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Supplementary Information

for

Interfacial tetrazine click chemistry mediated assembly of multifunctional colloidosomes

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1 General materials

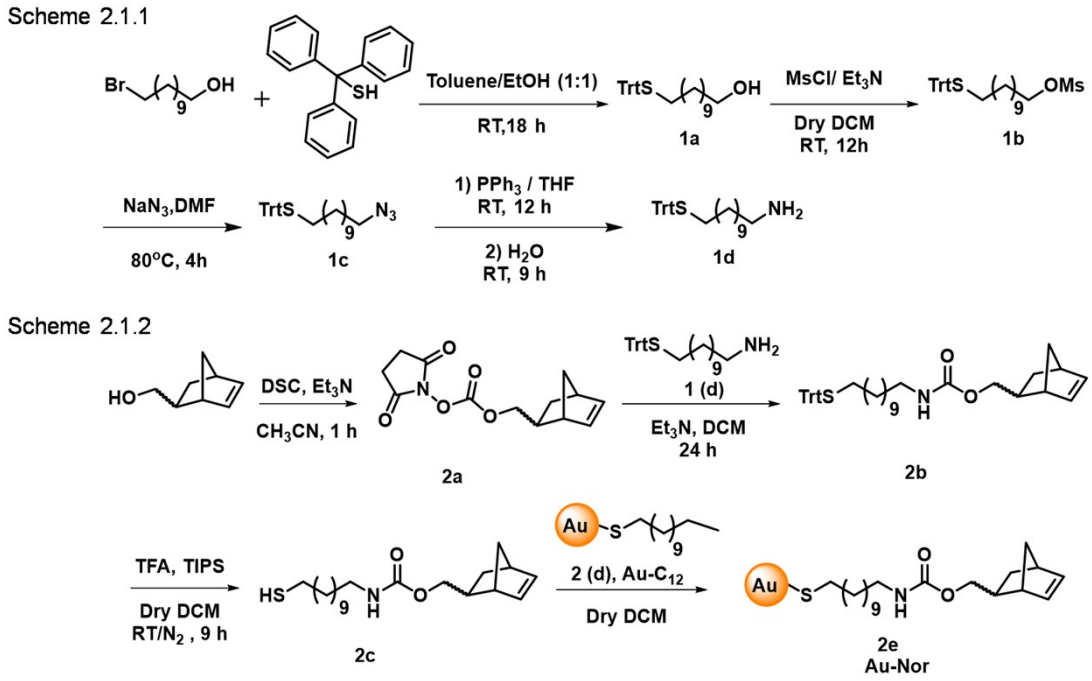
All chemicals were purchased from commercial sources and used without further purification. All reactions were performed at ambient conditions unless specified. Flash chromatography was carried on silica of 230-400 mesh and gravity column on silica of 60-120 mesh. ^{1}H-NMR was performed on 400 MHz Bruker AVANCE 400 FT-NMR spectrometer and data was analysed on spinworks_4.0.5. Mass analysis was performed on Electrospray Ionisation Mass Spectrometry (ESI-MS) from central mass spectrometry (Agilent 6538 UHD Accurate Mass Q-TOF LC-MS) facility at JNCASR. Optical and fluorescence microscopy images were captured on Zeiss Axio Vert A.1 inverted microscope. Zeba spin desalting column, used for nanoparticle purification (7K MWCO), was purchased from Thermo Scientific. Transmission Electron Microscopy (TEM) was performed on JEOL JEM-3010 operating at 300 kV. Atomic Force Microscopy (AFM) images were acquired on Bruker Innova instrument operating in tapping mode with cantilever resonant frequency 300 kHz, scan size 50 µm and scan rate 100 µm/s. All images obtained were analysed on Nanoscope Analysis software. MALDI experiment was performed in the reflection mode on Bruker Daltonics (Autoflex speed time-of-flight mass spectrometer) equipped with a Bruker smartbeam-II (Nd:YAG, 355 nm wavelength) laser. Amalgamator used here was commercially obtained from Unident Instruments (india) Pvt. Ltd. with power supply 220V-230V AC. SEM-EDAX experiment was performed on ZEISS Gemini SEM 500 at a probe current of 20 nA with EHT (Electron High Tension) 20KV. All Raman and SERS measurements were carried on Horiba LabRAM HR Evolution with a Nd-YAG laser of 532nm wavelength with 2-3 mW laser power at the sample.

Caution: Anhydrous hydrazine is explosive and not commercially available, therefore we used hydrazine hydrate. 1,2,4,5- tetrazine has a nitrogen rich environment, therefore can be highly reactive. Care should be taken when performing at high scale.

2 Synthetic method

Compound 1a and 1b[1], 2a[2], 2d[3], 3a[4], 4a and 4b[5], 4c[6], 5b[1] were synthesized according to the reported procedure.

Scheme 2.1



Scheme 2.1: Synthesis of norbornene functionalised gold nanoparticles **(Au-Nor)**

Compound 1c

In a 250 ml RB flask, compound 1b (3 g, 5.716 mmol) was dissolved in 25 ml dry DMF followed by subsequent addition of NaN_3 powder (743 mg, 11.432 mmol) to the solution. The mixture was stirred at 80^0C for 4 h. Reaction mixture was then cooled down to RT and solution was poured into 120 ml ice-water. The reaction mixture was extracted by DCM (3 x 50 ml). The organic layers were combined and washed with brine and dried over Na_2SO_4. The crude product was adsorbed on silica gel and purified by flash chromatography (eluent: 5% EtOAc in hexane). Yield 1g (2.119 mmol, 37%). ^{1}H-NMR (400 MHz, $CDCl_3$): 7.34 (m, 6H, $TrtH_{Ar}$), 7.2 (m, 6H, $TrtH_{Ar}$), 7.13 (m, 3H, $TrtH_{Ar}$), 3.17 (t, 2H, CH_2 -N_3, J =6.8 Hz), 2.06 (t, 2H, S-CH_2, J =7.2 Hz), 1.51 (m, 2H, **CH_2**-CH_2 -N_3, J =6.8 Hz), 1.1-1.4 (m, 18H, -CH_2-).

Compound 1d

In a 100 ml RB flask, compound 1c (1 g, 2.119 mmol) was dissolved in 20 ml dry THF. To this, PPh_3 (556 mg, 2.119 mmol) was added and stirred overnight at RT in N_2 atmosphere. After that 40 ml H_2O was added and then again stirred for 9 h at RT. Reaction mixture was filtered to remove solid and filtrate was extracted with DCM (3 times), washed with brine solution (2 times) and concentrated under reduced pressure. The TLC was checked in 10% MeOH in DCM. Crude sample was charged

on silica gel and purified by column chromatography (eluent: 10% MeOH in DCM). Compound 1d was thus obtained as yellow colour liquid. Yield 400 mg (0.898 mmol, 42%). ^{1}H-NMR (400 MHz, $CDCl_3$): 7.34 (m, 6H, $TrtH_{Ar}$), 7.20 (m, 6H, $TrtH_{Ar}$), 7.12 (m, 3H, $TrtH_{Ar}$), 2.61 (t, 2H, CH_2 –NH_2, J = 6.8 Hz), 2.05 (t, 2H, SCH_2, J = 7.2 Hz), 1.1-1.4 (m, 18, -CH_2-).

Compound 2b

In a 25 ml RB flask, compound 2a (5-Norbornene-2-methanol, mixture of endo and exo isomer) (77 mg, 0.292 mmol) was dissolved in 4 ml dry DCM. To it compound 1d (65 mg, 0.146 mmol) was added followed by subsequent addition of Et_3N (0.06 ml, 0.43 mmol) and reaction mixture was stirred at RT for 24 h and was monitored by TLC (20% EtOAc in Hexane). Then DCM and Et_3N were evaporated under reduced pressure. The residue obtained was adsorbed on silica gel and purified by flash column chromatography (eluent: 20% EtOAc in Hexane v/v). Compound 2b was obtained as yellow liquid. Yield 33 mg (0.056 mmol, 40%). HRMS (ESI) calculated for $C_{39}H_{49}NO_2S$ was 595.3484, found 618.3364 $[M+Na]^+$. ^{1}H-NMR (400 MHz, $CDCl_3$): 7.33 (m, 6H, $TrtH_{Ar}$), 7.20 (m, 6H, $TrtH_{Ar}$), 7.12 (m, 3H, $TrtH_{Ar}$), 6.06-5.87 (m, 2H, -CH=CH-, mixture of isomer), 4.54 (s, 1H, -NH-), 4.05,3.76 (d, 1H, -OCH_2, mixture of isomer), 3.87,3.54 (d, 1H, -OCH_2, mixture of isomer), 3.07 (t, 2H, -NCH_2-), 2.79 (m, 1H, C1-Nor), 2.73 (m, 1H, C4-Nor), 2.28 (m, 1H, C5-Nor), 2.05 (t, 2H, S-CH_2), 1.45 -1.0 (m, 22H, C6-Nor + C7-Nor + -CH_2-).

Compound 2c

In a 25 ml RB flask, compound 2b (25 mg, 0.042 mmol) was taken then 20% v/v TIPS in DCM (0.2 ml, 0.209 mmol, 2.5 eq) was added into it. After this, 20% v/v TFA in DCM (0.2ml, 0.524 mmol, 12.5 eq) was added to the reaction mixture. During addition of TFA, the solution slowly turned yellow which after sometime resulted in a colourless solution. The reaction mixture was stirred at RT for 2 h under N_2 atmosphere and was monitored by TLC (10% EtOAc in Hexane, Rf = 0.5, checked in iodine chamber). The volatile components (solvent, TFA, TIPS) were removed under reduced pressure. Thus, compound 2c was obtained as a colourless liquid. Yield 14 mg (0.039 mmol, 94%). HRMS (ESI) calculated for $C_{20}H_{35}NO_2S$ was 353.2389, found 354.2442 $[M+H]^+$. ^{1}H-NMR (400 MHz, $CDCl_3$): 6.14-5.95 (m, 2H, -CH=CH-, mixture of isomer), 4.60 (s, 1H, -NH-), 4.12, 3.84 (d, 1H, -OCH_2, mixture of isomer), 3.95, 3.62 (d, 1H, -OCH_2, mixture of isomer), 3.16 (t, 2H, -NCH_2-), 2.87 (m, 1H, C1-Nor), 2.80 (m, 1H, C4-Nor), 2.52 (t, 2H, S-CH_2), 2.37 (m, 1H, C5-Nor), 1.6 -1.27 (m, 22H, C6-Nor + C7-Nor + -CH_2-).

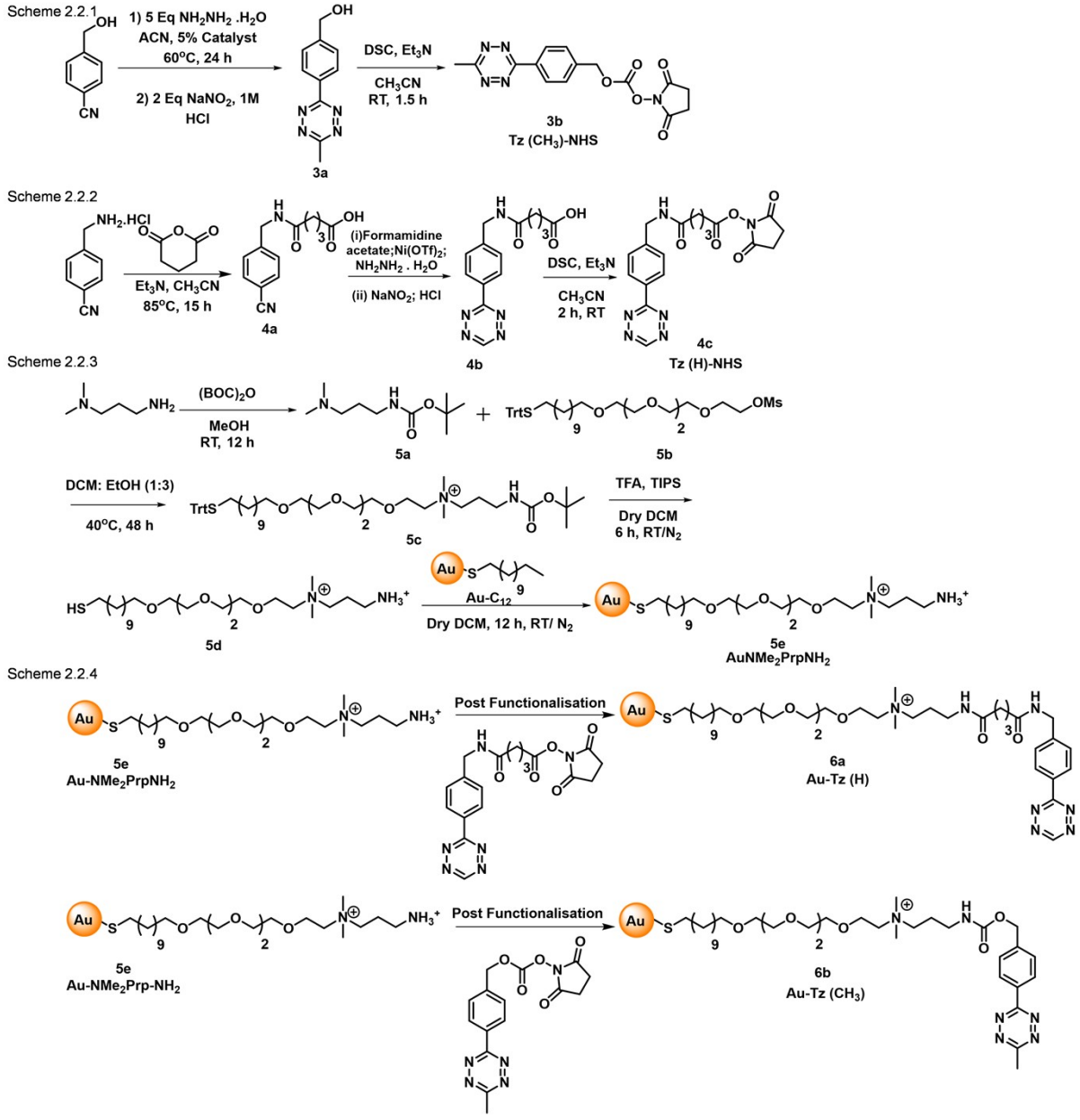
Compound 2e

To fabricate AuNPs, we followed two step procedure. Firstly, we synthesised dodecanethiol capped gold nanoparticle (Au-C_{12}, ~ 4.65 nm core diameter) by heat induced size evolution method. TEM images show the average size of **Au-C_{12}** nanoparticles to be 4.65 ± 0.16 nm. (Figure S9).

Norbornene functionalised gold nanoparticles (**Au-Nor**) were prepared by place exchange of dodecanethiol capped 4.65 nm gold nanoparticles (**Au-C_{12}**). In a 20 ml vial **Au-C_{12}** (7 mg) was

dissolved in nitrogen purged dry DCM. In another vial norbornene thiol ligand (compound 2c) was dissolved in nitrogen purged dry DCM (7 mg, 1:1 mass ratio) and transferred to the first vial. The reaction was stirred at RT for 48 h under N_2 atmosphere. The solvent was evaporated under reduced pressure and the nanoparticles were washed 3 times with methanol. Nanoparticles were recovered by centrifugation and the supernatant was discarded. After that, the nanoparticles were redispersed in toluene. Thus, we obtained **HS-Nor** decorated nanoparticles with 1 µM concentration.

Scheme 2.2



Scheme 2.2: Synthesis of post functionalised gold nanoparticles (**Au-Tz (H)** and **Au-Tz (CH_3)**)

Compound 3b

In a 25 ml RB flask, compound 3a (60 mg, 0.296mmol) was dissolved in 4.5 ml CH_3CN. To this, DSC (152 mg, 0.593 mmol) was added followed by subsequent addition of Et_3N (0.08 ml, 2.5 mmol). Reaction mixture was stirred at RT for 1.5 h. The TLC was checked in 50% EtOAc in Hexane. The volatile components (solvent, Et_3N) were evaporated under reduced pressure and redissolved in DCM and washed with IN HCl, brine solution and dried over Na_2SO_4. Pink colour compound was charged on silica gel and purified by flash chromatography (eluent: 20% EtOAc in hexane to 50% EtOAc in hexane, Rf = 0.54). Compound 3b was obtained as a pink colour solid. Yield 32 mg (0.093 mmol, 32%). ^{1}H-NMR (400 MHz, $CDCl_3$): 8.64 (d, 2H, H_{Ar}, J= 8.4 Hz), 7.62 (d, 2H, $H_{Ar,}$ J= 8.4 Hz) 5.43 (s, 2H, CH_2-benzyl), 3.11 (s, 3H, -CH_3-), 2.85 (s, 4H, CH_2 –CH_2).

Compound 5a

In a 25 ml RB flask, N,N-Dimethyl-1,3-propandiamine (2 ml, 16 mmol) was dissolved in 15 ml CH_3OH and reacted with $(BOC)_2O$ (3.50 g, 16 mmol) for 1 h at 0^0C and overnight (12 h) at RT. After completion of reaction, solvent was evaporated under reduced pressure. The residue was dissolved in 35 ml of distilled water and extracted with DCM and dried over Na_2SO_4. Thus, compound 5a was obtained as a colourless liquid. Yield 2.75 g (13.5 mmol, 85%). ^{1}H-NMR (400 MHz, $CDCl_3$): 5.13 (s, 1H, -NH-), 3.14 (d, 2H, -CH_2-NH-, J= 6.4 Hz), 2.28 (d, 2H, -CH_2-N-, J= 7.2 Hz), 2.18 (s, 6H, -CH_3-N-), 1.61 (q, 2H, –CH_2-, J= 6.8 Hz), 1.41 (s, 9H, -C-CH_3-).

Compound 5c

In a 25 ml RB flask, compound 5b (250 mg, 0.356 mmol) was dissolved in 2 ml DCM: EtOH (1:3) under N_2 atmosphere. To this, compound 5a (721 mg, 3.56 mmol) was added. Reaction Mixture was stirred at 40^0C for 48 h and monitored using TLC in 1:1 Hexane: EtOAc. The reaction mixture was then washed with hexane (5 times) and diethyl ether (2 times). The supernatant, after sonication and centrifugation, was discarded. Thus, Compound 5c was obtained as a colourless liquid. Yield 265 mg (0.328 mmol, 92.18%). ^{1}H-NMR (400 MHz, $CDCl_3$): δ 7.33 (m, 6H, TrtH_{Ar}), 7.20 (m, 6H, TrtH_{Ar}), 7.13 (m, 3H, TrtH_{Ar}), 5.90 (br, 1H, -NH-), 3.88 (t, 2H, -O-**CH_2**-CH_2-N-), 3.69 (t, 2H, -O-**CH_2**-), 3.60-3.49 (m, 12H, -CH_2-O-CH_2-), 3.30-3.21 (m, 12H, -N-**CH_3**- + -N-**CH_2**- + -NH-**CH_2**-), 2.68 (s, 3H, OMs), 2.10 (t, 2H, -SCH_2-), 1.96 (m, 2H, **CH_2**-CH_2-NH-), 1.48 (m, 2H, -CH_2-), 1.36 (s, 9H, -C-CH_3-), 1.25 - 1.02 (m, 16H, -CH_2-).

Compound 5d

In a 10 ml RB flask, compound 5c (265 mg, 0.328 mmol) was taken. To this, 20% v/v TFA in DCM (2.5 ml, 6.56 mmol, 20 eq) was added. During the addition of TFA, solution slowly turned yellow. After this 20% v/v TIPS in DCM (0.84 ml, 0.82 mmol, and 2.5 eq) was added to the reaction mixture which resulted in a colourless solution. Then the reaction mixture was stirred at RT for 6 h under N_2 atmosphere. The volatile components (solvent, TFA, TIPS) were evaporated under reduced pressure. The yellow residue was washed repeatedly with hexane and diethyl ether via sonication and centrifugation. Compound 5d (HS-C_{11}-TEG-prpNH_2) was obtained as a colourless liquid. Yield 109 mg

(0.234 mmole, 71.4%). HRMS (ESI) calculated for [$C_{24}H_{53}N_2O_4S^+$] was 465.3721, found 465.3772 [M]$^+$ (100%), 466.3725 [M+H]$^+$ (26%). ^{1}H-NMR (400 MHz, $CDCl_3$) δ 8.24 (br, 3H, -NH_3-), 3.91 (t, 2H, -O-**CH_2**-CH_2-N-), 3.62 (m, 14H, -CH_2-O-CH_2-), 3.44 (t, 2H, CH_2-N-), 3.19 (m, 8H, -N-CH_3- + -N-CH_2-), 2.72 (t, 2H, -**CH_2**-NH-), 2.50 (t, 2H, -SCH_2-), 1.56 (m, 4H, **CH_2**-CH_2-NH_3- + -CH_2-), 1.31-1.26 (m, 16H, -CH_2-).

Compound 5e

A place exchange reaction of dodecanethiol coated 4.65 nm gold nanoparticles (**Au-C_{12}**) was performed with HS-C_{11}-TEG-prpNH_2 ligand. In a 20 ml vial, Au-C_{12} (4.8 mg) was dissolved in nitrogen purged dry DCM. In another vial, compound 5e was dissolved in nitrogen purged dry DCM (4.8 mg, 1:1 mass ratio) and transferred to the first vial. The reaction mixture was stirred at RT for 12 h under N_2 atmosphere. The solvent was evaporated under reduced pressure and the nanoparticles were washed 3 times with hexane and DCM respectively. Nanoparticles were recovered by centrifugation and the supernatant was discarded. The residue was dissolved in small amount of milli Q water and dialysed (membrane MWCO= 10,000, purchased from thermo scientific) for 2 days to remove the excess ligands. Thus, we obtained HS-C_{11}-TEG-prpNH_2 decorated nanoparticles with a concentration of 3 μM.

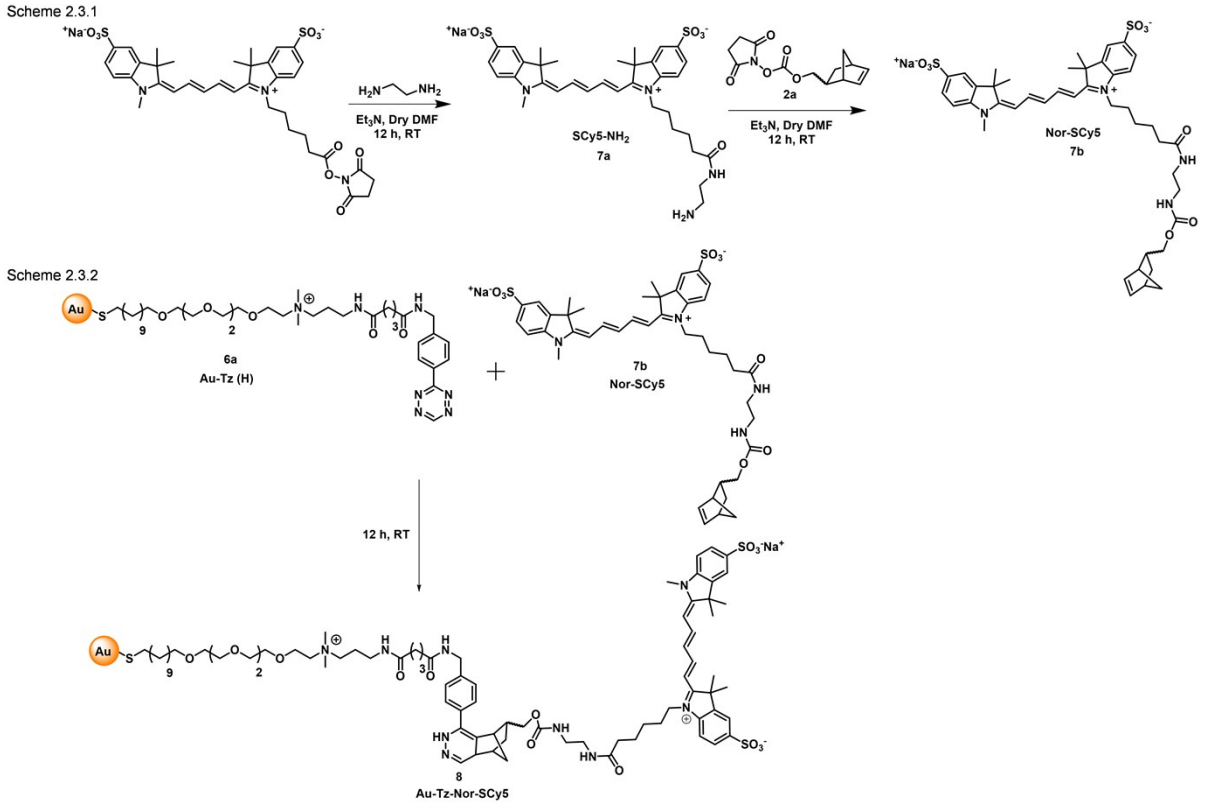
Compound 6a

Au-Tz (H) functionalised gold nanoparticle**:** Compound 5e (**AuNM$e_2$prpNH_2**) (85 μl, 3 μM) was taken in a 1.5 ml microcentrifuge tube. To this $NaHCO_3$ (10 μl, 1 M) was added and vortexed. Stock solution of Tz (H)-NHS i.e. compound 4c was prepared, (10mg/ml, 25.10 mM) in DMF. Excess of Tz (H)-NHS (5 μl, 500 eq) solution was added into the nanoparticle solution. After each addition, the mixture was vortexed. Solution of nanoparticles was vortexed for 3h at RT. The obtained nanoparticles were purified by 0.5 ml zeba spin desalting Column (7K MWCO). The resultant UV-Vis spectrum after subtracting **Au-Tz (H)** absorbance from **AuNM$e_2$prpNH_2** in Figure S10 shows the resultant absorbance around 270 nm which is matching with only Tz (H)-NHS (inset, compound 4c). Therefore, depicts the post functionalization of tetrazine moiety on **AuNM$e_2$prpNH_2** NPs.

Compound 6b

Au-Tz (CH_3) functionalised gold nanoparticle: Compound 5e, **AuNM$e_2$prpNH_2** (85 μl, 3 μM) was taken in a 1.5 ml microcentrifuge tube. To this, $NaHCO_3$ (10 μl, 1 M) was added and vortexed. Stock solution of Tz (CH_3)-NHS i.e. compound 3b was prepared (10mg/ml, 29.12 mM) in DMF. Excess of Tz (CH_3)-NHS (5 μl, 500 eq) solution was added into the nanoparticle solution. After each addition, the mixture was vortexed. Solution of nanoparticles was vortexed for 3h at RT. The obtained nanoparticles were purified by 0.5 ml zeba spin desalting Column (7K MWCO).

Scheme 2.3



Scheme2.3: Synthesis of **Nor-SCy5** and **Au-Tz (H)** cycloaddition reaction

Compound 7a

In 0.5 ml microcentrifuge tube, solution of ethylene diamine (47.33 µg, 0.788 umol) in dry DMF was taken. To this, Et_3N (79.73 µg, 0.788 umol) solution in dry DMF was added. After that, stock solution of SCy5 NHS ester (purchased from lumiprobe) was prepared (5.675 mg/ml, 7.45 mM) in DMF. 20 µl of SCy5 NHS ester solution (200 µg, 0.262 µmol) was finally added to the reaction mixture, mixed properly and stirred for 12 h at room temperature. The product was purified by reverse phase HPLC and lyophilized. Thus, we obtained Compound 7a (**SCy5-NH_2**) with 0.869 mM concentration. LCMS (ESI) calculated for $C_{34}H_{43}N_4O_7S_2^-$ was 683.26, found 685.40 $[M+2H]^+$.

Compound 7b

Stock solution of Nor-NHS (i.e., compound 2a) was prepared (10 mg/ml, 37.69 mM) in dry DMF. Taken out 3.4 µl of Nor-NHS (33.88 µg, 0.127 µmol) and mixed with Et_3N (12.85 µg, 0.127 µmol) solution in dry DMF. This mixed solution was then added to **SCy5-NH_2** (49 µl, 0.869mM) solution in dry DMF. Reaction mixture was stirred for 12 h at room temperature. The product was purified by reverse phase HPLC and lyophilized. Thus, we obtained Compound 7b (**Nor-SCy5**) with 0.68 mM concentration. LCMS (ESI) calculated for $C_{45}H_{53}N_4O_9S_2^-$ was 833.33, found 835.30 $[M+2H]^+$.

Compound 8

Compound 6a, **Au-Tz (H)** (7.43 µl, 0.68 µM) was taken in a 0.5 ml microcentrifuge tube. To this, **Nor-SCy5** (1 µl, 0.68 mM, 100 eq) and H_2O (1.56 µl) was added and stirred at RT for 12 h. Finally, the product was purified by G25 sephadex column (7K MWCO).

3 Experimental sections

3.1 Colloidosome fabrication

3.1.1 Au-Tz (H) vs. Au-Nor and Au-Tz (CH_3) vs. Au-Nor

Au-Tz (H) vs. Au-Nor

In a 1.5 ml microcentrifuge tube, 20 µl of stock solution of **Au-Nor** (1 µM) was taken and diluted with 180 µl of toluene (final concentration = 0.1 µM). In another microcentrifuge tube 8 µl of **Au-Tz (H)** (2.55 µM) was taken and diluted with 2 µl of milli-Q water (final concentration = 2 µM). In the next step, 10 µl solution of this was added to the toluene solution. The heterogeneous mixture was vigorously shaken for 20 s. After this, the supernatant liquid was washed several times with toluene. The colloidosomes formed by this technique were polydispered. The average size of colloidosome for **Au-Tz (H)** is 46.92 ± 3.41 µm. Assuming that equal water was distributed to each colloidosome, estimation of number of colloidosomes are as followed:

Total number of colloidosomes in 10 µl of aqueous phase:

Average diameter of colloidosome = 46.92 µm

$N_{colloidosome} = V_{water} / V_{colloidosome}$

$= V_{water} / [4\ \pi\ (d / 2)^3 / 3)$

$= 10\ \mu l / (4\ \pi(46.92\ \mu m / 2)^3 / 3)$

$N_{colloidosome} = 184989$

$= 1.8 \times 10^5$

Au-Tz (CH_3) vs. Au-Nor

Similar procedure was followed for **Au-Tz (CH_3)** with the same concentration for all. But the colloidosomes formed by these nanoparticles had average size of 231.62 ± 19.15 µm. Assuming that equal water was distributed to each colloidosome, estimation of number of colloidosomes are as followed:

Total number of colloidosomes in 10 µl of aqueous phase:

Average diameter of colloidosome = 231.62 µm

$N_{colloidosome} = V_{water} / V_{colloidosome}$

$= V_{water} / [4 \pi (d / 2)^3 / 3)$

$= 10 \mu l / (4 \pi(231.62 \mu m / 2)^3 / 3)$

$N_{colloidosome} = 1538$

$= 1.5 \times 10^3$

3.1.2 Control experiments

Four control experiments were performed to support the fact that crosslinking of self-assembled nanoparticles is due to conjugation of tetrazine AuNPs with norbornene AuNPs.

Control a: Au-Nor (In oil phase only without **Au-Tz (H)**

In a 1.5 ml microcentrifuge tube 20 µl of stock solution of **Au-Nor** was taken (1 µM) and diluted with 180 µl of toluene (final concentration = 0.1 µM). 10 µl milli-Q water was then added to toluene phase. The heterogeneous mixture was vigorously shaken for 20 s. (Figure S11a)

Control b: Au-Tz (H) (In water phase only without **Au-Nor**)

In a 1.5 ml microcentrifuge tube, 200 µl of toluene was taken. In another microcentrifuge tube 8 µl of **Au-Tz (H)** (2.55 µM) was taken and diluted with 2 µl of milli-Q water (final concentration= 2 µM).10 µl of this solution was added to the toluene solution. The heterogeneous mixture was vigorously shaken for 20 s. (Figure S11b)

Control c: Au-Nor: **AuNMe$_2$prpNH$_2$**

In a 1.5 ml microcentrifuge tube, 20 µl of stock solution of **Au-Nor** was taken and diluted with 180 µl of toluene (final concentration = 0.1 µM). In another microcentrifuge tube 8 µl of **AuNMe$_2$prpNH$_2$** (2.55 µM) and 2 µl of milli-Q water was taken (final concentration= 2 µM).10 µl of this solution was added to the 200 µl of toluene solution. The heterogeneous mixture was vigorously shaken for 20 s. (Figure S11c)

Control d: Au-C$_{12}$: **Au-Tz (H)**

In a 1.5 ml microcentrifuge tube, 4 µl of stock solution of **Au-C$_{12}$** was taken and diluted with 196 µl of toluene (final concentration= 0.1 µM). In another microcentrifuge tube 8 µl of **Au-Tz (H)** (2.55 µM) and 2 µl of milli-Q water was taken (final concentration= 2 µM). In the next step, 10 µl of this solution was added to the 200 µl toluene solution. The heterogeneous mixture was vigorously shaken for 20 s. (Figure S11d)

3.2 TEM studies

TEM images of crosslinked nanoparticles were taken after washing the microcapsules with toluene to remove excess nanoparticles. Colloidosomes were drop casted on TEM grid (carbon coated copper grid) and allowed to dry for 24 h. The low magnification image of TEM showing thin film like

morphology depicted that spherical nature of capsule is retained, while densely packed and well dispersed nanoparticles is revealed by high magnification TEM Image.

3.3 AFM studies

The AFM images of colloidal microcapsules were recorded by drop casting toluene washed microcapsules on a clean mica surface and drying for 4 h in a vacuum. The AFM image revealed the microcapsule shell thickness as height profile when determined from the height difference between the mica background and fold free region of the microcapsule (white dotted lines in Figure S12). The thickness of collapsed flat regions on a dried capsule were measured from the height profile of the AFM image. From these measurements, the capsule single wall thickness was determined as half of the height of the collapsed flat regions of dried capsules. The average height profile was found to be 16.67 nm which includes the two layers of the collapsed shell. The average height profile resembles to the theoretical hydrodynamic diameter of two surface functionalised nanoparticles (**Au-Tz (H)** = 13.63 nm and **Au-Nor** = 9.29 nm), indicating their monolayer nature.

Fabrication of colloidosomes: In a 1.5 ml microcentrifuge tube, 22.09 µl of stock solution of **Au-Nor** (0.905 µM) was taken and diluted with 177.90 µl of toluene (final concentration = 0.1 µM). In another microcentrifuge tube 10 µl of **Au-Tz (H)** (1.715 µM) was taken (final concentration = 1.715 µM). In the next step, 10 µl solution of this was added to the toluene solution. The heterogeneous mixture was vigorously shaken for 20 s. After this, the supernatant liquid was washed several times with toluene.

3.4 MALDI characterisation

The microcapsules (1 µl) were drop casted on 384 MTP ground steel MALDI plate. After then, the sample was gently mixed with 1 µl MALDI matrix (saturated solution of CHCA in 70% ACN: 30% water with 0.1% TFA) and air dried before analysis. The MALDI spectrum showed peak at m/z = 844.96 (observed) which corresponds to the calculated fragmented mass value (m/z = 844.56) of thiol ligand of tetrazine crosslinked to norbornene moiety (Figure S13).

Fabrication of colloidosomes: In a microcentrifuge tube, 22.09 µl of stock solution of **Au-Nor** (0.905 µM) was taken and diluted with 177.90 µl of toluene (final concentration = 0.1 µM). In another microcentrifuge tube 10 µl of **Au-Tz (H)** (1.715 µM) was taken (final concentration = 1.715 µM). In the next step, 10 µl solution of this was added to the toluene solution. The heterogeneous mixture was vigorously shaken for 20 s. After this, the supernatant liquid was washed several times with toluene.

3.5 Fluorophore encapsulated studies

3.5.1 Single cargo encapsulation

FITC-functionalised dextran polymer (M. wt. = 10 kDa), BSA-Rh (M. wt. = 66.5 kDa), DNA-FAM (M. wt. = 2.1 kDa) were encapsulated in the colloidosomes.

Synthesis of Rhodamine conjugated BSA: To 90 µl of BSA solution (160 µM prepared in PBS) in a 1.5 ml microcentrifuge tube, 10ul of $NaHCO_3$ (1 M) solution was added followed by the addition of 7.5 µl of Rhodamine NHS (18.94 mM in DMSO). Reaction mixture was allowed to stir at room

temperature under dark. Conjugate was purified by passing through the zeba column. Concentration was measured based on absorption.

FITC- Dextran encapsulation - In a 1.5 ml microcentrifuge tube, 20 µl of stock solution of **Au-Nor** (1 µM) was taken and diluted with 180 µl of toluene (final concentration = 0.1 µM). In another microcentrifuge tube 10 µl of **Au-Tz (H)** (2.55 µM) and 2 µl of FITC- Dextran (final concentration= 66.6 µM) in aqueous phase was taken. Finally, 12 µl of this prepared solution was added to 200 µl of toluene solution. The heterogeneous mixture was vigorously shaken manually for 20 s to form colloidosomes.

BSA-Rhodamine (BSA-Rh) encapsulation -In a 1.5 ml microcentrifuge tube, 20 µl of stock solution of **Au-Nor** (1 µM) was taken and diluted with 180 µl of toluene (final concentration = 0.1 µM). In another microcentrifuge tube 8 µl of **Au-Tz (H)** (2.55 µM) and 2 µl of BSA-Rh (final concentration= 91 µM) in aqueous phase was taken. 10 µl of this prepared solution was added to the 200 µl of toluene solution (Figure 2b). The heterogeneous mixture was vigorously shaken for 20 s to form colloidosomes. Same experiment was performed for Figure 3a (main paper), but for continuous phase, toluene solvent was removed and microcapsules were redispersed in 200 µl aqueous phase.

DNA-FAM encapsulation - In a 1.5 ml microcentrifuge tube, 20 µl of stock solution of **Au-Nor** (1 µM) was taken and diluted with 180 µl of toluene (final concentration = 0.1 µM). In another microcentrifuge tube 8 µl of **Au-Tz (H)** (2.55 µM) and 2 µl of DNA-FAM (final concentration = 10 µM) in aqueous phase was taken. 10 µl of this prepared solution was added to the 200 µl of toluene solution. The heterogeneous mixture was vigorously shaken for 20 s.

After this supernatant liquid was washed with toluene several times and images were taken using ZEISS inverted microscope.

3.5.2 Dual cargo encapsulation

Simultaneous encapsulation of BSA-Rh and DNA-FAM in colloidosomes.

In a 1.5 ml microcentrifuge tube, 20 µl of stock solution of **Au-Nor** (1 µM) was taken and diluted with 180 µl of toluene (final concentration = 0.1 µM). In another microcentrifuge tube 8 µl of **Au-Tz (H)** (2.55 µM), 1 µl of BSA-Rh (final concentration= 44.5 µM) and 1 µl of DNA-FAM (final concentration= 5 µM) in aqueous phase were taken. 10 µl of this prepared solution was added to the 200 µl of toluene solution. The heterogeneous mixture was vigorously shaken for 20 s.

3.6 Permeability test:

To check the release kinetics of dual cargo (BSA-Rh and DNA-FAM) encapsulated microcapsules in continuous phase, microcapsules were formed exactly in same way as described in section 3.5.2. However, after the formation of microcapsules in oil-water phase, supernatant liquid was taken out and microcapsules were washed with toluene one time. Consequently, the maximum toluene phase was taken out and microcapsules were redispersed in 200 µl of water. After solvent exchange

process, diffusion of cargo to outer aqueous environment was monitored by fluorescence microscope which showed fluorescence intensity change over time**.**

3.7 Live cell encapsulation:

In a 1.5 ml microcentrifuge tube, 6.8 µl of stock solution of **Au-Nor** (2.96 µM) was taken and diluted with 193.2 µl of toluene (final concentration = 0.1 µM). In another microcentrifuge tube 9 µl of **Au-Tz (H)** (final concentration= 1.53 µM) and 1 µl of SVEC-RFP cell line (9.85 x 10^5 cells) in DMEM media was taken. 10 µl of this prepared solution was added to 200 µl of toluene solution. The heterogeneous mixture was vigorously shaken by hand for 20 s to form colloidal microcapsules. After this, the supernatant liquid was washed two times with toluene and images were taken using ZEISS inverted microscope as shown in Figure S14.

3.8 Cytotoxicity assay of colloidosomes and docetaxel loaded colloidosomes.

HeLa cells were used for the experimental study. The cells were cultured in a humidified atmosphere (5% CO_2) at 37°C and grown in Dulbecco's Modified Eagle's Medium (DMEM, high glucose) supplemented with 10% fetal bovine serum (FBS) (Gibco, USA), 2 mM Glutamax (Invitrogen, USA) and 1% antibiotics (100 U/ml penicillin, 100 µg/ml streptomycin and 0.25 µg/ml amphotericin) (Gibco, USA).

Prior to the experiment, the cells were seeded at ~10,000 cells/100µl in a 96-well plate. After 24 h, the cells were washed with 1X DPBS and incubated for 4 h with aqueous phase dispersed colloidosomes alone and docetaxel loaded colloidosomes in serum free culture media. The cells were then washed, and fresh complete media was added to each well. After 24 h, the cells were washed and incubated with media containing 1x Alamar blue solution. After 4 h incubation, absorbance was measured at 570 nm (experimental wavelength) and 600 nm (reference wavelength) and the data was analysed. Furthermore, statistical analysis was performed from the dataset and one-way ANOVA, Multiple Comparisons (Tukey's multiple comparisons test) was done using GraphPad Prism software. The toxicity profile is shown in the Figure 3c.

Fabrication of colloidosomes: In a 1.5 ml microcentrifuge tube, 22 µl of stock solution of **Au-Nor** (0.905 µM) was taken and diluted with 178 µl of toluene (final concentration = 0.1 µM). In another microcentrifuge tube 10 µl of **Au-Tz (H)** (final concentration = 0.465 µM) was taken. In the next step, 10 µl solution of this was added to the toluene solution. The heterogeneous mixture was vigorously shaken for 20 s. After this, the supernatant liquid was washed several times with toluene.

Fabrication of Docetaxel loaded colloidosomes: In a 1.5 ml microcentrifuge tube, 22 µl of stock solution of **Au-Nor** (0.905 µM) was taken and diluted with 178 µl of toluene (final concentration = 0.1 µM). In another microcentrifuge tube 9 µl of **Au-Tz (H)** (final concentration = 0.465 µM) and 1 µl of Docetaxel (final concentration = 3 µM in 1% DMSO in water) in aqueous phase was taken. In the next step, 10 µl solution of this was added to the toluene solution. The heterogeneous mixture was vigorously shaken for 20 s. After this, the supernatant liquid was washed several times with toluene.

Toxicity study: 3 x 10^4 number microcapsule was dispersed in 200 µl of serum free cell culture media and subsequently incubated with the cells. Both control microcapsules without drug molecules and Docetaxel loaded microcapsules were used in the assay, as described above.

3.9 Colloidal microcapsule fabrication using commercial amalgamator.

Similar protocol was followed to fabricate microcapsules as described in section 3.1.1, however this time the heterogeneous mixture was stirred utilising a commercially available amalgamator for 8 s. After this, the supernatant liquid was washed several times with toluene. The colloidal microcapsules fabricated by this technique were imaged via optical microscopy (Figure S15a). Using amalgamator for emulsification, the average size of microcapsule for **Au-Tz (H)** was obtained ~58.62 ± 2.43 µm (Figure S15b). Assuming that equal water was distributed to each colloidal microcapsules, estimation of number of colloidosomes are as followed:

Fabrication of colloidosomes: In a 1.5 ml microcentrifuge tube, 22 µl of stock solution of **Au-Nor** (0.905 µM) was taken and diluted with 178 µl of toluene (final concentration = 0.1 µM). In another microcentrifuge tube 10 µl of **Au-Tz (H)** (final concentration = 0.465 µM) was taken. In the next step, 10 µl solution of this was added to the toluene solution. The heterogeneous mixture was vigorously shaken for 20 s. After this, the supernatant liquid was washed several times with toluene.

Total number of colloidosomes in 10 µl of aqueous phase:

Average diameter of colloidosome = 58.62 µm

$N_{colloidosome} = V_{water} / V_{colloidosome}$

$= V_{water} / [4\ \pi\ (d / 2)^3 / 3)$

$= 10\ \mu l / (4\ \pi\ (58.62\ \mu m / 2)^3 / 3)$

$N_{colloidosome} = 94860$

$= 9.48 \times 10^4$

4 SERS studies on R6G encapsulated colloidosome

4.1 Sample preparation

Rhodamine 6G (**R6G**) encapsulated colloidosomes- In a 1.5 ml microcentrifuge tube, 4 µl of stock solution of **Au-Nor** (3.1 µM) was taken and diluted with 196 µl of toluene i.e., 0.06 µM. In another microcentrifuge tube 9 µl of **Au-Tz (H)** (final concentration = 0.4 µM) and 1 µl of R6G solution was taken (final concentration= 10 µM). 10 µl of this prepared solution was added to the 200 µl of toluene solution. The heterogeneous mixture was vigorously shaken by hand for 20 s to form microcapsules.

Control (blank colloidosomes) - In a 1.5 ml microcentrifuge tube tube, 4 µl of stock solution of **Au-Nor** (3.1 µM) was taken and diluted with 196 µl of toluene i.e., 0.06 µM. In another microcentrifuge tube 9 µl of **Au-Tz (H)** was taken and diluted with 1 µl of milli-Q water (final concentration = 0.4 µM). 10 µl of

this prepared solution was added to the 200 µl of toluene solution. The heterogeneous mixture was vigorously shaken by hand for 20 s to form microcapsules.

4.2 SERS detection

Experimental details- All Raman and SERS measurements were carried on Horiba LabRAM HR Evolution with a Nd-YAG laser of 532nm wavelength. The laser power used was 2-3 mW at the sample. Light was focussed onto the sample through 50X LWD objective.

10 µl of R6G (10 µM) encapsulated colloidosomes (R6G@colloidosomes) were drop-casted on a hydrophobic glass substrate. Spectra were recorded and averaged from at least 15 different regions of the dried sample. The spectral acquisition time was kept 60 seconds. All spectra are background subtracted and plotted due to fluorescence from R6G.

4.3 Normal Raman evaluation of R6G

Normal Raman spectrum was recorded of 1 mM R6G which was drop-casted and dried on a hydrophobic glass substrate. The acquisition time used was 1s (Figure S16).

4.4 Enhancement factor calculation

The most intense peak, 612 cm^{-1} is chosen for the enhancement factor calculation. It corresponds to the C-C-C in-plane bending mode of the ring.[7]

The enhancement factor, G is given by,

$$G = \frac{I_{SERS} / N_{SURF}}{I_{RAMAN} / N_{BULK}}$$

Where I_{SERS} and I_{RAMAN} are time-normalized intensities, N_{SURF} and N_{BULK} are the number of moles of analyte at the surface and in the bulk.

$$N_{SURF} = \mu_s \mu_m A A_{eff} \qquad N_{BULK} = \frac{A_{eff} h \rho}{M}$$

Where μ_s is the surface density of the molecule on the metal [m^{-2}]; μ_m is the surface density of individual nano-structure with respect to the main plane forming the substrate [m^{-2}]; **A** is the metallic surface area [m^2]; A_{eff} is the effective laser spot area; **h** is the penetration depth of the laser spot; ρ is the density of the molecule and **M** is the molecular weight of the analyte. We consider a uniform sphere forms after drying of the sample.

SERS experiments were performed at least twice to assure reproducibility. Hence after multiple SERS experiments, average intensities were chosen from the experiment with most data points possible (approx. 30).

$$NSURF = \mu_s \mu_m A A_{eff}$$

$$= 3.4X10^{-18}\, moles \qquad = 2.5\ X\ 10^{13} molecules\ cm^{-2} X\ 1.8\ X\ 10^{13}\ cm^{-2} X\ 3.5\ X\ 10^{-13} cm^2$$

$$NBULK = \frac{Ah\rho}{M}$$

$$= \frac{1.3X10^{-8}\ cm^2\ X\ 100\ X\ 10^{-4} cm\ X\ 1.26g\ cm^{-3}}{479.02g\ mol^{-1}}$$

$$= 3.4X10^{-13}\, moles$$

$$G = \frac{180}{1440} X \frac{3.4\ X10^{-13}}{3.4\ X\ 10^{-18}} \approx 10^4$$

In conclusion, a SERS enhancement factor of 10^4 was achieved from the **Au-Tz (H) - Au-Nor** crosslinked colloidosomes.

5 UV-Visible spectral changes during colloidosome fabrication

Red shift was observed due to plasmonic changes of crosslinked AuNPs in colloidosomes. (Figure S17).

6 UV-Visible absorbance spectrum of Nor-SCy5- Au-Tz (H) cycloaddition reaction

Au-Tz-Nor-SCy5 conjugation

Compound 6a, **Au-Tz (H)** (7.43 µl, 0.68 µM) was taken in a 0.5 ml microcentrifuge tube. To this, **Nor-SCy5** (1 µl, 0.68 mM, 100 eq) and H_2O (1.56 µl) was added and stirred at RT for 12 h. Finally, the product was purified by G25 sephadex column (7K MWCO) (Figure S18).

Au- NMe_2prpNH_2 -Nor-SCy5 (Control)

Compound 5e, **Au-NMe_2prpNH_2** (1.78 µl, 0.68 µM) was taken in a 0.5 ml microcentrifuge tube. To this, **Nor-SCy5** (1 µl, 0.68 mM, 100 eq) and H_2O (7.21 µl) was added and stirred at RT for 12 h. Finally, the product was purified by G25 sephadex column (7K MWCO).

7 Fluorescence studies to determine surface coverage of Au-Nor and Au-Tz (H) gold nanoparticles via cycloaddition reactions with respective SCy5 fluorophore.

7.1 To determine the number of surface ligands in respective gold nanoparticles, following experiments were performed:

In one case, **Au-Nor** (25 nM) and **Tz-SCy5** fluorophore (12.5 µM, 500 eq, synthesised according to the reported literature[8]) was taken in total 50 µl of acetonitrile. Similarly, only Tz-SCy5 fluorophore (12.5 µM) as control was taken in 50 µl of ACN solution. Both the reaction mixture was stirred in thermomixer at 37˚C for 12 h. After that, fluorescence spectrum from the supernatant was recorded (20x diluted solution in water) at excitation wavelength of 620 nm and emission wavelength scan from 650 nm – 750 nm (Figure S19a).

Calculation of the total number of norbornene surface ligands per Au-Nor gold nanoparticle:

The percentage fluorescence quenched due to the reaction of Tz-Cy5 with the AuNP anchored Nor ligand = 19%.

Therefore, 19% of 12.5 µM Tz-SCy5 reacted with norbornene ligands that are present on the surface of Au-Nor

Means, 2.375 µM of Tz-SCy5 reacted with 25 nM of Au-Nor

Number of Nor ligand/particle = Concentration of Tz-SCYy5 reacted / Concentration of Au-Nor taken

= 2375 nM / 25nM

= 95 ligands of norbornene per **Au-Nor** nanoparticle

In another case, **Au-Tz (H)** (25 nM) and **Nor-SCy5** fluorophore (12.5 µM, 500 eq) was taken in total 50 µl of water. Similarly, mixture of **Au-NMe$_2$-prp-NH$_2$** (25 nM) with **Nor-SCy5** fluorophore (12.5 µM, 500 eq) and only Nor-SCy5 fluorophore (12.5 µM) were taken as controls in 50 µl water. Given the dye is negatively charged, a cationic control nanoparticle (**Au-NMe$_2$-prp-NH$_2$**) was used to eliminate the binding contribution from negatively charged surface. The reaction mixtures were stirred in thermomixer at 37˚C for 12 h. After that, fluorescence spectrum was recorded (20x diluted solution in water) at excitation wavelength of 620 nm and emission wavelength scan from 650 nm - 750 nm (Figure S19b).

Calculation of the total number of tetrazine surface ligands per Au-Tz (H) gold nanoparticle:

Total percentage of fluorescence quenched in case of Au-Tz (H) + Nor-SCy5 = 44.80%.

Therefore, 44.80% of 12.5 µM Nor-SCy5 reacted with tetrazine ligands of Au-Tz (H)

Means, 5.6 µM of Nor-SCy5 reacted with 25 nM of Au-Tz (H)

= Concentration of Nor-SCy5 reacted / Concentration of Au-Tz (H) taken

= 5600 nM / 25nM

= 224 ligands of tetrazine per **Au-Tz (H)** nanoparticle

7.2: To determine the number of crosslinked ligands per Au-Tz (H) NP during microcapsule fabrications

To determine the number of ligands of Au-Tz (H) engaged in crosslinking during microcapsules, initially microcapsules were fabricated with **Au-Nor** (20 µl, 0.905 µM) in 180 µl toluene and Au-Tz (H) (10 µl, 0.179 µM) in water. After then, the microcapsules were let to be dried with toluene solution. After 24h, the dried microcapsules were resuspended in 6% water in ACN (total 120 µl solution). Therefore, the final concentration of Au-Nor was 150.83 nM and 14.9 nM for Au-Tz (H) after resuspension. To determine the remaining Au-Tz (H) free ligands, **Nor-SCy5** fluorophore (7.45 µM, 500 eq, 1.45 µl) was added to the reaction mixture. Similarly, only **Nor-SCy5** fluorophore (1.45 µl, 7.45 µM) was taken as control in 6% water in ACN (total 120 µl solution). Both the reaction mixtures were stirred in thermomixer at 37^0C for 12 h. After that, fluorescence spectrum was recorded of 20x time diluted sample in water at excitation wavelength of 620 nm and emission wavelength scan from 650 nm – 750 nm (Figure S19c).

Calculation of the free tetrazine ligands in crosslinked microcapsules:

Total percentage fluorescence quenched in case of Au-Tz (H) + Nor-SCy5 = 12.71%.

Therefore, 12.71% of 7.45 µM Nor-SCY5 reacted with free tetrazine ligands of Au-Tz (H)

Means, 0.946 µM of Nor-SCy5 reacted with free ligands of Au-Tz (H) nanoparticles

= Concentration of Nor-SCy5 reacted / Concentration of Au-Tz (H) taken

= 946 nM / 14.9 nM

= 63 ligands (free Tz(H) ligand)

Reacted Tz(H) ligands = 224 total ligands - 63 free ligands

= 161 ligands

Therefore, 161 ligands of tetrazine out of 224 ligands means 71% per **Au-Tz (H)** nanoparticle were crosslinked during microcapsule fabrication.

8 Field Emission Scanning Electron Microscopy- Energy Dispersive X-Ray Analysis (FESEM-EDAX) of microcapsules:

The existence of Au element in microcapsules was proved by FESEM-EDAX analysis. The colloidal microcapsules were drop casted on a clean silicon wafer and vacuum dried for 4 h. After that, the sample was examined on SEM instrument equipped with elemental microanalyzer (EDAX). The SEM image and element mapping image of microcapsules revealed the uniform distribution of gold nanoparticles on the microcapsules as shown in Fig S20.

Fabrication of colloidosomes: In a 1.5 ml microcentrifuge tube, 22.09 µl of stock solution of **Au-Nor** (0.905 µM) was taken and diluted with 177.90 µl of toluene (final concentration = 0.1 µM). In another microcentrifuge tube 10 µl of **Au-Tz (H)** (1.715 µM) was taken (final concentration = 1.715 µM). In the next step, 10 µl solution of this was added to the toluene solution. The heterogeneous mixture was vigorously shaken for 20 s. After this, the supernatant liquid was washed several times with toluene.

9 ^{1}H-NMR spectra

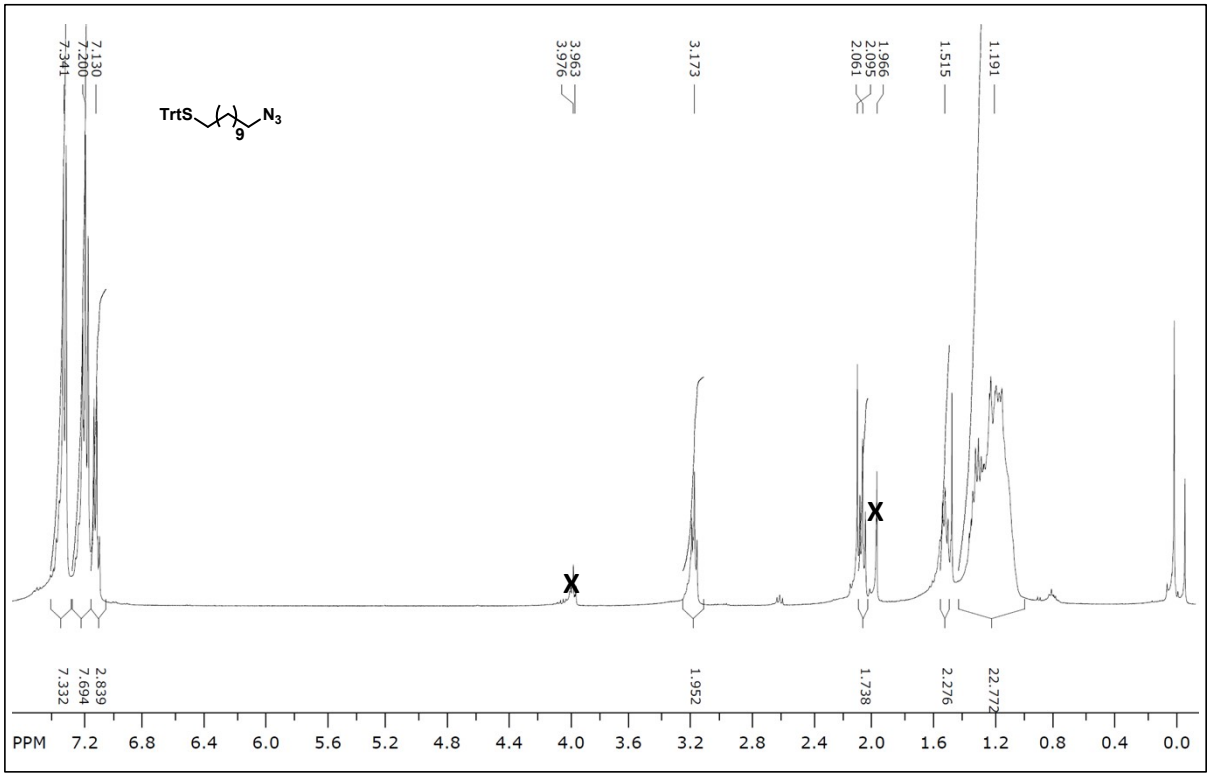


Figure S1: 400 MHz ^{1}H NMR spectrum of compound 1c in $CDCl_3$. (X refers to solvent impurities)

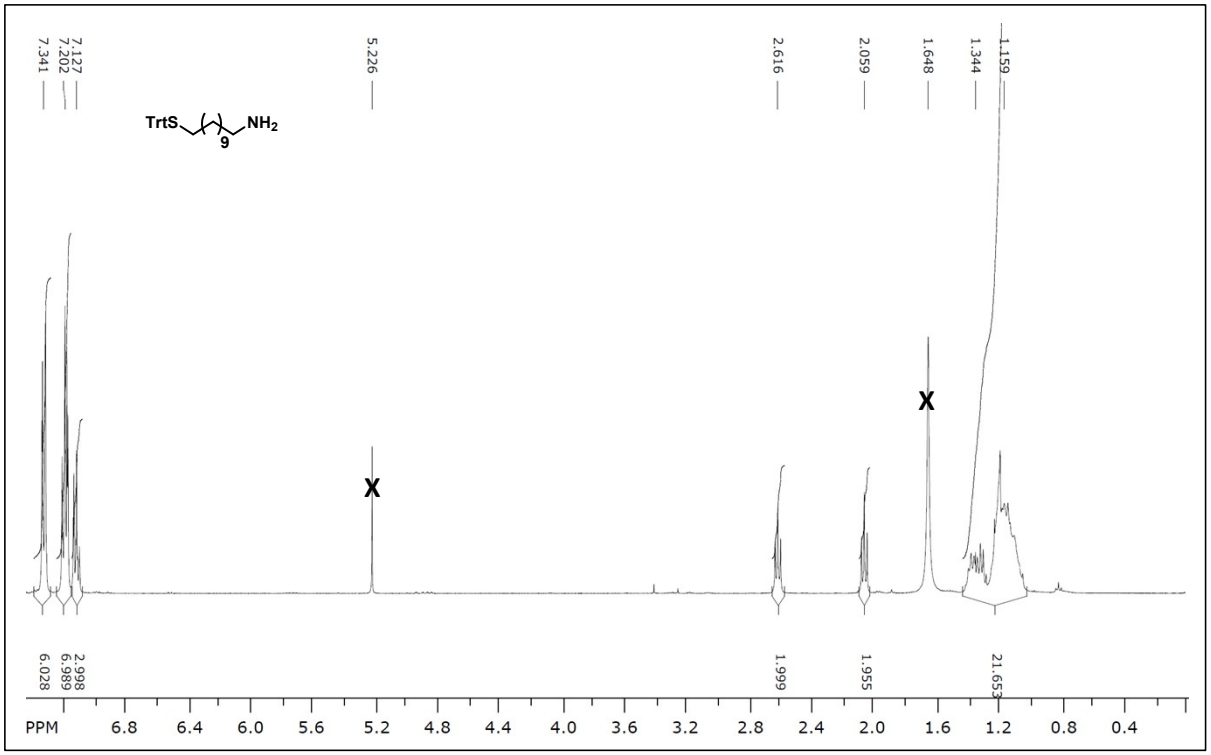


Figure S2: 400 MHz ^{1}H NMR spectrum of compound 1d in $CDCl_3$. (X refers to solvent impurities)

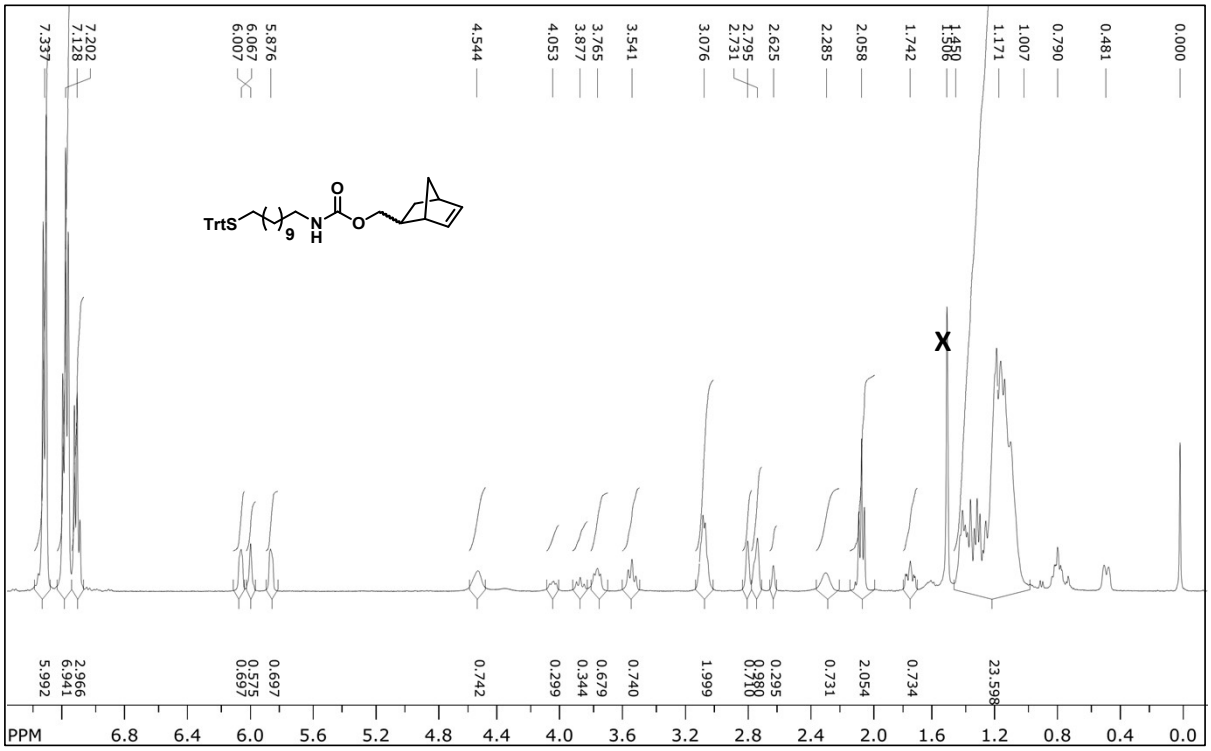


Figure S3: 400 MHz ^{1}H NMR spectrum of endo-rich mixture (70%) compound 2b in $CDCl_3$. (X refers to solvent impurities)

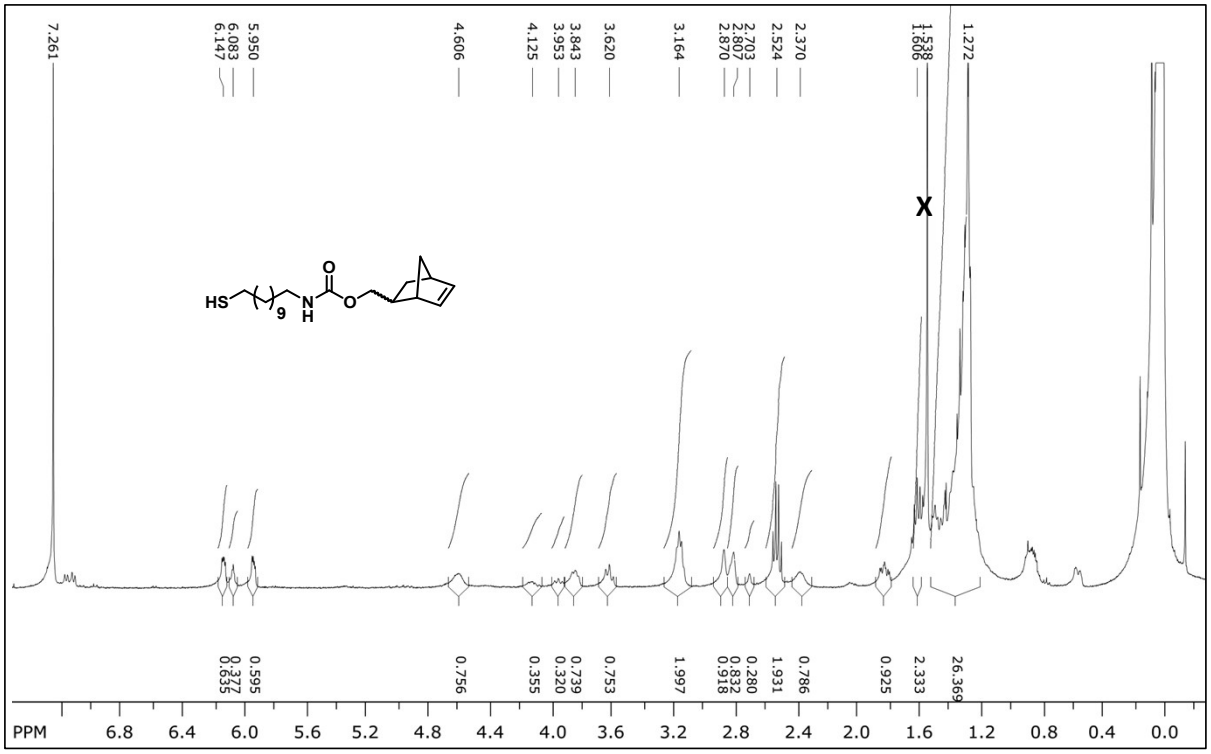


Figure S4: 400 MHz ^{1}H NMR spectrum of endo-rich mixture (70%) compound 2c in $CDCl_3$. (X refers to solvent impurities)

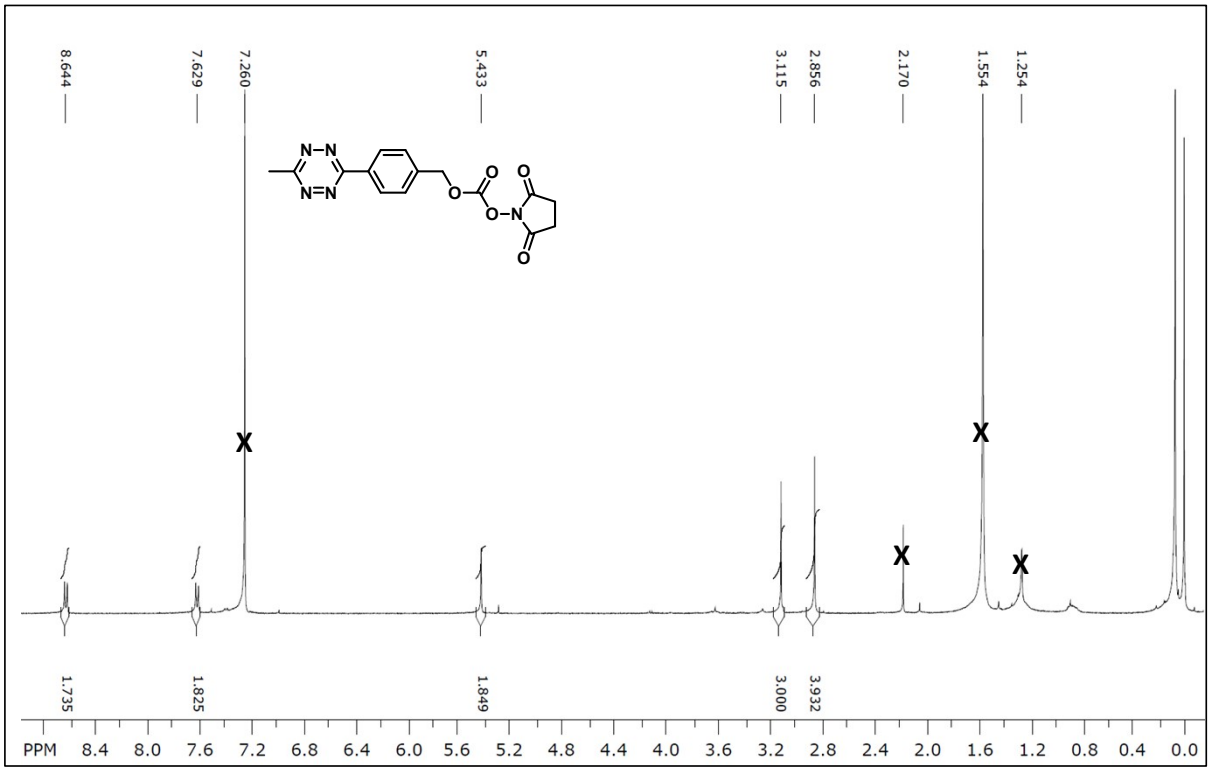


Figure S5: 400 MHz ^{1}H NMR spectrum of compound 3d in $CDCl_3$. (X refers to solvent impurities)

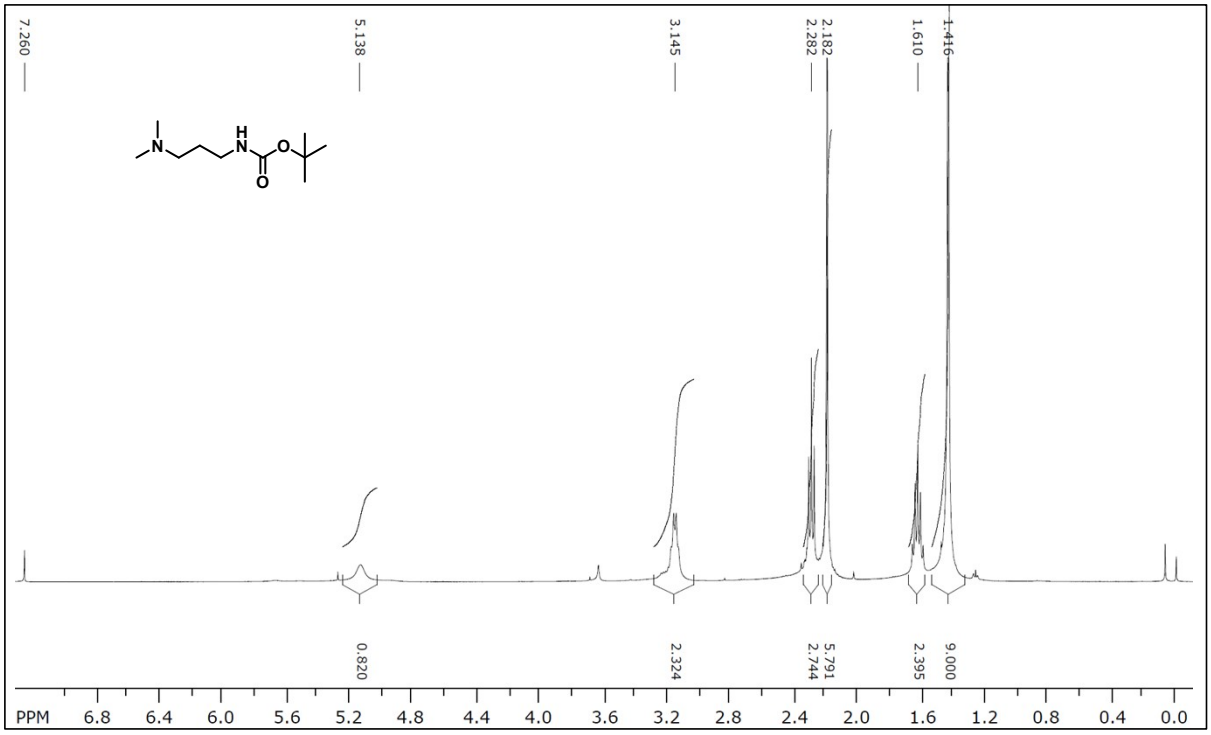


Figure S6: 400 MHz ^{1}H NMR spectrum of compound 5a in $CDCl_3$.

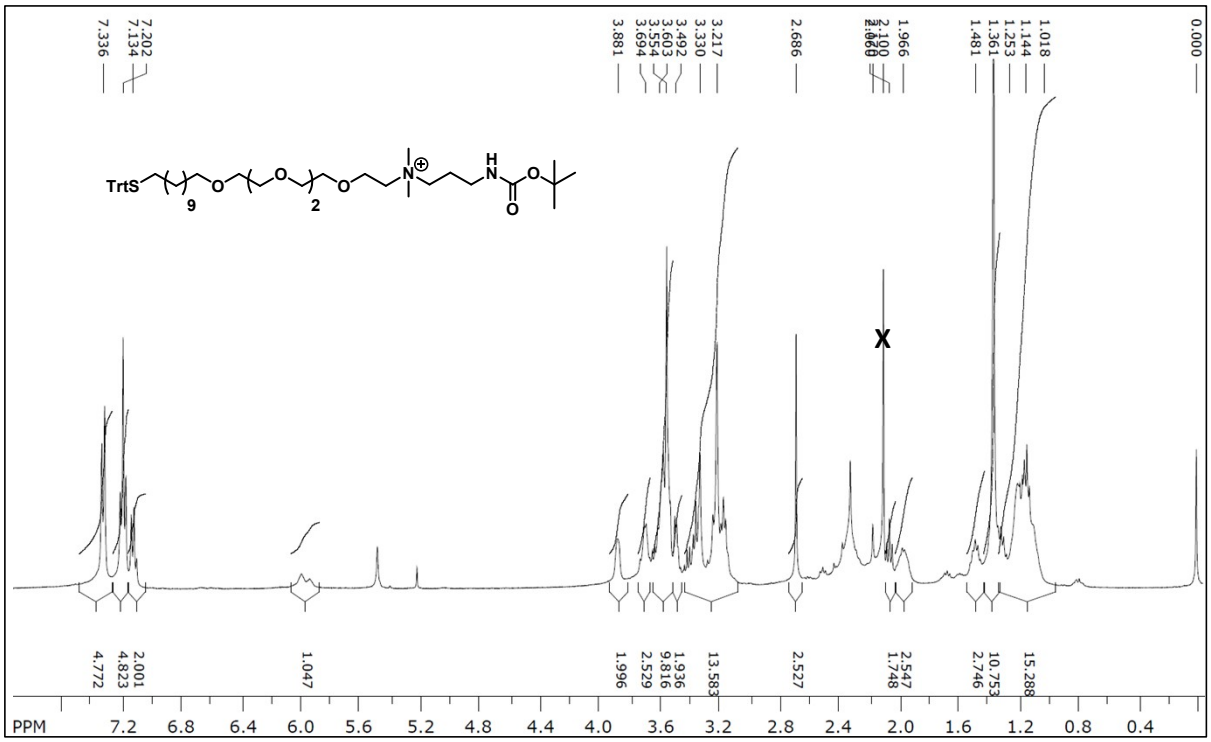


Figure S7: 400 MHz ^{1}H NMR spectrum of compound 5c in $CDCl_3$. (X refers to solvent impurities)

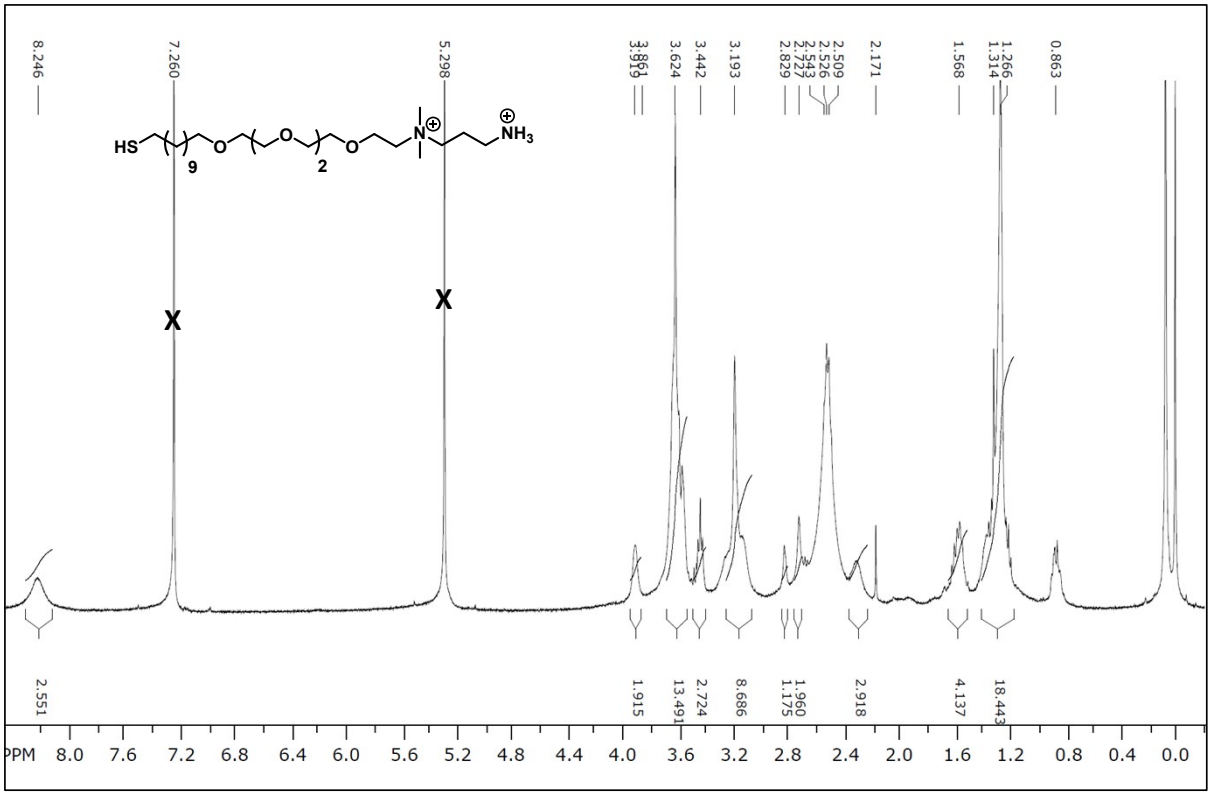


Figure S8: 400 MHz ^{1}H NMR spectrum of compound 5d in $CDCl_3$. (X refers to solvent impurities)

10 Supplementary figures

Figure S9

TEM image and size distribution analysis of Au-C_{12}

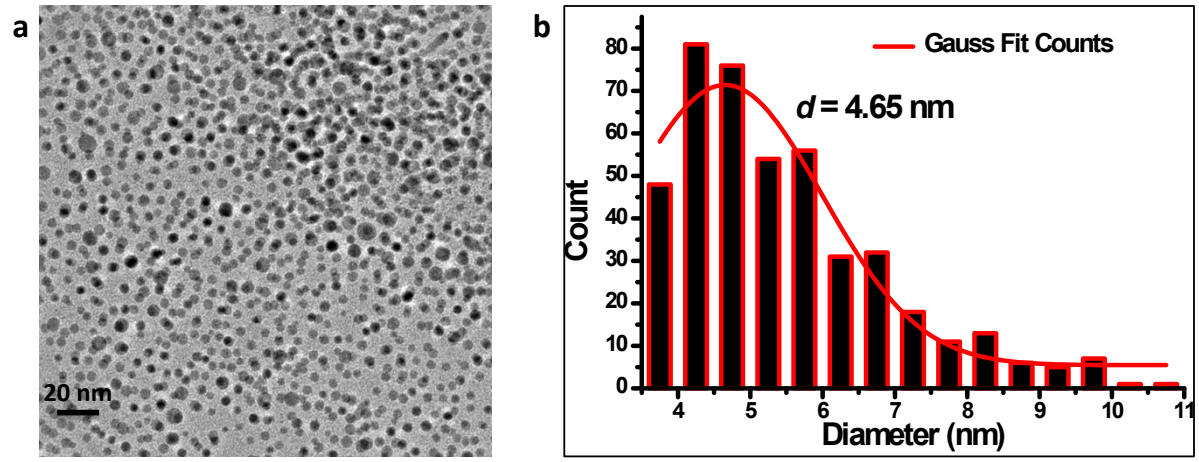


Figure S9: a) High resolution TEM image of **Au-C_{12}** (4.65 nm) nanoparticles b) Size distribution analysis of **Au-C_{12}**.

Figure S10

UV-Visible spectrum of Au-Tz (H)

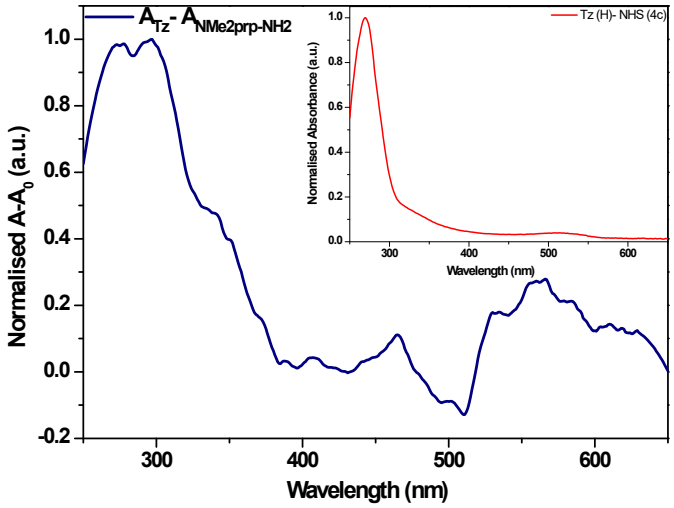


Figure S10: UV-Visible spectrum showing resultant absorbance around 270 nm which indicates the presence of tetrazine moiety, inset: UV-Vis spectrum of Tz (H)-NHS (compound 4c).

Figure S11

Control experiments to demonstrate that the successful formation of the stabilized microcapsules critically depends on interfacial crosslinking between Au-Nor and Au-Tz nanoparticles

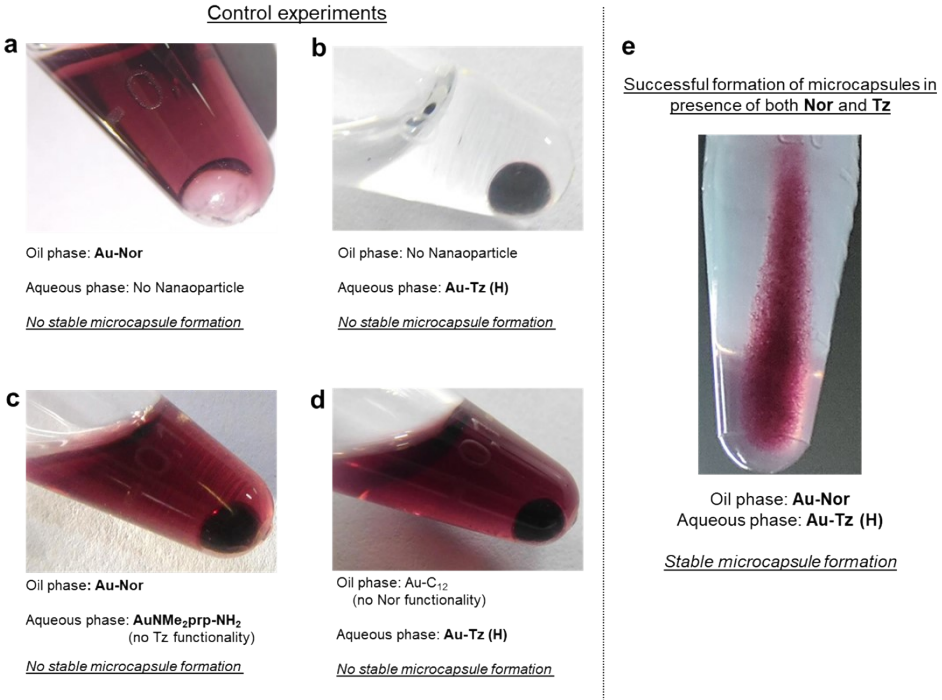


Figure S11: (a-d) Control experiments where none of the combinations contain both Nor and Tz functionalized AuNPs in their respective phases. As a result, no cross-linking was possible for these

control combinations. In the absence of cross-linking, we did not observe any form of stable microcapsules. After emulsification, these control sets quickly formed a phase-separated solution, where a 10 µl water phase visually appeared as a settled droplet at the bottom of the tube containing 200 µl of toluene phase. e) Successful fabrication of microcapsules via Au-Nor (toluene phase) and Au-Tz(H) (aqueous phase) after vigorous shaking of emulsion. A visually distinct appearance of the stabilized microcapsules was observed as compared to the control sets.

Figure S12

AFM image of a microcapsule

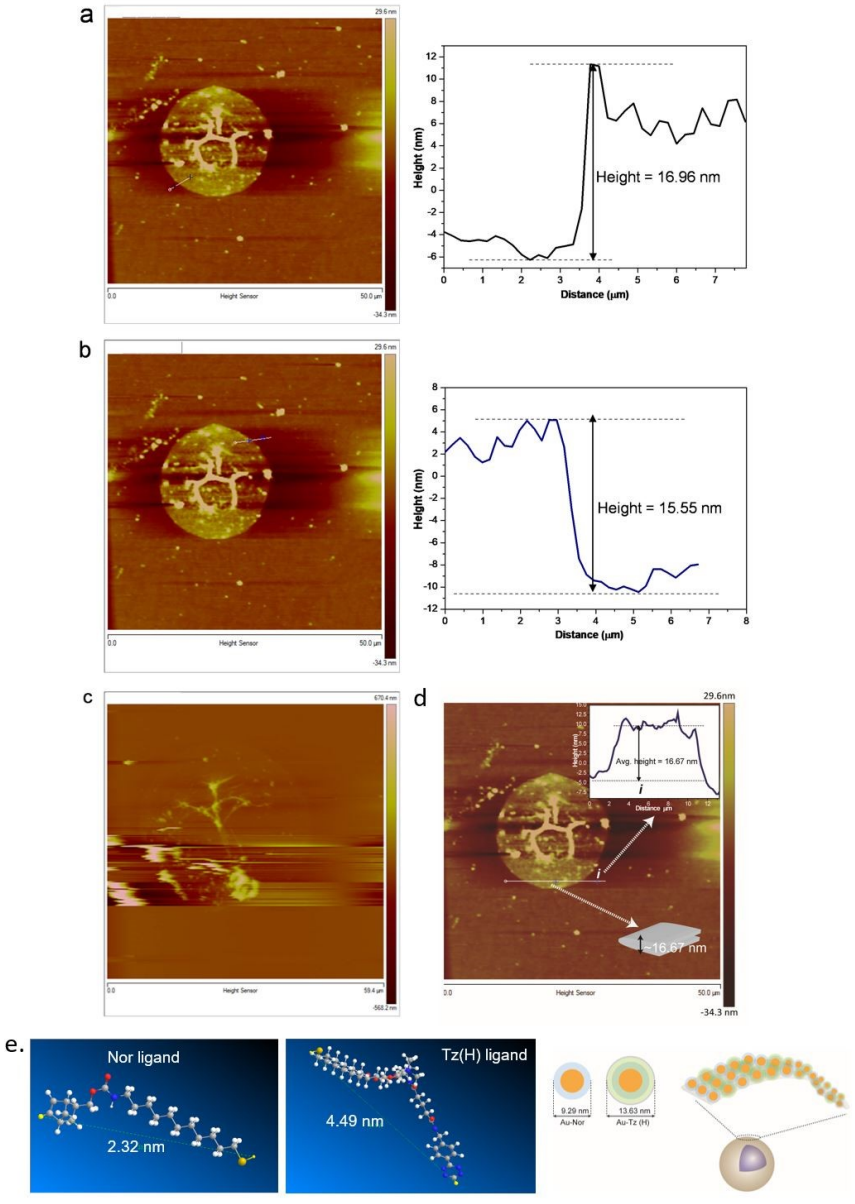


Figure S12: (a and b) Representative AFM images of the microcapsules. Height profiles are given along with the microcapsule images, which represent the height difference between the mica background and fold free region of the microcapsule (white dotted lines). The height profile was found to be (a) 16.96 nm and (b) 15.55 nm which includes the two layers of the collapsed shell. (c)

Additional AFM image of a microcapsule. (d) Schematic showing that the height profile includes two layers of the collapsed capsule. The capsule single wall thickness was determined as half of the height of the collapsed flat regions of dried capsules. (e) Based on the overall size of the nanoparticle (core + ligand), a laterally crosslinked structure was predicted from the measurement.

Figure S13

MALDI characterisation of microcapsule

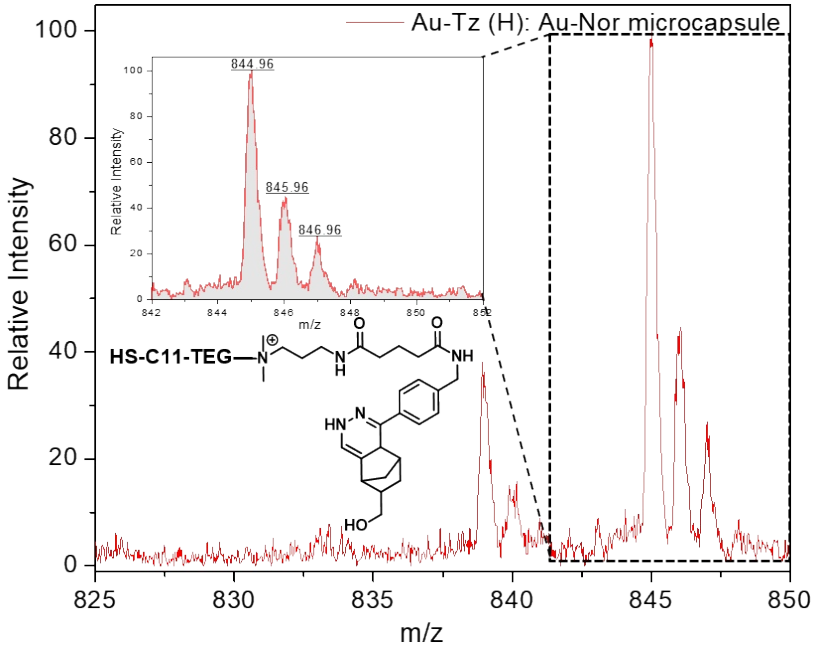


Figure S13: MALDI spectrum of microcapsules showing peak at m/z = 844.96 (observed) and isotopic pattern [844.96 (100.0%), 845.96 (44.7%), 846.9684 (24.7%)] which corresponds to the theoretical mass value (m/z = 844.56) and isotopic pattern [844.5616 (100.0%), 845.5650 (49.8%), 846.5684 (12.1%)] of thiol ligand of tetrazine crosslinked to norbornene moiety.

Figure S14

SVEC-RFP cell line encapsulation in colloidosome

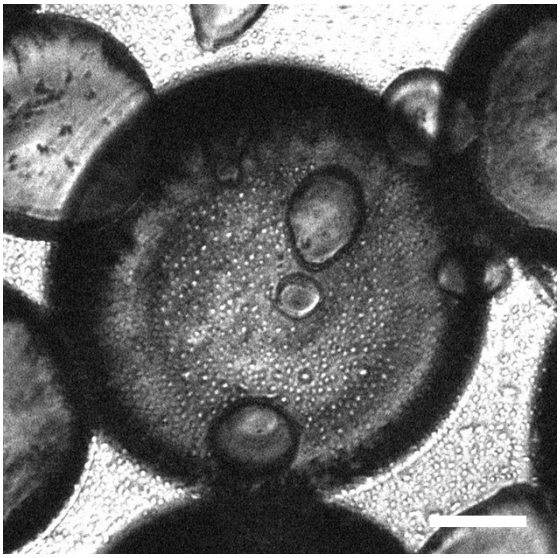


Figure S14: Optical micrograph of SVEC-RFP cell line encapsulated colloidosome (scale bar = 50 µm).

Figure S15

Microcapsule fabrication by amalgamator

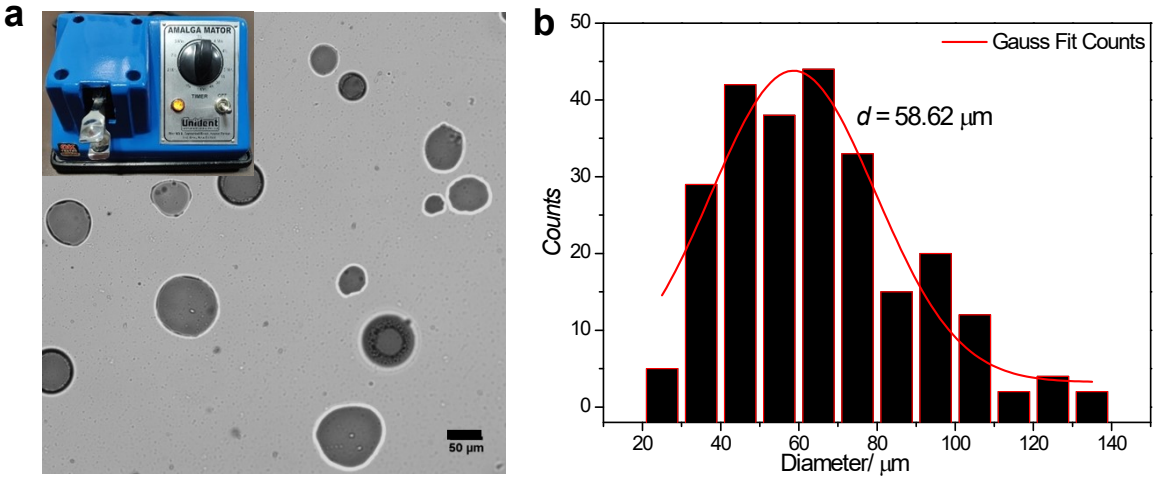


Figure S15: (a) Optical micrograph of **Au-Tz (H)** and **Au-Nor** microcapsules fabricated via amalgamator in 8s, scale bar (50 µm) (b) size distribution analysis of microcapsule depicting the average size of 58.62 ± 2.43 µm through gauss fit curve (red curve).

Figure S16

Raman spectrum of R6G

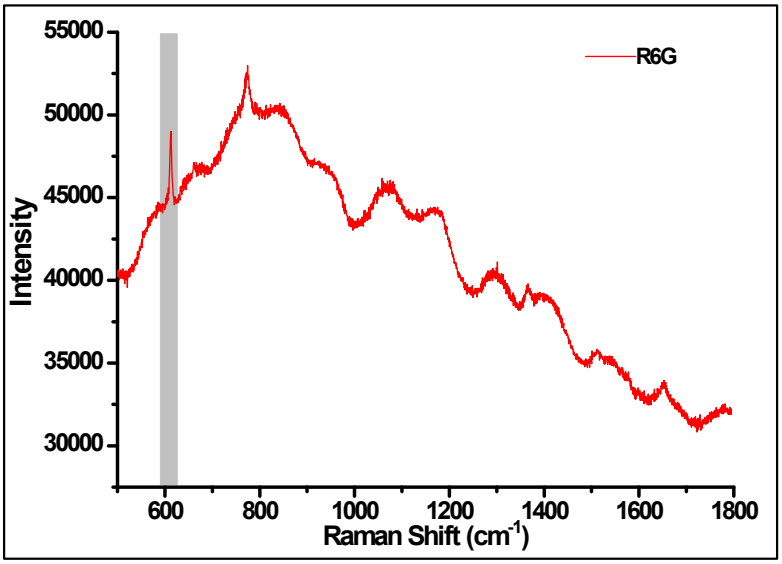


Figure S16: Normal Raman spectrum of aqueous R6G solution with an acquisition time of 1s.

Figure S17

UV-Visible spectral changes during colloidosome fabrication

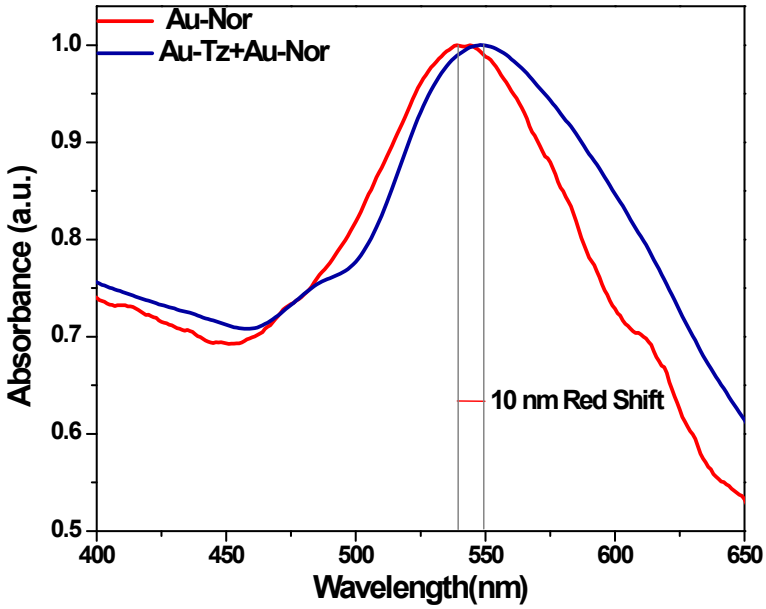


Figure S17: Plasmonic shift (red shift) of crosslinked gold nanoparticles during colloidosome fabrication.

Figure S18

UV-Vis spectrum of Au-Tz (H) -Nor-SCy5 conjugation

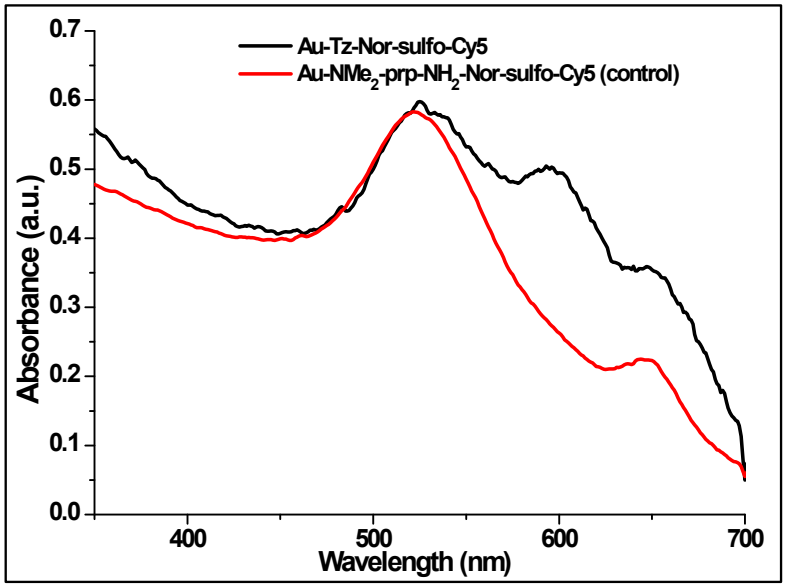
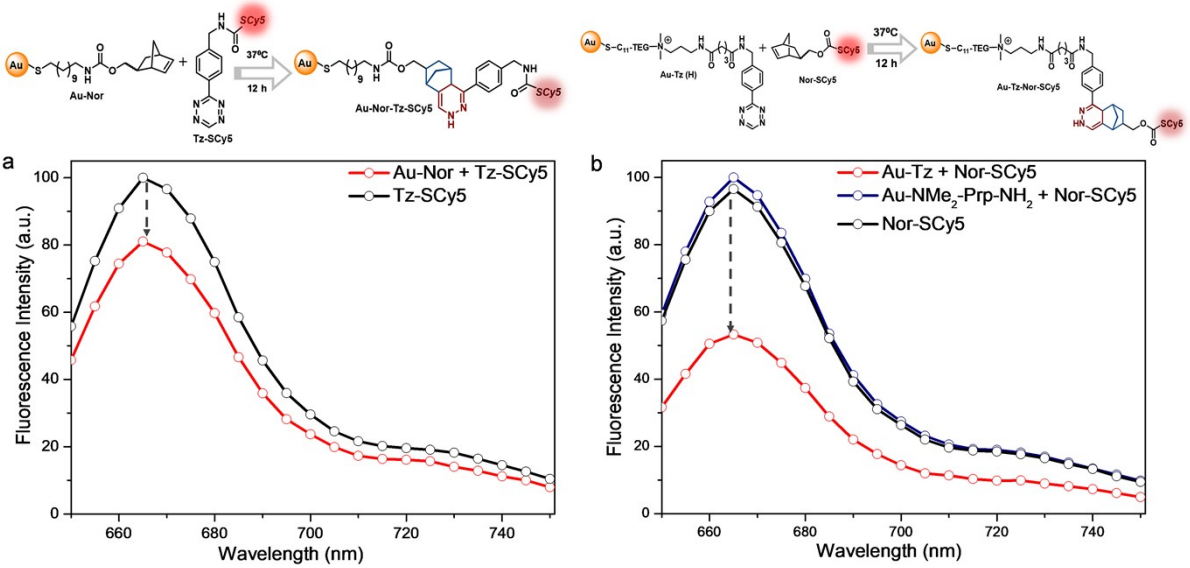


Figure S18: UV-Visible absorbance spectrum of **Au-Tz-Nor-SCy5** conjugation and Au- NMe_2prpNH_2 -Nor-SCy5 as control.

Figure S19

Fluorescence studies to determine surface coverage of Au-Nor, Au-Tz (H) gold nanoparticles and Au-Tz (H)-Au-Nor crosslinked nanoparticles in microcapsules



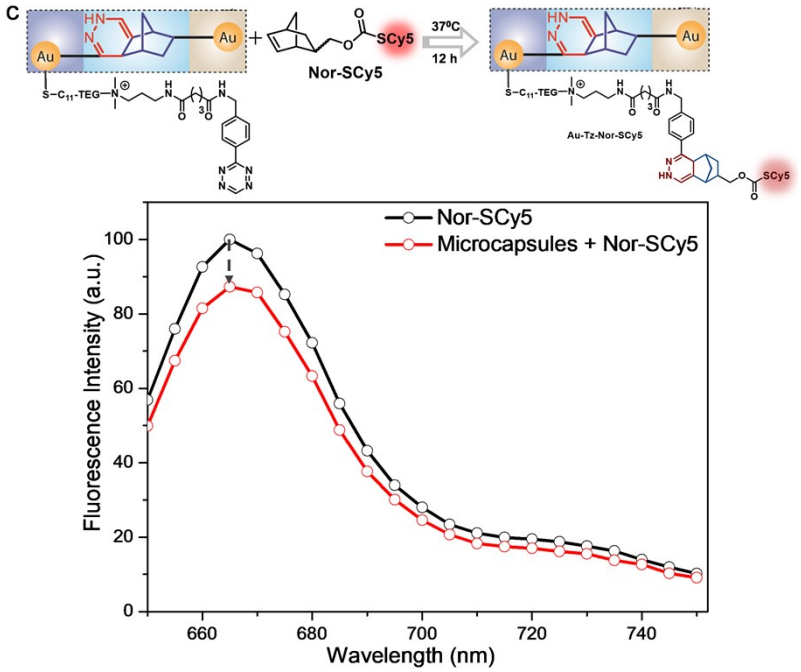


Figure S19: Fluorescence spectra of (a) **Tz-SCy5** fluorophore to determine number of norbornene ligands per **Au-Nor** gold nanoparticle (b) **Nor-SCy5** fluorophore to determine number of tetrazine ligands per **Au-Tz (H)** gold nanoparticle and (c) **Nor-SCy5** fluorophore to determine total surface ligands of **Au-Tz(H)** crosslinked with Au-Nor gold nanoparticles in microcapsules.

Figure S20

Field Emission Scanning Electron Microscopy- Energy Dispersive X-Ray Analysis (FESEM-EDAX) of microcapsules:

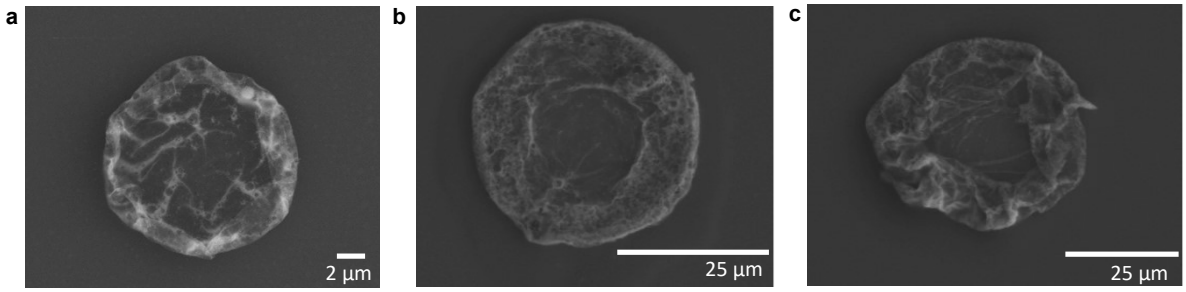


Figure S20: (a-c) Additional FESEM images of assembled microcapsules, generated via cross-linking between Au-Tz (H) and Au-Nor. These were taken from different parts of the sample-drop-casted silicon wafer. These images show the overall uniform spherical morphology of the fabricated microcapsules.

11 References:

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