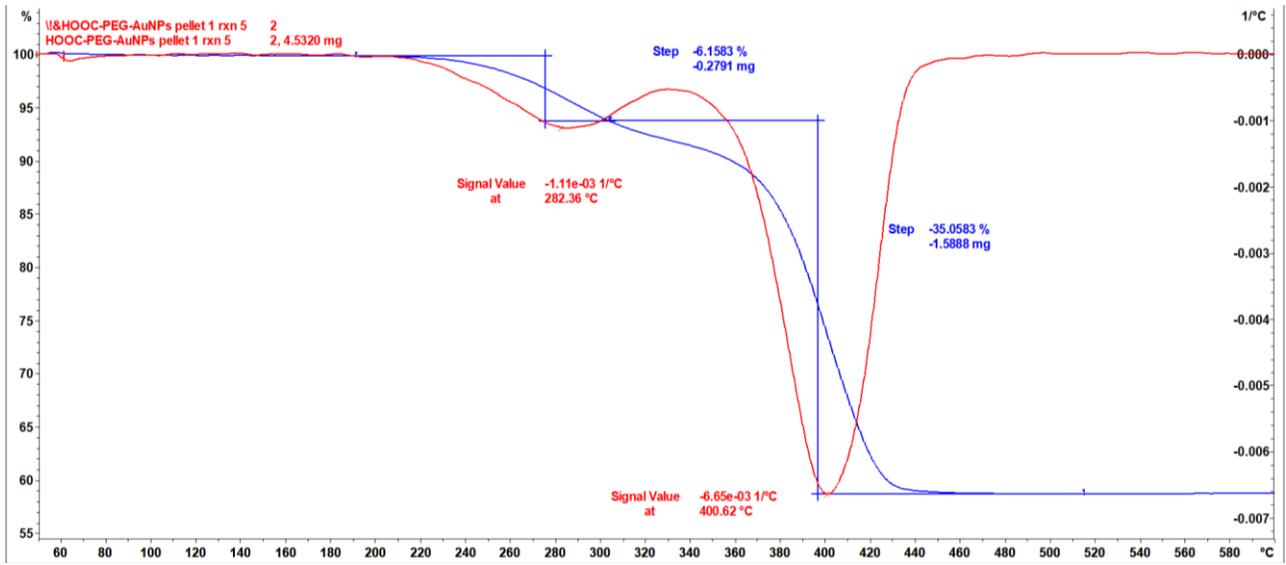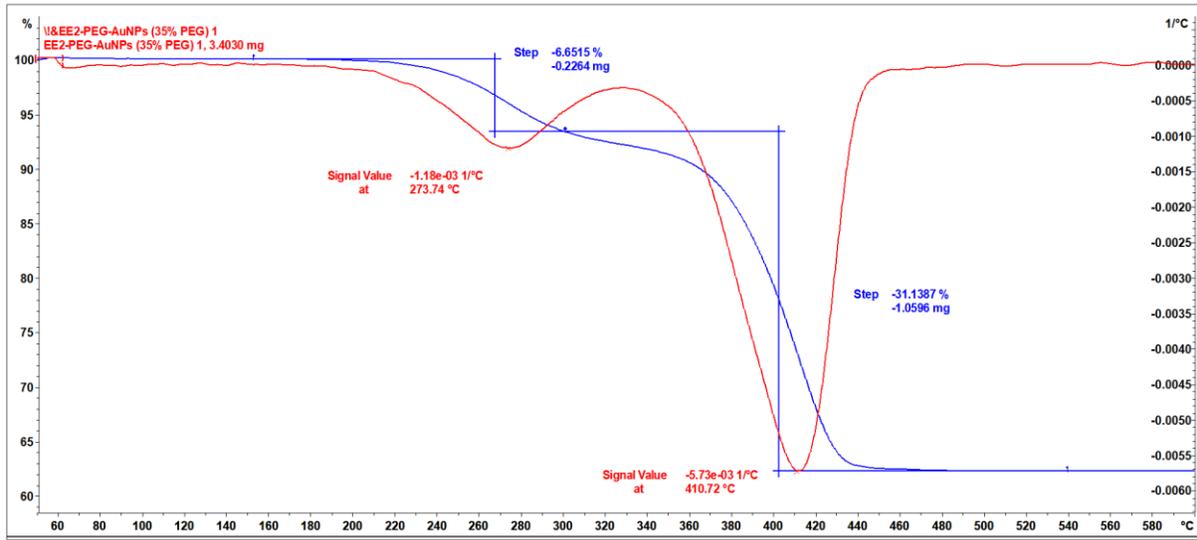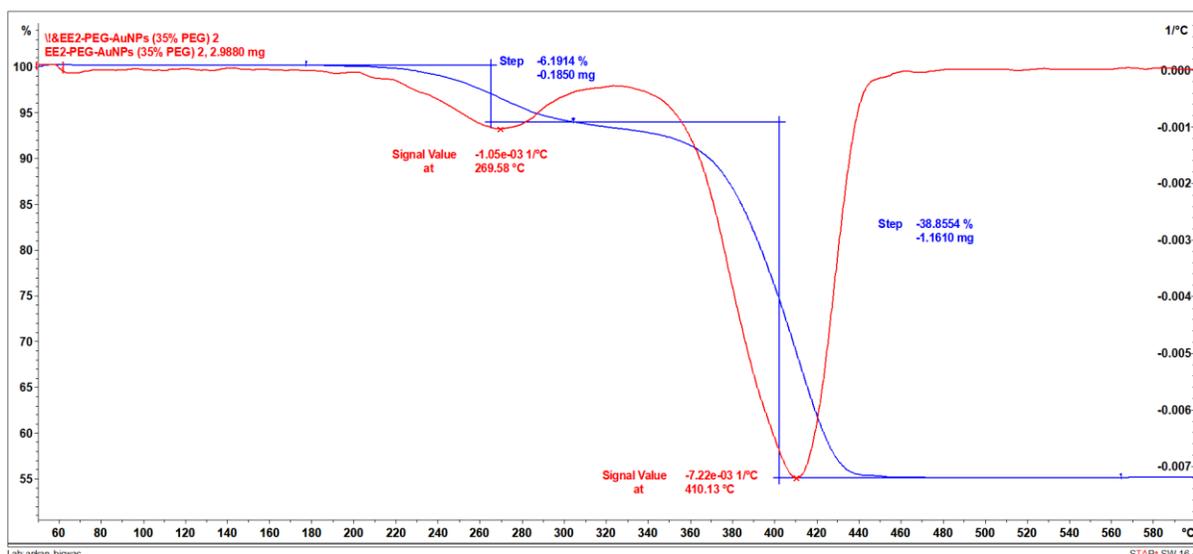| Sample	Decomp. T1 (ºC)	Weight Loss 1 (%)	Decomp. T2 (ºC)	Weight Loss 2 (%)
1	277.9	5.9	404.5	33.6
2	282.4	6.2	400.6	35.1
Ave	**280** ± 3	**6.0** ± 0.2	**403** ± 3	**34.3** ± 1.0

Figure S17. TGA graphs of **purified HOOC-PEG-AuNPs** (new batch).

Cumulative **TGA Table** for *EE2*-**PEG-AuNPs**

Sample	Decomp. T1 (ºC)	Weight Loss 1 (%)	Decomp. T2 (ºC)	Weight Loss 2 (%)
1	273.7	6.7	410.7	31.1
2	269.6	6.2	410.1	38.9
Ave	**272** ± 3	**6.4** ± 0.3	**410** ± 0	**35.0** ± 5.5

TGA of *E2-EG$_4$*-PEG-S-AuNPs #1

TGA of *estradiol-EG₄*-PEG-AuNPs #2

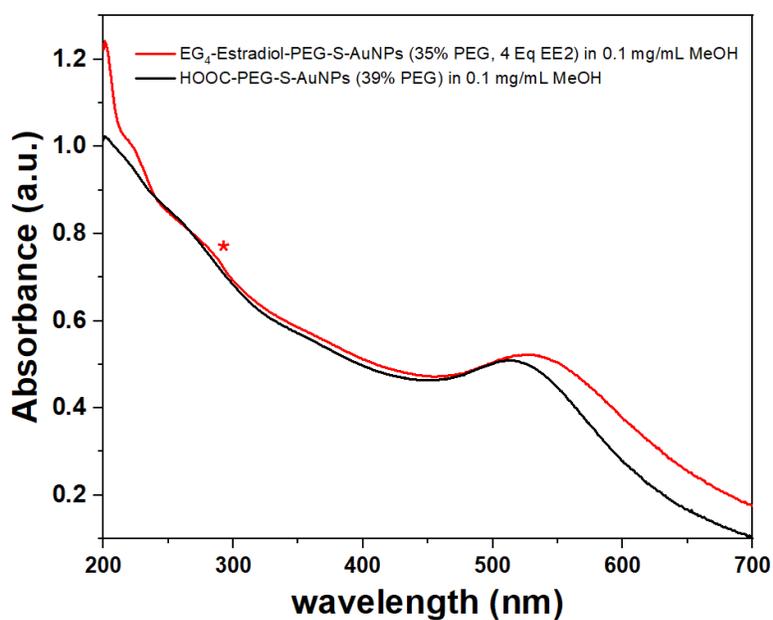**Figure S18.** TGA graphs of *EE2*-PEG-AuNPs.

Figure S19. UV-Vis spectra of (a) *EE2*-PEG-AuNPs (red color) and HOOC-PEG-AuNPs at 0.1 mg/mL both in MeOH. The *EG₄-estradiol* peak at 280 nm is shown with an asterisk. The other estradiol peak at 230 nm is also evident.
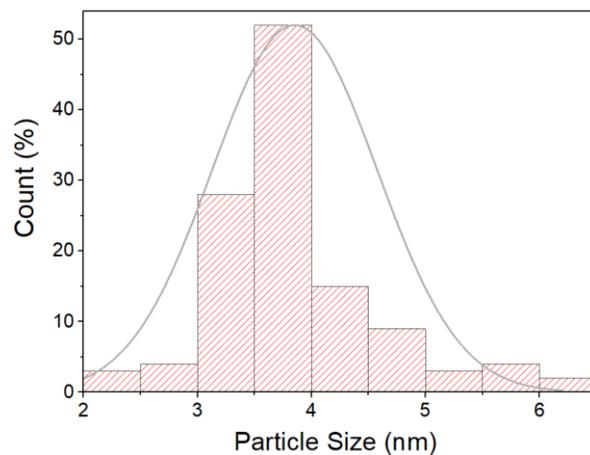
Figure S20. Size distribution of *EE2*-PEG-AuNPs with average particle size of **3.9 ± 0.7 nm** obtained from TEM images.
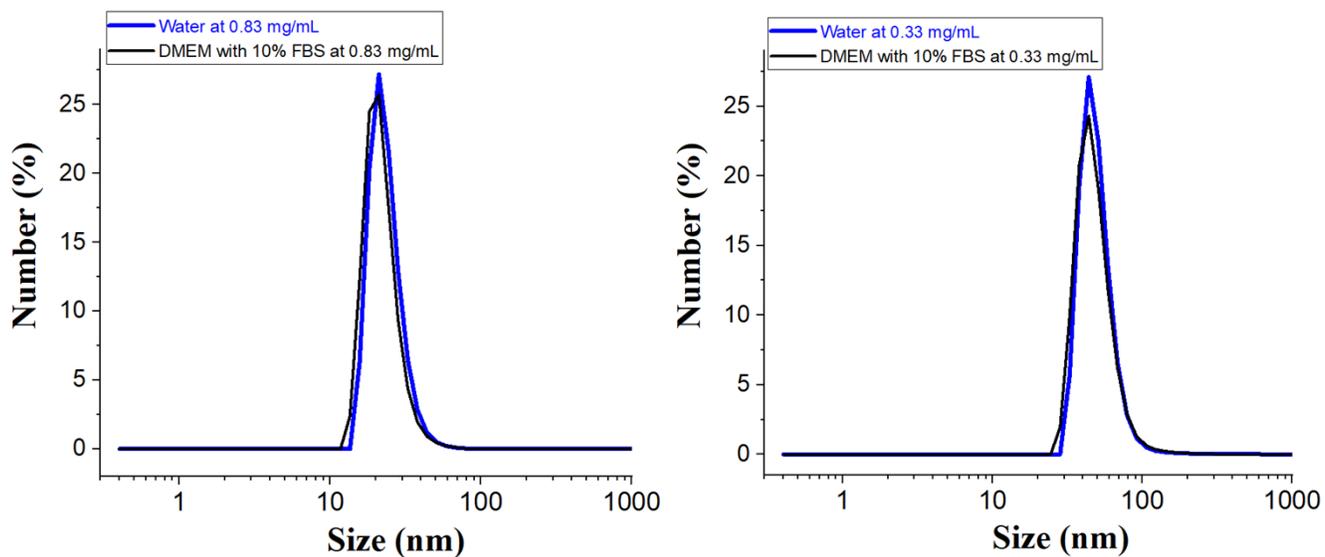

Figure S21. DLS spectra of *EE2*-PEG-AuNPs at 0.83 mg/mL(left) and 0.33 mg/mL (right). The *EE2*-coated NP in water is shown blue, while in DMEM containing 10% FBS is shown black.
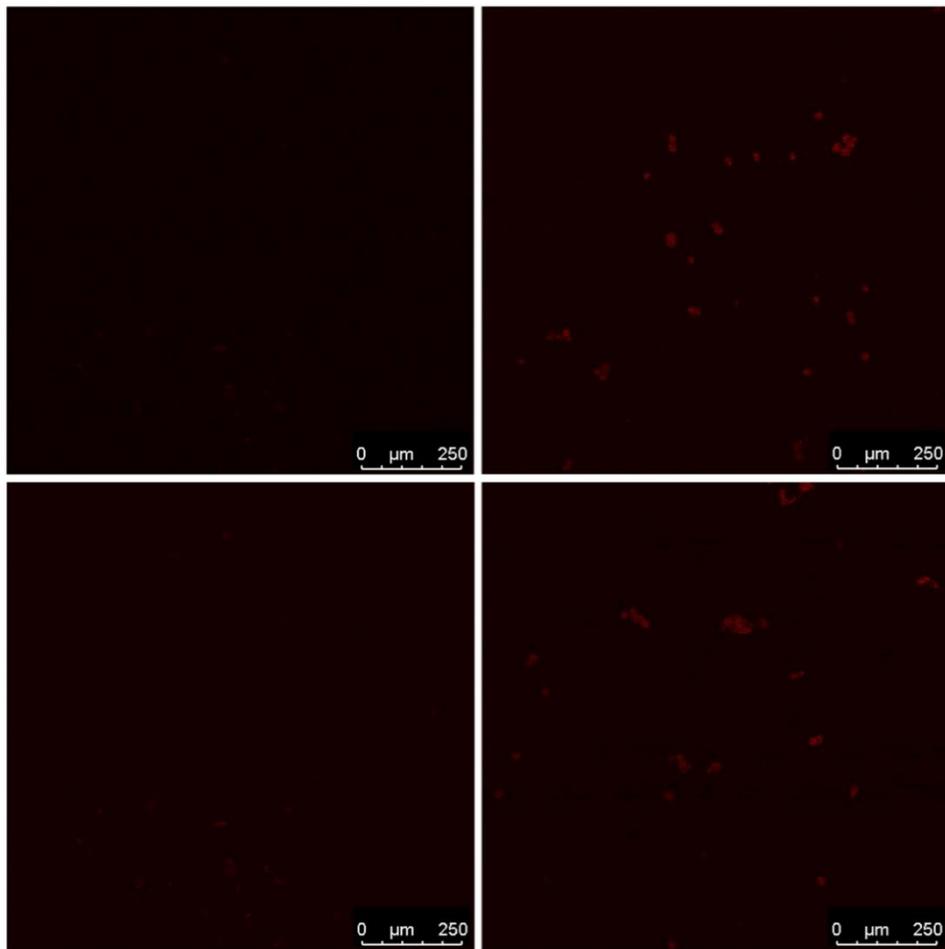
Figure S22. Confocal fluorescence of ER+ human MCF-7 breast cancer cells incubated with (a) medium only and (b) (Alexa 594-EG$_2$)/*EE2*-PEG-AuNPs at 220 µg Au after 24 h (upper panel) and 48 h (lower panel). Laser power was 1% and gain was 694 for all the images.
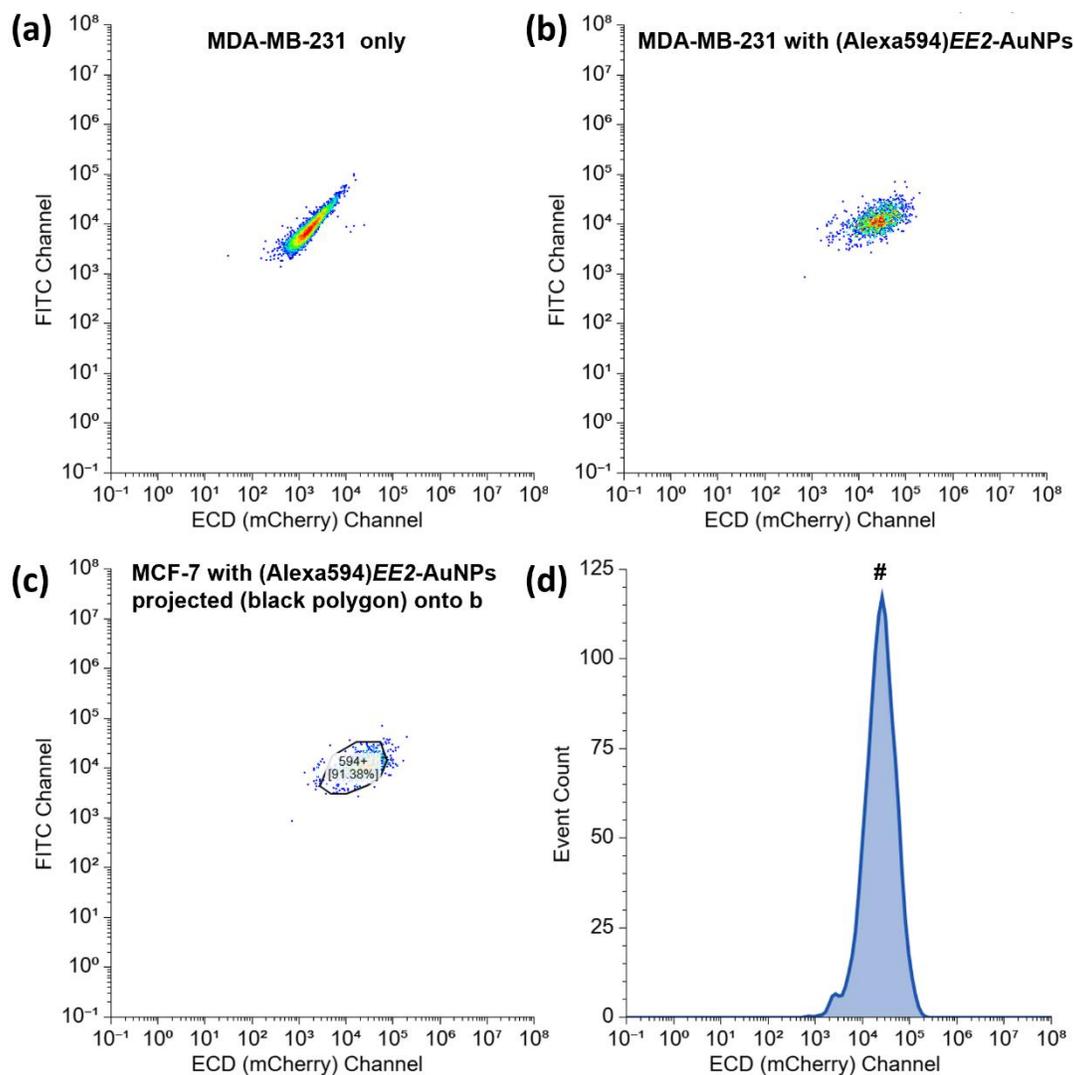
Figure S23. (a-d) Flow cytometry binding studies of MDA-MB-231 cells incubated with (a) medium only and (b) (Alexa 594)/*EE2*-PEG-AuNPs at 119 µg Au/mL showing some fluorescence; (c) binding of (Alexa 594)/*EE2*-PEG-AuNPs at to MCF-7 cells (polygon black line) at the same concentration projected onto the same fluorescent-NP with MDA-MB-231 cells to show that the fluorescence signal stems from the same area; (d) histogram of b, showing a ~5.6 times weaker signal intensity than that of MCF-7 cells (Figure 4d), using the same polygon (gating area). Results are shown after removal of media and washings to remove unbound dye. Fluorescence in the mCherry channel (exc./em. 587/610 nm) is shown over the FITC channel (reference; no fluorescence).