Electronic Supplementary Information

of

Peptide-decorated gold nanoparticles via strain-promoted azide alkyne cycloaddition and post assembly deprotection

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Contents

1. Materials and methods

All chemicals and solvents were used as received unless specified. All solvents were peptide synthesis grade, except water (18.2 MΩ cm). N-Fluorenyl-9-methoxycarbonyl (Fmoc) protected L-amino acids (Fmoc-L-Arg(Pbf)-OH, Fmoc-L-Gly-OH, Fmoc-L-Asp(OtBu)-OH), Fmoc-L-Lys(Boc)-OH, Fmoc-L-Cys(Trt)-OH, [2-(2-(Fmoc-amino)ethoxy)ethoxy]acetic acid (AEEA linker), rink amide MBHA resin (100-200 mesh, loading 1.4 meq/g), 2-chlorotrityl chloride resin (100-200 mesh, loading: 1.5 meq/g), o-(benzotriazol-1-yl)-N,N,N',N'-tetramethyluronium hexafluorophosphate (HBTU), diisopropylethylamine (DIEA), trifluoroacetic acid (TFA), trethylsilane (TES) and piperidine were commercially available. All the materials for preparation of azide gold nanoparticles were purchased and used as previous reported. [1]

^{1}H NMR spectrum was recorded using a Mercury 400 spectrometer with CD_3CN, D_2O, $CDCl_3$ as solvents and the residual solvent was used as reference. Thermogravimetric analysis (TGA) was recorded by placing the sample into a 70 μL ceramic crucible and heating it from 25-750 ºC at a rate of 10ºC min^{-1}. The TGA was run under a flow of nitrogen of 70 mL/min in a Mettler Toledo TGA/SDTA 851 instrument.

Transmission electron microscopy (TEM) images were recorded from a TEM Philips CM10. Infrared spectra were recorded using a Bruker Vector33 spectrometer and making a thin film of the sample onto a KBr disk. High resolution mass spectra (HRMS) were obtained using electrospray ionization (ESI).

2. Synthesis of "DBCO"-RGD peptide

The "DBCO"-RGD peptide was synthesized manually via standard Fmoc SPPS procedure by using rink amide MBHA resin as solid support. Briefly, the peptide chain including RGD and a linker was grown on the resin and DBCO acid was coupled to terminate the peptide. The coupling of each residue used 5 eq. (relative to the loading of resin) Fmoc-protected amino acids, 5 eq. of HBTU and 10 eq. of DIEA in a DMF solution for 4 hours. During the synthesis, Fmoc protecting group was deprotected using 20% piperidine/DMF (v/v) for 15 minutes. In the end of the synthesis, DBCO acid was conjugated to the peptide following the above-mentioned procedure except that 1.1 eq. DBCO acid, 1.1 eq. HBTU and 2.2 eq. DIEA were used. After the completion of the synthesis, the resin was washed with DMF and DCM (each for four times) and dried under vacuum for 24 hours. Cleavage of the expected peptide and the removal of side chain protecting groups from the dried resin were performed by suspending the resin in cleavage cocktail containing TFA (95%) and TES (5%) for 1 hour. The filtration was concentrated to a viscous solution by flushing N_2. After the precipitation in ice cold ether, the crude product was collected, dissolved in MQ water water and freeze-dried.

The characterization of "DBCO"-RGD peptide with calculated mass 849.3657 was performed by ESI-MS and found 849.3395 [1+]. Purity: 98.7% determined by high-performance liquid chromatography (HPLC) with a C18 column and using a linear gradient of acetonitrile and MQ water containing 0.05% TFA.

3. Synthesis of DBCO-(PG)RGD peptide

The DBCO-(PG)RGD peptide was synthesized manually via standard Fmoc SPPS procedure by using 2-chlorotrityl chloride resin as solid support. The coupling of the first residue used 1.5 eq. Fmoc-protected amino acid and 4 eq. of DIEA in a DCM solution for 2 hours. Other amino acid couplings and DBCO acid coupling were carried out followed by the above-mentioned procedure. Cleavage of the protected peptide from the dried resin was performed by suspending the resin in cleavage cocktail containing TFA (5%), TES (5%) and DCM (90%) for 1 hour. The filtration was concentrated to a viscous solution by flushing N_2. The crude product was dissolved in acetonitrile and purified by HPLC. The characterization of DBCO-(PG)RGD peptide with calculated mass 1259.5784 was performed by ESI-MS and found 1260.5776 [1+]. Purity: 96.5% determined by high-performance liquid chromatography (HPLC) with a C18 column and using a linear gradient of acetonitrile and MQ water containing 0.05% TFA.

4. Synthesis of DBCO-(PG)CRGDK

The synthesis was performed as described for DBCO-(PG)RGD. Cleavage of the protected peptide was performed by suspending the resin in cocktail containing 0.5%TFA/DCM for 15 min. The filtration was concentrated to a viscous solution by flushing N_2. The crude product was dissolved in acetonitrile and purified by HPLC. The characterization of DBCO-(PG)CRGDK with calculated mass 1659.7393 was performed by ESI-MS and found 1660.6294 [1+]. Purity: 94.8% determined by high-performance liquid chromatography (HPLC) with a C18 column and using a linear gradient of acetonitrile and MQ water containing 0.05% TFA.

5. Synthesis of azide AuNPs

In a typical synthesis, 50.0 mg of Me-EG_3-AuNP were transferred into a clean 25 mL round bottom flask. This compound was dissolved in 10 mL of acetone. Then 10.0 mg (42.5 μmol) of N_3-EG_4-AuNP were transferred into this solution. The reaction was stirred vigorously for 20 min. After this time, the acetone was immediately evaporated off. The thin film of nanoparticles was washed first with hexanes (in which AuNP are not soluble) and the solvent was removed under vacuum. Subsequently the film was quickly rinsed with isopropanol three times. This entire washing procedure was repeated three times until the smell of the thiol was gone. 44.9 mg of nanoparticles were obtained. The nanoparticles were redissolvable readily in H_2O, acetone, acetonitrile, methanol,

ethanol, DMF, DMSO and DCM with little to no aggregation. and characterized by ^{1}H NMR and IR. ^{1}H NMR (CD_3CN, 400 MHz): δ_H (ppm): 3.60, 3.49, 3.39, 3.31. ^{1}H NMR (D_2O, 400 MHz): δ_H (ppm): 3.66, 3.57, 3.43, 3.32. IR (KBr disk, cm^{-1}): 2921, 2871, 2101, 1443, 1349, 1292, 1244, 1198, 1119, 1033.

6. Bioconjugation of azide AuNPs with RGD peptide via SPAAC-PAD

DBCO-(PG)RGD (1.2 eq. of DBCO relative to azide substitution on AuNPs) and azide AuNPs were mixed in 3 ml acetonitrile at room temperature for 1 hour. After that, the AuNPs were purified by using centrifugal filter devices (10KDa MWCO) and washed with 60% MeOH/H_2O until no unreacted peptide can be found be ESI-MS. The resulting AuNPs were treated with 90% TFA/DCM at room temperature overnight and the solvent removed by flushing N_2. The obtained AuNP-RGD were purified by dialysis against water using Cellulose ester dialysis membranes MWCO of 10 kDa for 2 days and characterized by IR, TEM and ESI-MS. IR (KBr disk, cm^{-1}): 3370, 2930, 2110, 1685, 1109.

7. Bioconjugation of azide AuNPs with DBCO-(PG)CRGDK

The bioconjugation was performed as described for AuNP-RGD. The obtained AuNP-CRGDK after SPAAC-PAD was characterized by IR and ESI-MS. IR (KBr disk, cm^{-1}): 3370, 2930, 2692, 2110, 1685, 1446, 1346, 1109.

8. Calculation of nanoparticle formula

Assuming that the AuNP are spherical and that their size is monodisperse (3 nm) it is possible to calculate an approximate molecular formula for azide AuNPs. The number of gold atoms (N_{Au}) can be calculated using the following formula:

$$N = \frac{\pi \rho d^3 N_A}{6 M_{Au}}$$

Wherer is the density of the face centered cubic (fcc) gold lattice (19.3 g cm^{-3}), *d* is the average diameter of the nanoparticles in centimeters found from the TEM images, M_{Au} is the mole atomic weight of gold (196.9665 g mol^{-1}), and N_A is Avogadro constant.

The number of thiol ligands surrounding the gold core (N_L) can be calculated using the following formula:

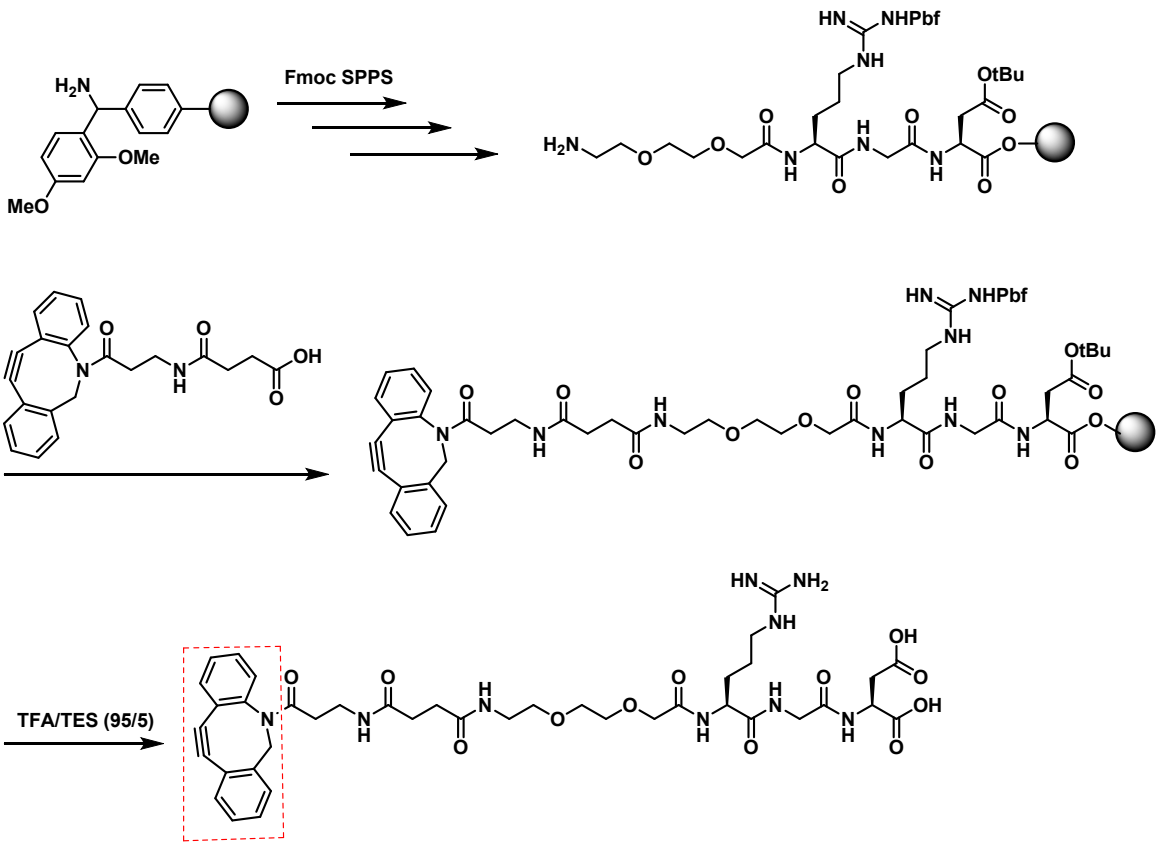
$$N_L = \frac{N M_{Au} W_{\%}}{(1 - W_{\%})[MW_{N_3 - EG_4 - S} M_{\%} + [MW_{Me - EG_3 - S}(1 - W_{\%})]]}$$

Where W% is the percentage of mass loss due to the organic ligands found through TGA measurements, $MW_{Me\text{-}EG3\text{-}S}$ is the molecular weight of the thiolate ligand, $MW_{N3\text{-}EG4\text{-}S}$ is the molecular weight of the azide thiolates ligand, and M% is the mole percentage of azide ligand.

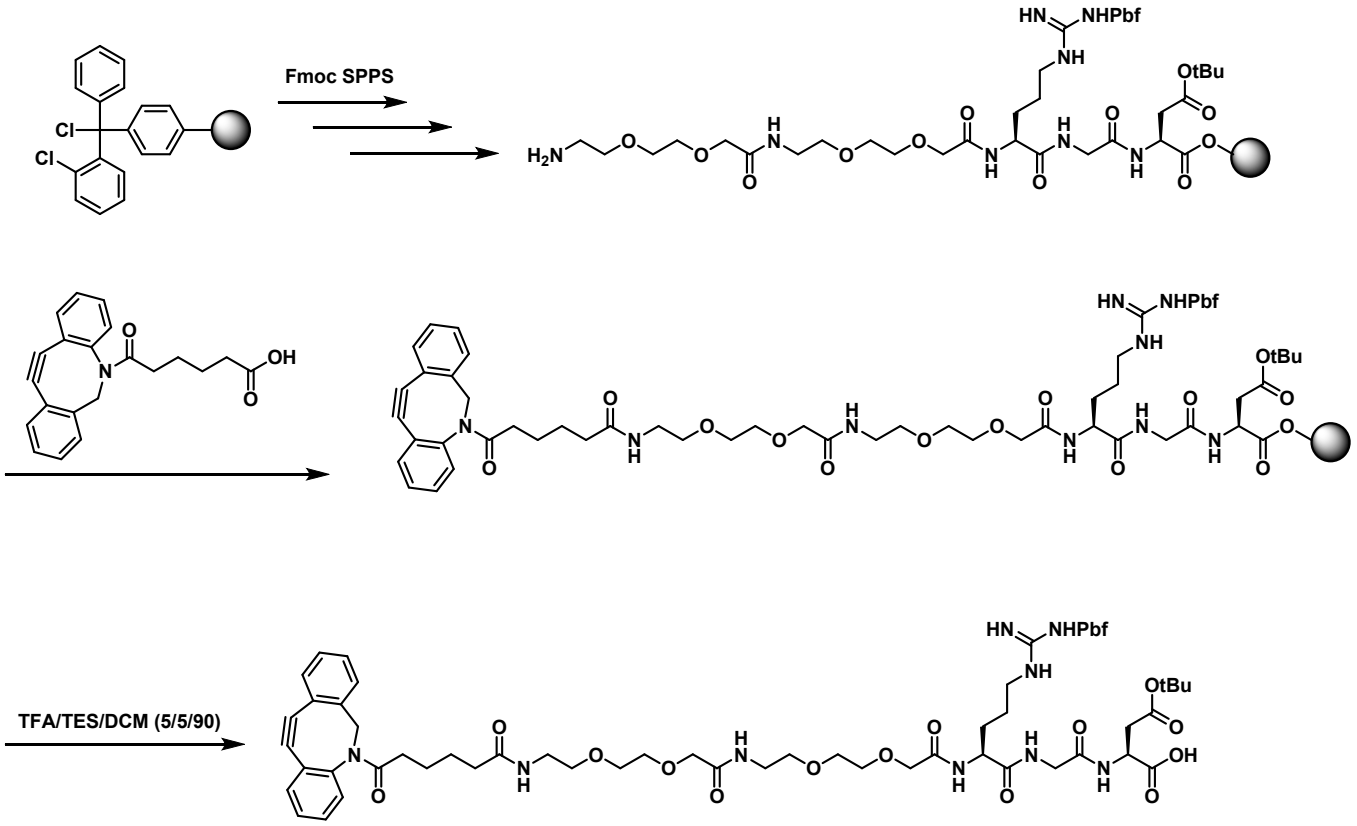
Supplementary References

[S1] P. Gobbo, S. Novoa, M. C. Biesinger, M. S. Workentin, *Chem Commun. (Camb)*, **2013**, *49*, 3982.

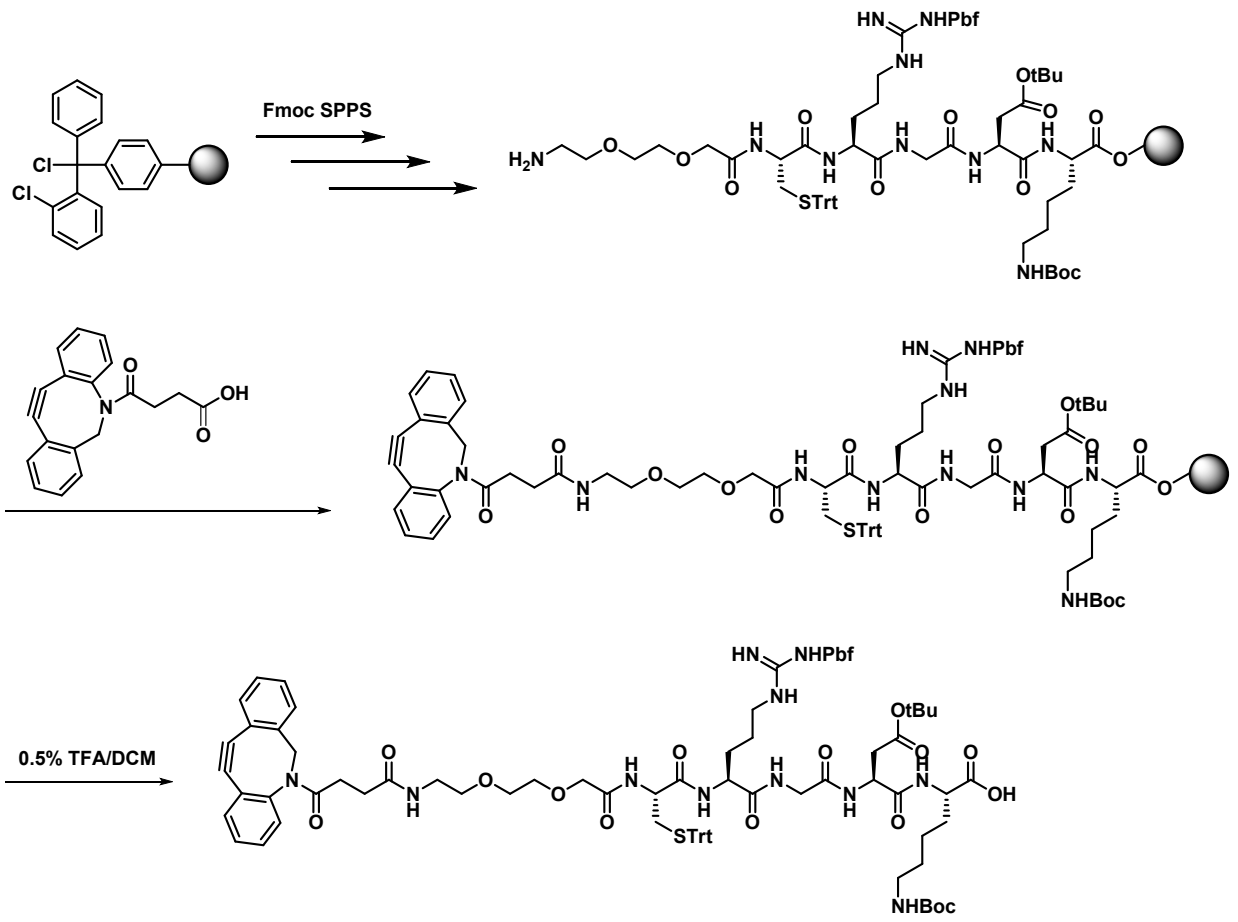
Supplementary Schemes and Figures



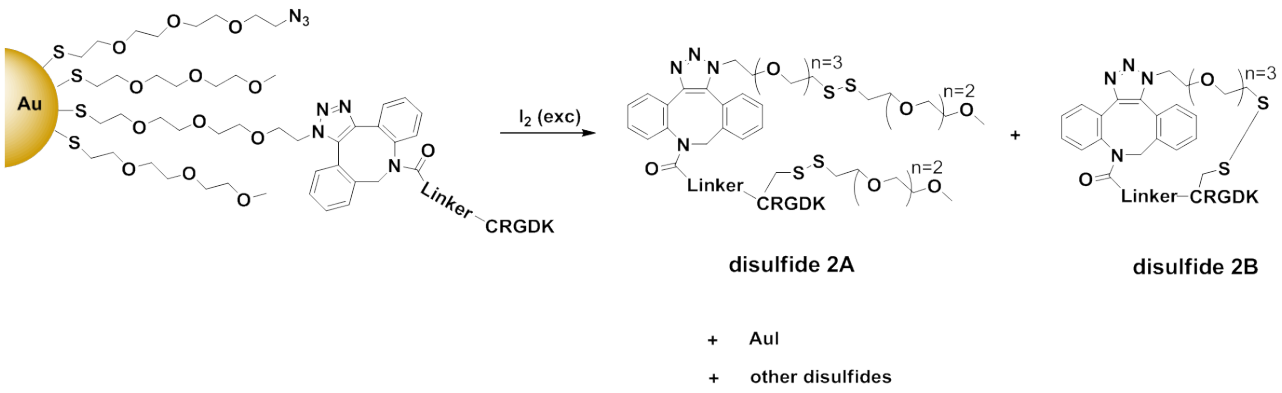
Scheme S1. Synthesis of DBCO-RGD peptide.



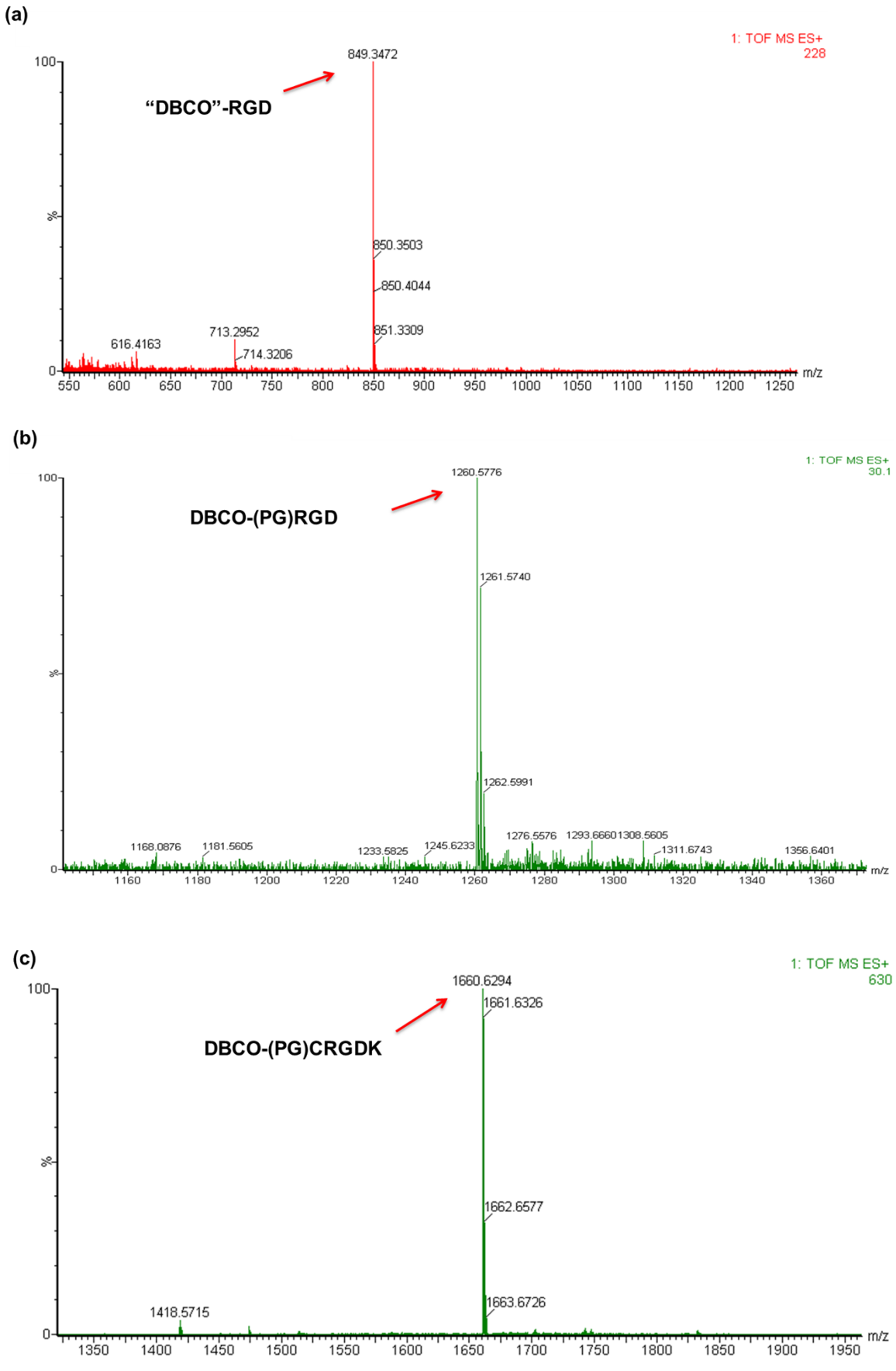
Scheme S2. Synthesis of DBCO-(PG)RGD peptide.



Scheme S3. Synthesis of DBCO-(PG)CRGDK peptide.



Scheme S4. Re-oxidation of CRGDK-AuNPs by iodine. Disulfides **2A** and **2B** was characterized by ESI-MS. **2A** calculated 1600.6645 and found 800.2318 [2+] m/z. **2B** calculated 1242.5162 and found 621.6824 [2+] m/z.


(a)
1: TOF MS ES+
228
100
%
0
849.3472
"DBCO"-RGD
850.3503
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713.2952
714.3206
616.4163
550 600 650 700 750 800 850 900 950 1000 1050 1100 1150 1200 1250
m/z
(b)
1: TOF MS ES+
30.1
100
%
0
1260.5776
DBCO-(PG)RGD
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1262.5991
1168.0876
1181.5605
1233.5825
1245.6233
1276.5576
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1356.6401
1160 1180 1200 1220 1240 1260 1280 1300 1320 1340 1360
m/z
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%
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m/z

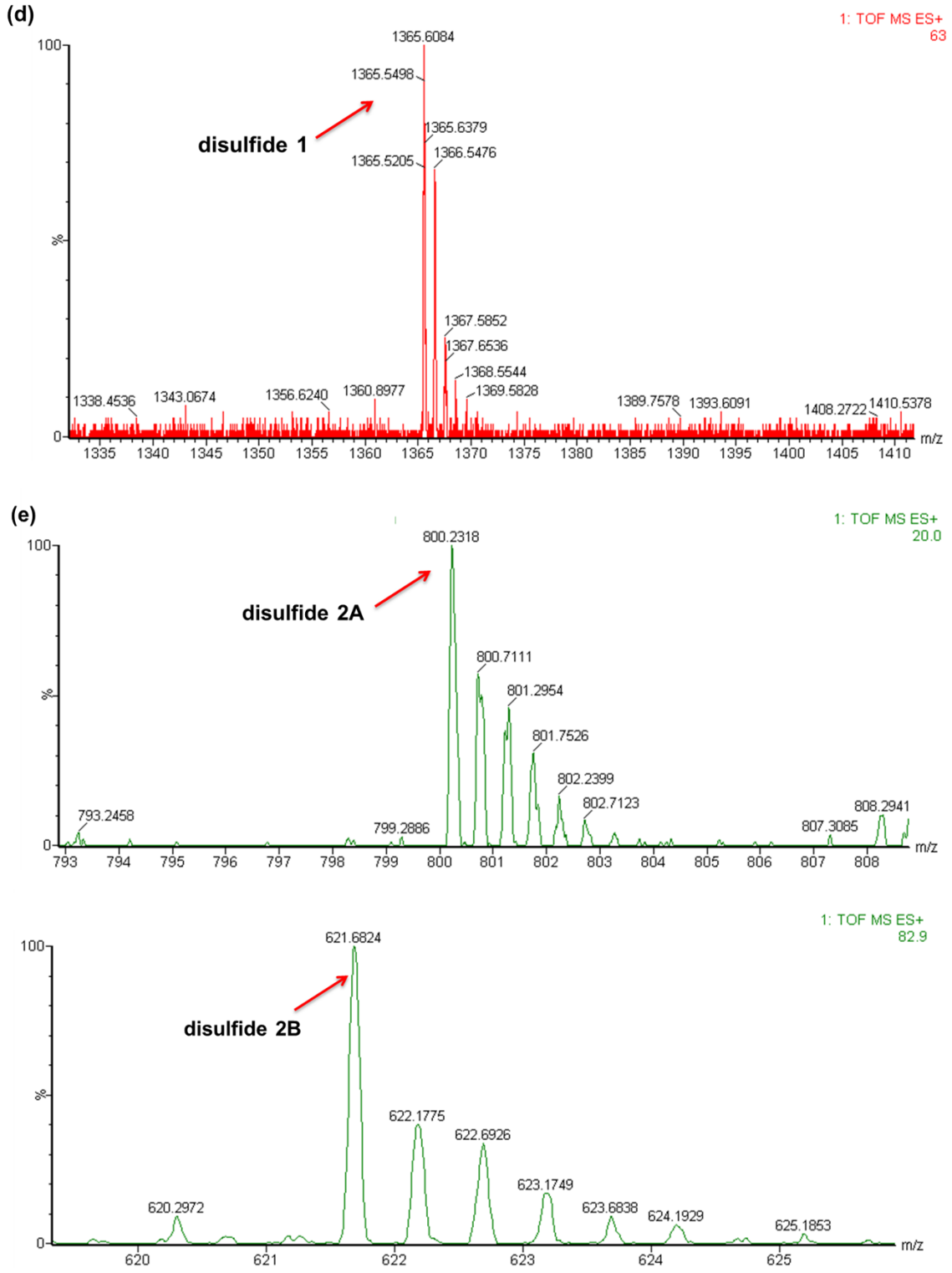
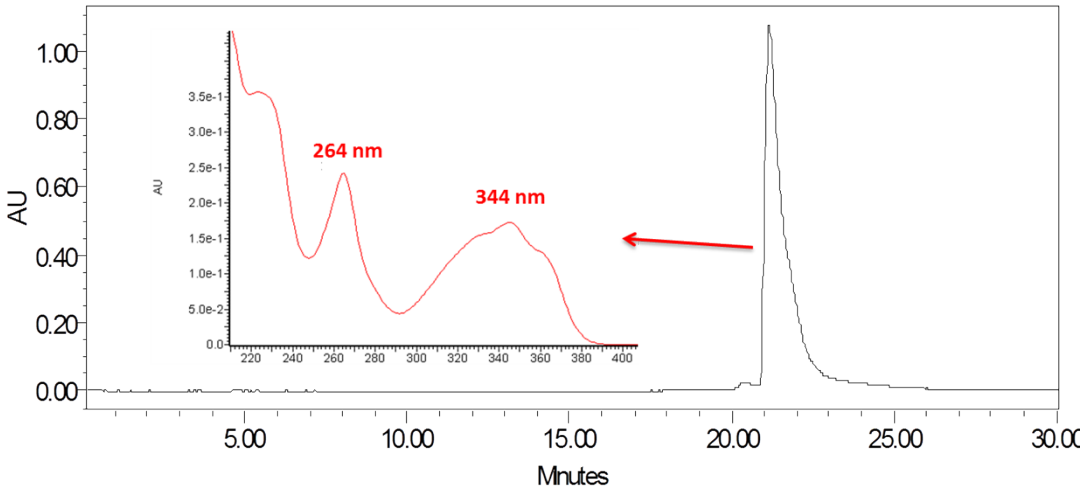


Figure S1. ESI-MS analysis of (a) "DBCO"-RGD (b) DBCO-(PG)RGD (c) DBCO-(PG)CRGDK (d) disulfide **1** from re-oxidation of AuNP-RGD by iodine (e) disulfide **2A** and **2B** from re-oxidation of AuNP-CRGDK by iodine.

(a)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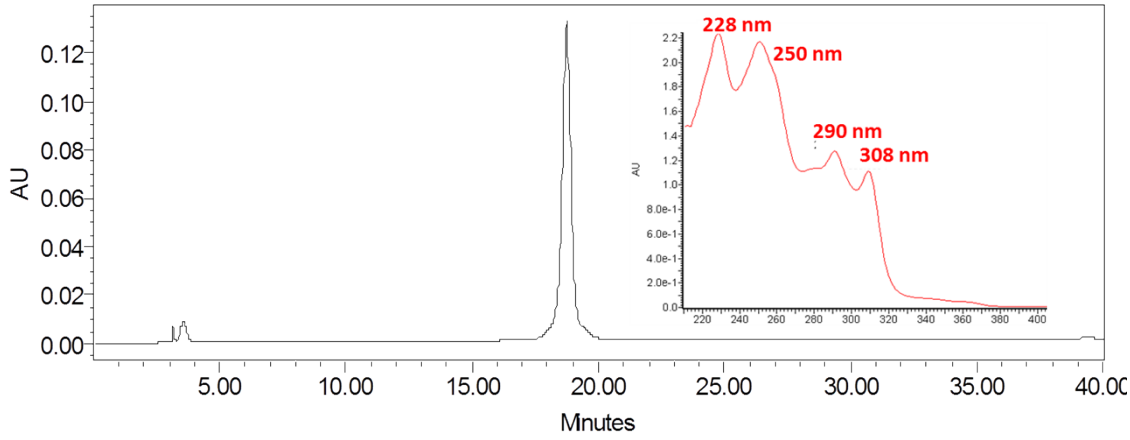
344 nm
AU
Minutes

(b)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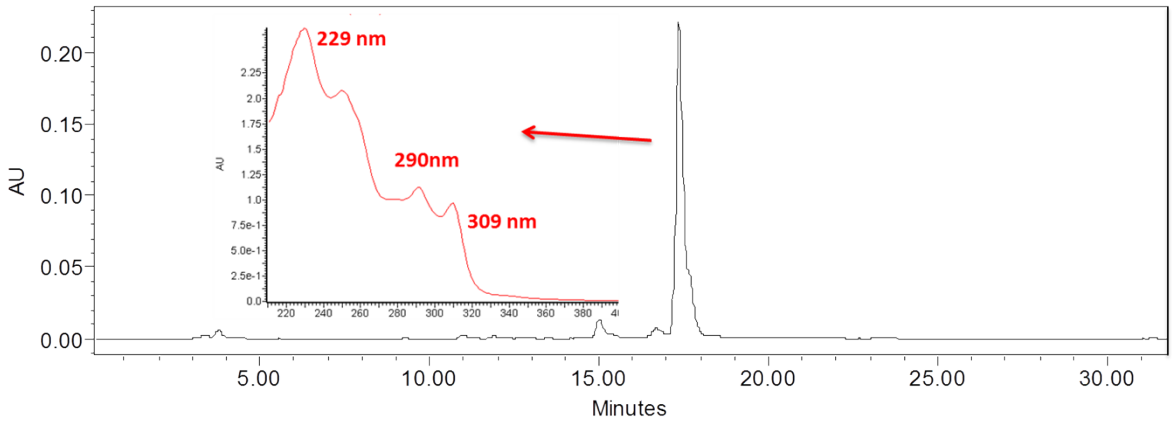
290 nm
308 nm
AU
Minutes

(c)


229 nm
290nm
309 nm
AU
Minutes

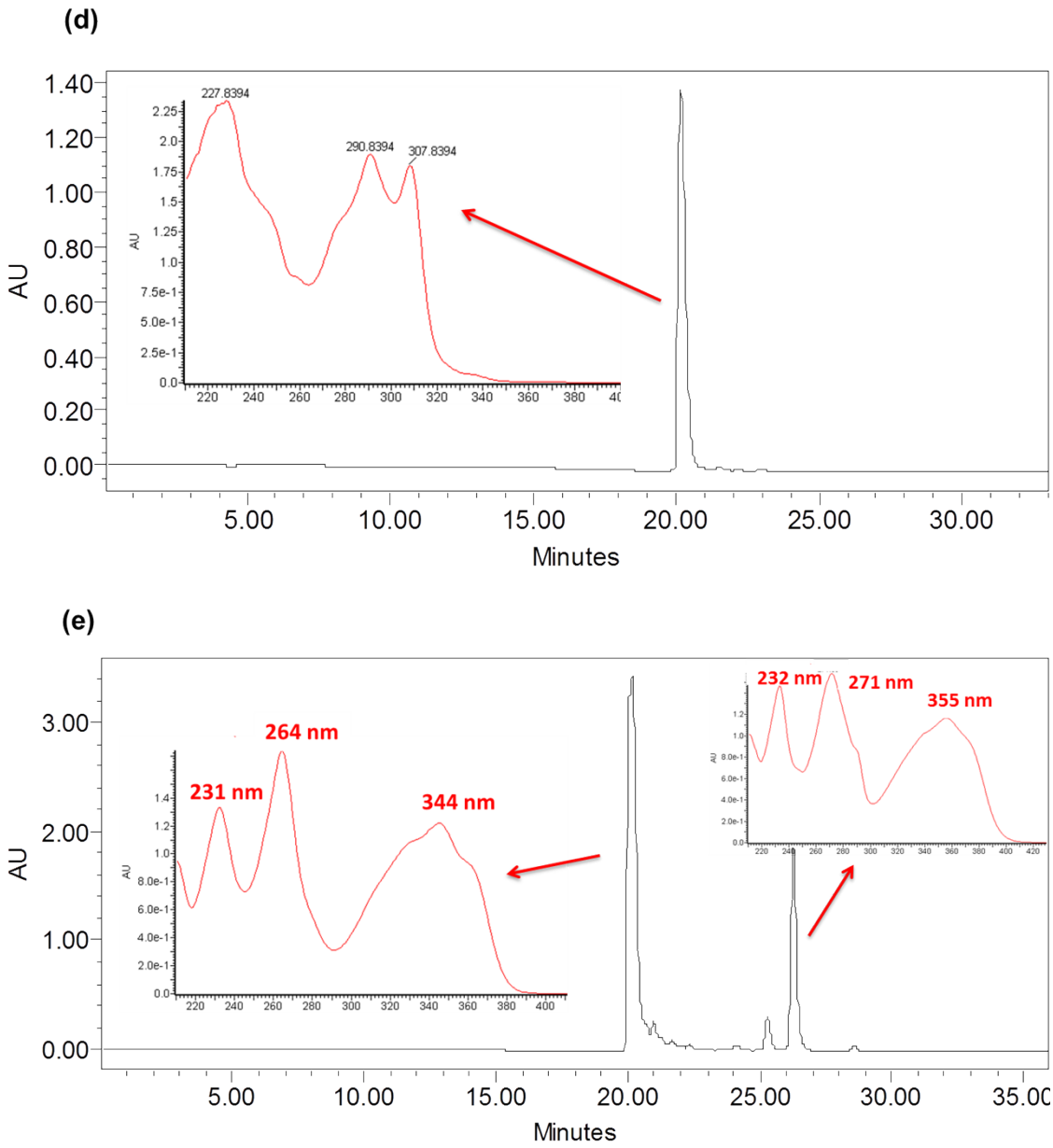


Figure S2. HPLC analysis at 265 nm of (a) "DBCO"-RGD (b) DBCO-(PG)RGD (c) DBCO-(PG)CRGDK (d) DBCO amine and (e) decomposition of DBCO after treatment with concentrated TFA.

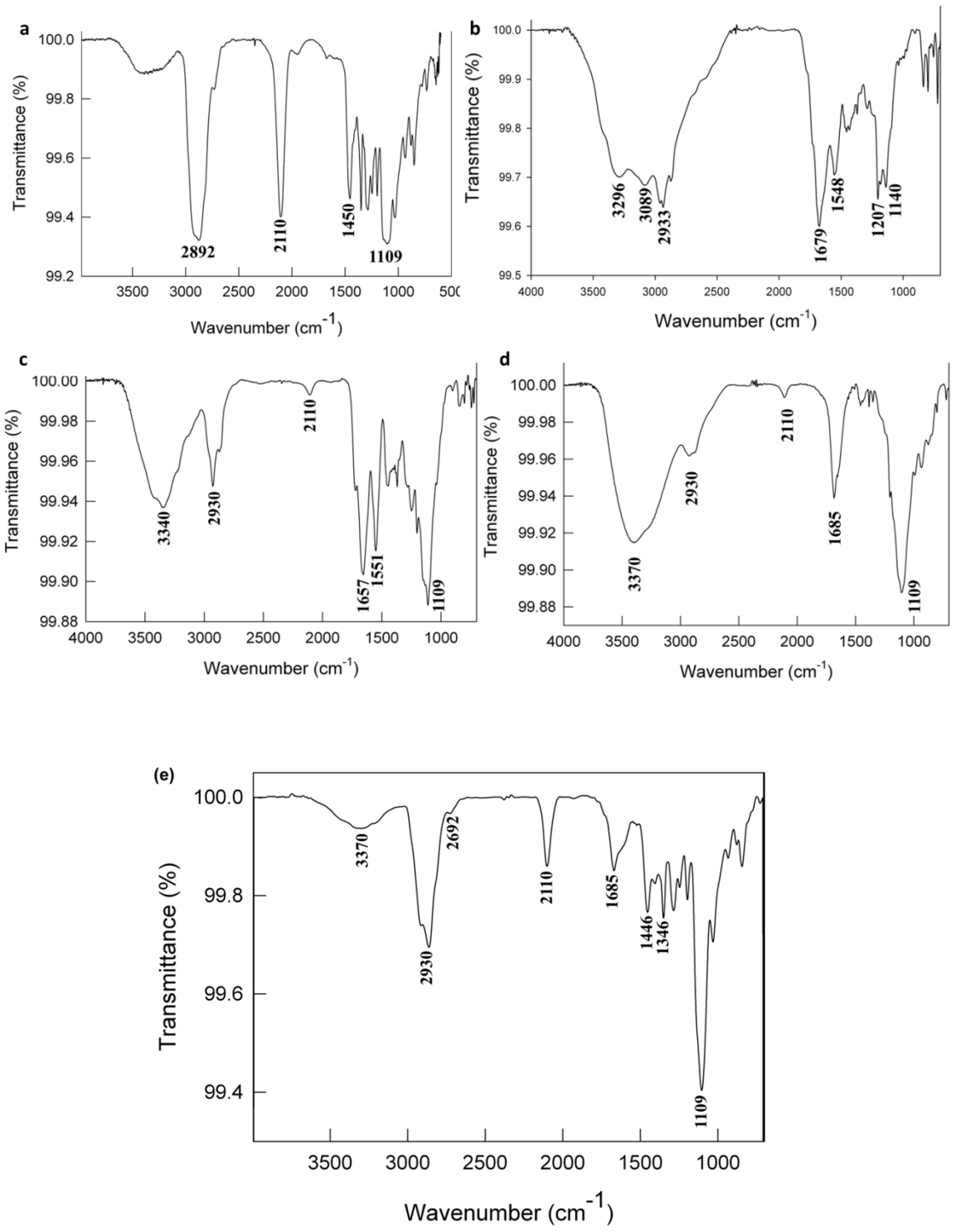


Figure S3. IR spectra of (a) azide AuNPs, (b) DBCO-(PG)RGD, (c) (PG)RGD-AuNPs (d) RGD-AuNPs (e) CRGDK-AuNPs.

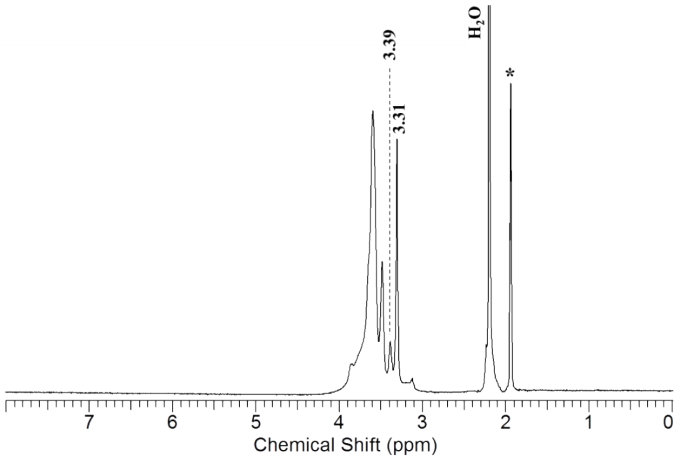


Figure S4. ^{1}H NMR spectra of azide AuNP recorded in acetonitrile-d_3 and calibrated against residual ACN (*).

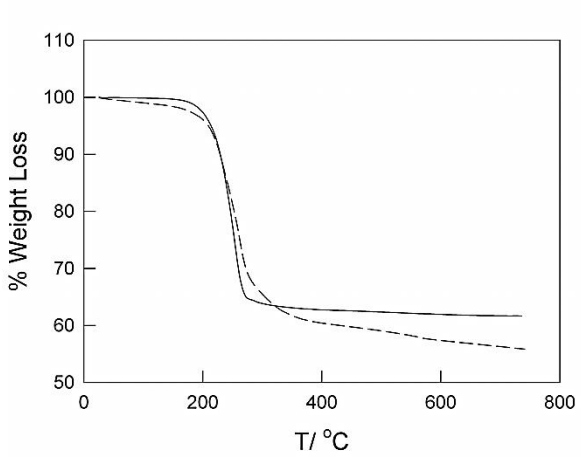


Figure S5. TGA of control Me-EG_3-AuNP (solid line) and of N_3-EG_4-AuNP (dashed line).

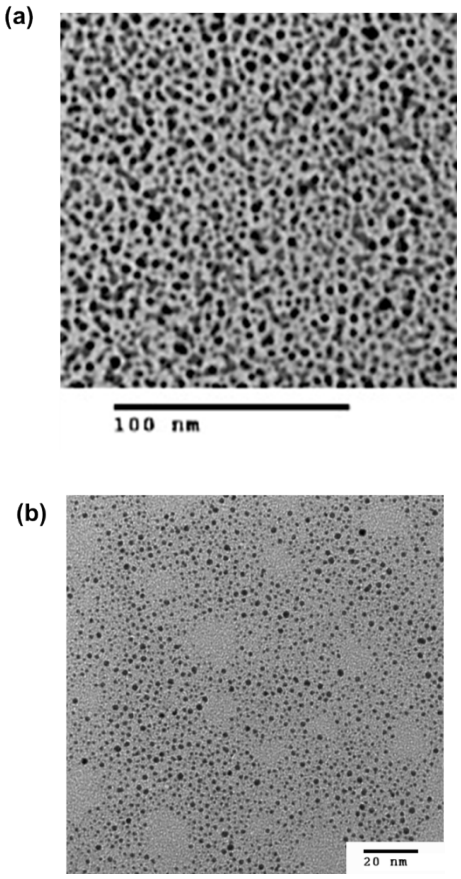
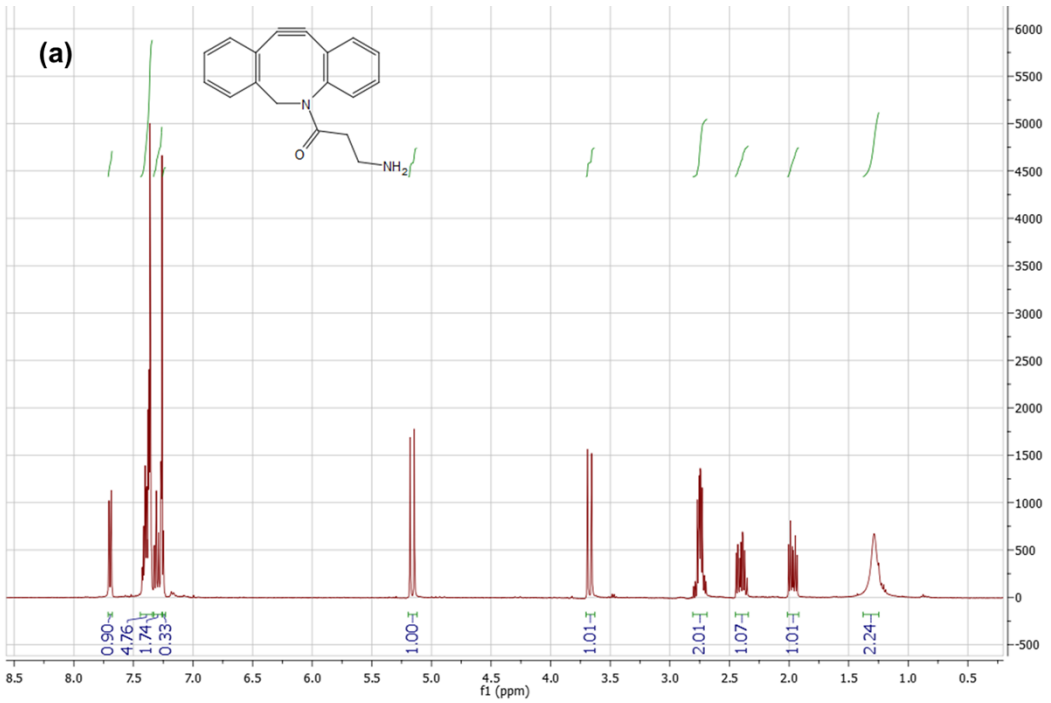


Figure S6. TEM image of (a) azide AuNPs (b) RGD-AuNPs.


(a)
N
O
NH2
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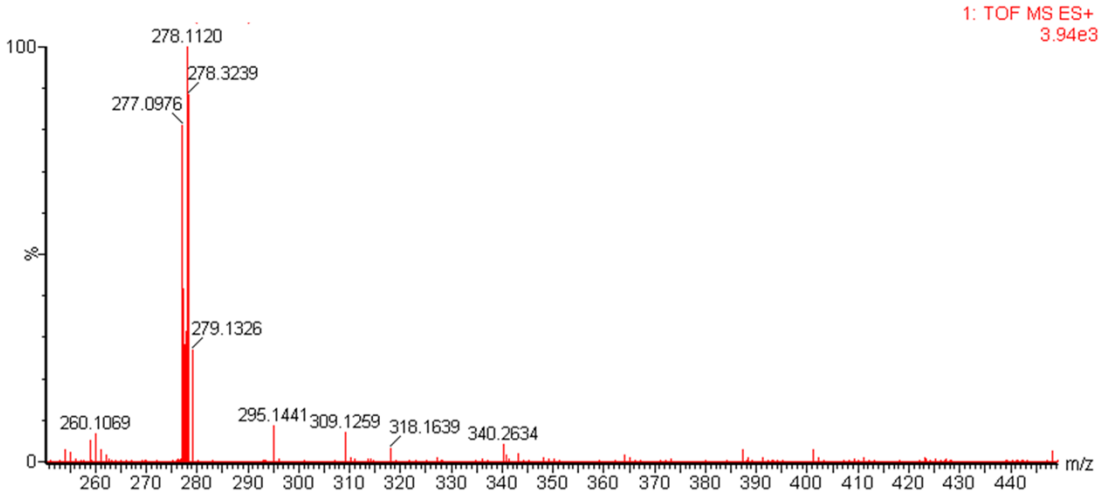
1.74
0.33
1.00
1.01
2.01
1.07
1.01
2.24
f1 (ppm)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100
%
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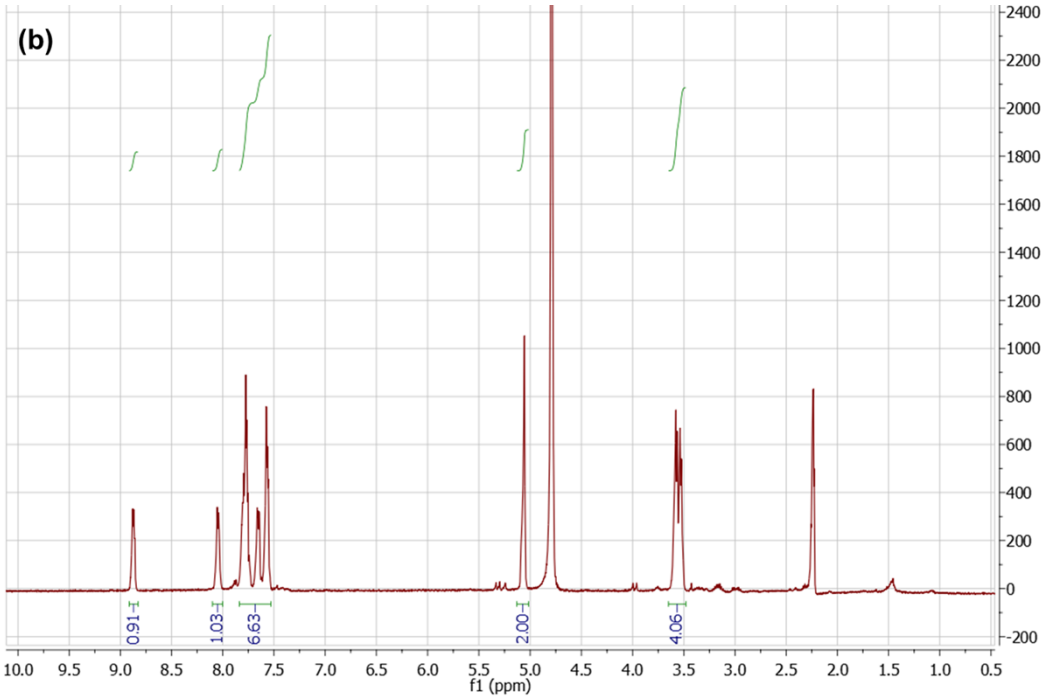
309.1259
318.1639
340.2634
m/z


(b)
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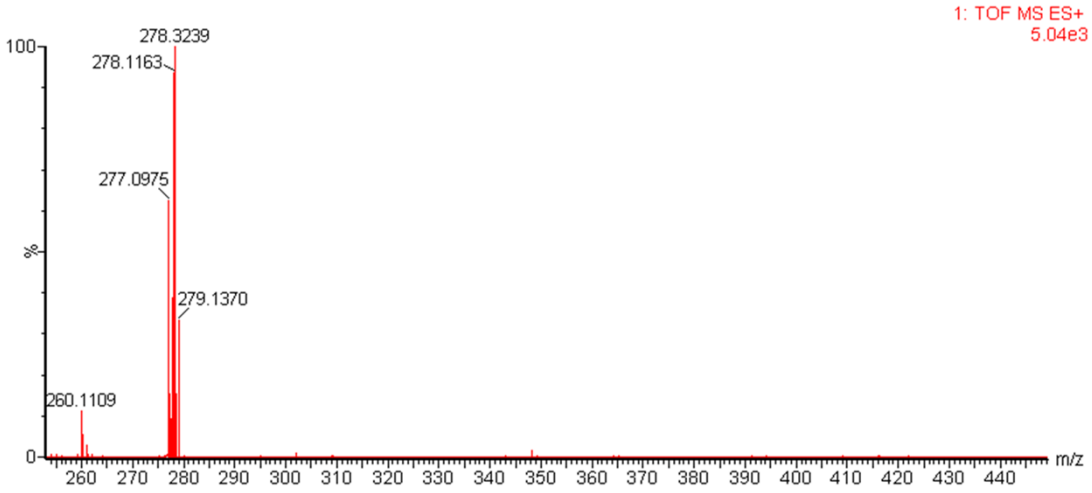
1.03
6.63
2.00
4.06
f1 (ppm)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%
m/z

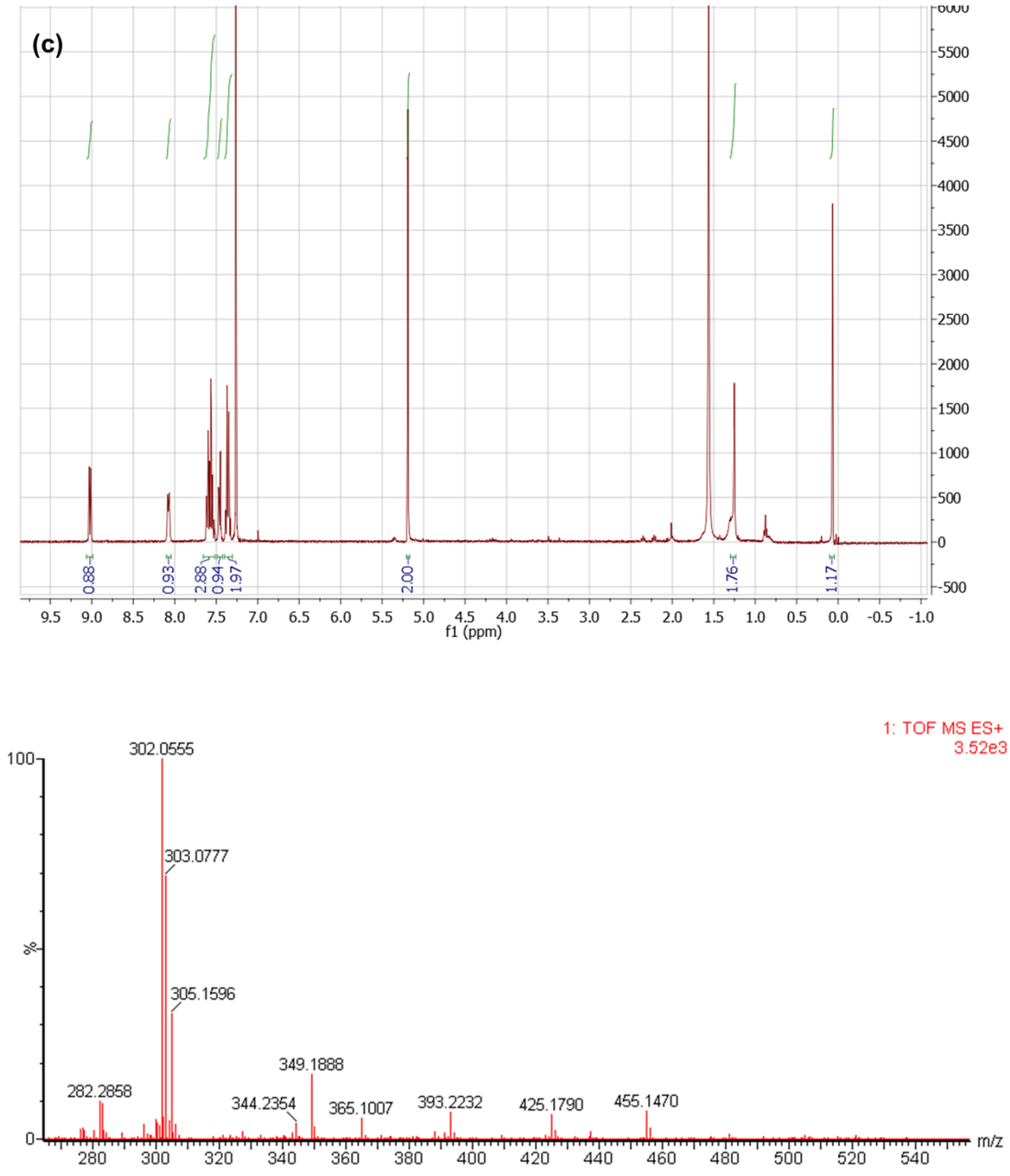

Figure S7. ^{1}H NMR (top) and positive mode ESI-MS (bottom) spectra of (a) DBCO amine (found mass: 278.1120 $[M+H]^{+}$); (b) isolated compound (found mass: 278.3239 [+1]) and (c) isolated compound (found mass: 302.0555 [+1]) after treatment of DBCO amine with concentrated TFA. ^{1}H NMR spectra were recorded in $CDCl_3$, D_2O/ACN-d_3 (2:1) and $CDCl_3$, respectively.