Supporting Information

PEGylated gold nanoparticles promoted rapid macromolecular chain-end transformation and formation of injectable hydrogels

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Materials

poly(ethylene glycol) (PEG, 4000 g/mol, sigma aldrich), 4-(chloromethyl)benzoyl chloride (Cl-Bz-Cl, >98%, TCI), triethylamine (>99%, TCI), methanesulfonyl chloride (>98%, TCI), sodium azide (>99%, TCI), cysteamine hydrochloride (>98%, Sigma Aldrich), poly(vinyl alcohol) (PVA, 98-99%, M_w~31,000), 1,1'-carbonyldiimidazole (CDI, >97%, TCI), propergylamine (>97%, TCI), sodium borohydrate ($NaBH_4$, SRL, 95%), tetra chloroauric acid (99.99%, Alfa Aesar), copper(II) sulfate pentahydrate (>98%,sigma aldrich), sodium ascorbate (sigma aldrich), 1-Hexyne (>97%, TCI), poly(ethylene glycol) diacrylate (750 g/mol, Aldrich), 1,6-hexanedithiol (96%, Aldrich), poly(ethylene glycol) bis(3-aminopropyl) terminated (1500 g/mol, Aldrich), pentaerythritol tetra(3-mercaptopropionate) (>98%, TCI), ε-poly(lysine) (M_w ~ 5500 Da, Aldrich), and dialysis membrane (D0530, MWCO 2000 g/mol, Sigma Aldrich) were used as received. All the solvents were from Spectro Chem, India, and were used as received.

Synthesis of prepolymers

Synthesis of α-ω-halide-terminated PEG (Cl-PEG-Cl)

Cl-PEG-Cl was synthesized by the esterification reaction of PEG with Cl-Bz-Cl. PEG (4000 g/mol, 10 g, 0.005 mol of hydroxyl groups) was dissolved in toluene (100 mL). About 20 mL of toluene was evaporated by rotary evaporator to remove moisture from the solution. Next, triethylamine (0.02 mol, 2.019 g) was added to round bottom flask by syringe. The temperature of the flask was kept at 5-10 ^oC by an ice bath. Subsequently, Cl-Bz-Cl (0.02 mol, 3.779 g) previously dissolved in toluene (10 mL) was added to the round bottom flask drop-wise via the syringe. The mixture was allowed to stir at room temperature for 6 h. After 6 h the insoluble mass was filtered off, and the solid mass was recovered by removing the toluene with a rotary evaporator at 60 ^oC. The solid mass was then dissolved in deionized water and centrifuged for

10 min at 5000 rpm. The transparent supernatant solution was collected and lyophilized to obtain the product. ^{1}H NMR ($CDCl_3$): δ=3.6 (158 H, methylene protons of PEG backbone), 4.6 (methylene protons of phenyl benzyl chloride), 7-8 (aromatic protons), 4.4 ppm.[1,2]

Synthesis of α-ω-Azide-terminated PEG (N_3-PEG-N_3)

The N_3-PEG-N_3 was prepared by a two-step reaction.[3] First, dried PEG (M_n=4000 g/mol, 5 g, 0.0025 mol of hydroxyl groups) was dissolved in dry pyridine (20 mL) and was cooled to 0 ^{o}C. The methanesulfonyl chloride (0.71 g, 0.00625 mol) separately dissolved in dry DCM (10 mL) was added drop wise to the above solution under continuous stirring for 10 min. After complete addition, the reaction mixer was allowed to stir at room temperature for 12 h. The solvent was removed by rotary evaporator, and the mass was treated with a saturated aqueous solution of $NaHCO_3$. After treatment, it was extracted with CH_2Cl_2. The organic phase was dried over Na_2SO_4 and precipitated in diethyl ether. The precipitated mass was dried under vacuum. PEG-dimesylate (4 g, 0.002 mol of mesitylene) and sodium azide (0.325 g, 0.005 mol) were dissolved in DMF (20 mL) and allowed to stir under nitrogen at 110 ^{o}C for 4 h. After that, it was allowed to stir at room temperature for another 18 h. The solid mass was removed by filtration and the filtrate obtained was concentrated by rotary evaporator. The concentrated mass was precipitated in diethyl ether. The precipitate obtained was partitioned between DCM and water. The organic phase was collected and dried over Na_2SO_4 and evaporated to dryness. The solid mass obtained was further dissolved in water and dialyzed against water for 24 h. The dialyzed solution was then freeze-dried and characterized by ^{1}H NMR spectroscopy (Fig. S1). ^{1}H NMR ($CDCl_3$): δ=3.6 (158 H, methylene protons of PEG backbone), 3.33 ppm (4H, t, CH_2-N_3).

Synthesis of alkyne-functional poly(vinyl alcohol) (PVA-alkyne)

PVA (M_n=31000, 1 g, 0.0195 mol of hydroxyl) was dissolved in dry DMSO (15 mL), and the solution was dried by addition of some amount of dry toluene followed by its removal.[3] The CDI (1.58 g, 0.00975 mol) was added in the above solution in one portion with continuous stirring under nitrogen atmosphere at room temperature. The reaction mixture was then allowed to stir for 3 h. After 3 h the solution of propargylamine (0.002785 mol, 0.153 g) prepared in 1 mL of DMSO was added and was allowed to stir for 20 h at room temperature. After that 10 mL of ammonia was added and allowed to stir for another 1 h. Next, the reaction mixture was diluted with 60 mL of water, followed by filtration. The filtrate was concentrated by rotary evaporator. The polymer was then precipitated in diethyl ether and ethanol mixture (80:20). The precipitated samples were dissolved in water and dialysed against water for 24 h. The dialysed solution was freeze-dried. ^{1}H NMR (D_2O): δ= 4.90 (m, methine protons of modified unit of PVA), 4.00-3.8 (1H, m, methane protons of unmodified unit of PVA), 1.9-1.5 (2H, m, polymer backbone methylene of PVA), 2.73 (1H, s, methane proton of propargyl unit), 3.73 ppm (2H, d, methylene protons of propargyl unit) (Fig. S2). Degree of alkyne substitution (DS) was calculated to be 0.03 by considering the intensity ratio of peaks at 2.73 ppm to peak at 4.00-3.8 ppm.

Characterization of Au NPs and assessment of stability

The stability of prepared $(PEG\text{-}SH)_n$ stabilized Au [Au-$(PEG\text{-}SH)_n$] NPs were assessed by using UV-Visible analysis. UV-Visible spectra were recorded in Shimadzu Spectrometer (UV-2700). First, UV-Visible spectrum of as prepared (solution) NPs was recorded. The lyophilized Au-$(PEG\text{-}SH)_n$ NPs (1 mg) were dispersed in deionized water, and UV-Visible spectrum was noted. The absorbance maxima (λ_{max}) was observed at 514 nm in both the experiments. The thermal stability of Au NPs was monitored by heating at 100 ^{o}C. The stability of Au NPs with a change in pH of aqueous media was determined by incubating the aqueous solutions at pH

7.4 and pH 5 respectively for 48 h and at temperature 37 ^{o}C. The UV-Visible spectra were noted after 48 h. The λ_{max} was found to be 514 nm. Similarly, stability in the presence of NaCl (100 mM aqueous solution) or BSA (400 μM) was verified by incubating the Au NPs in presence of respective extraneous reagents. Further, TEM analysis was performed to assess NPs stability.

Further, the isolated Au-(PEG-SH)$_n$ NPs were dispersed in xylene and λ_{max} was noted. Next, the dispersed Au-(PEG-SH)$_n$ NPs in xylene was allowed to heat at 100 ^{o}C for 48 h. The UV-Visible spectrum was collected after 48 h of incubation. TEM analysis was also performed to assess the stability of the NPs.

The hydrodynamic diameter (D_h) of the NPs dispersed in deionized water was determined by Dynamic Light Scattering (DLS). DLS measurements were performed in a Nano Biochem Technology Spectro Size 300. The solution (0.1 mg/mL of NPs) in deionized water was filtered through a membrane (pore size 0.45 μm) for DLS analysis. The D_h was found to be 10 nm by DLS measurements.

The size of the NPs was obtained by TEM analysis. The Au NPs dispersed in deionized water (0.1 mg/mL) was drop-cast on a copper grid and dried at room temperature for 24 h followed by storage inside a calcium chloride filled desiccator to remove moisture.

The TEM images of the Au-(PEG-SH) NPs loaded gels were also recorded. The Au NPs (0.1 mg/mL) were dispersed in dilute prepolymer solution (QPDMA: Cl-PEG-Cl =1:1, total polymer concentration 0.1 %) and solution was casted on copper grid. The grid was allowed to dry for 24 h at room temperature and further stored for 24 h in calcium chloride desiccator. TEM images were recorded in a JEOL, JEM 2100 machine operating at an accelerating voltage 120 KV (Fig. 1B showing 5 nm size).

Evaluation of effect of Au-(PEG-SH)$_n$ NPs on the rate of AAC reaction at macromolecular backbone and chain-ends

AAC reaction between sodium azide and PVA-alkyne was performed in the presence and absence of Au-(PEG-SH)$_n$ NPs or Cu catalyst. The solution of propargylamine-functionalized PVA (PVA-alkyne) (0.007 g in 2 mL of D_2O, DS of alkyne=0.03) was prepared, and Au-(PEG-SH)$_n$ NPs (0.01% w/w with respect to total reactant) was dispersed in it. The solution of NaN_3 (0.0113 g, $1.73x10^{-4}$ mol in 2 mL of D_2O) was then added in the previously prepared solution. The ^1H NMR spectrum was recorded after 24 h. The formation of triazole was confirmed by ^1H NMR spectroscopy. ^1H NMR (D_2O): δ =8.01 (1H, s) triazole, 4.90 (1H, m) polymer backbone of modified PVA unit, 3.73, (H, m) methine proton of PVA backbone of unmodified unit, 2.00-1.35, (2H, m) polymer backbone, 3.66 (2H) methylene protons of unreacted and reacted propargylamine, and 2.73 ppm (H, methine) of unreacted propargylamine (Fig. S8). The unreacted alkyne groups of PVA were calculated by comparing the intensity ratio of $I_{2.73}$ (unreacted methane of propargyl moiety) to $I_{3.66}$ of starting reaction mixture and after reaction. This gives conversion of alkyne moieties of PVA. Similarly, the reaction mixture containing no catalyst and containing $CuSO_4$ (1.4% w/v, 95 mM) and sodium ascorbate (0.59 g, 490 mM) was performed under similar concentrations of substrates. The Cu catalyst was added in PVA-alkyne solution for the reaction.

Conversion of chain end-functionality of N_3-PEG-N_3 by AAC reaction was performed by reacting N_3-PEG-N_3 and 1-hexyne in DMSO at 25 ^oC for 24 h in the presence of Au-(PEG-SH)$_n$ NPs. The triazole terminated PEG was then isolated by dialysis and freeze drying. The ^1H NMR spectrum ($CDCl_3$) of isolated functionalized PEG was taken. The conversion was calculated accordingly by comparing the intensity ratio of methylene proton of reacted alkyne moiety to methylene proton of unreacted PEG termini.

Evaluation of the effect of Au-(PEG-SH)$_n$ NPs on the rate of thiol-acrylate Michael addition reaction at the macromolecular chain-ends

The reaction between acrylate-terminated PEG (AA-PEG-AA) and 1,6-hexanedithiol was performed in the presence and absence of Au-(PEG-SH)$_n$ NPs. The solution of AA-PEG-AA (0.02 g, 5.3x10^{-5} mol of acrylate groups in 2 mL methanol-d4) was prepared, and then Au-(PEG-SH)$_n$ NPs (0.01% w/w with respect to total substrate) was dispersed in it. Solution of 1, 6-hexanedithiol (0.0039 g, 5.3x10^{-5} mol of thiol in 0.4 mL of methanol-d4) was added in the above solution. The concentration of individual AA-PEG-AA, and 1, 6-hexanedithiol was 0.023 M. The ^{1}H NMR spectra were recorded after a specific time interval. The conversion (mol/mol) of acrylate functional groups was calculated by the following formula:

$$Conversion\ of\ acrylate\ functional\ groups = \frac{I_{5.5-6.5(0)}/I_{3.6} - I_{5.5-6.5(t)}/I_{3.6}}{I_{5.5-6.5(0)}/I_{3.6}} \times 100\ (\%, {}^{mol}/_{mol}) \quad (1)$$

where $I_{5.5\text{-}6.5(0)}$ and $I_{5.5\text{-}6.5(t)}$ are the integral areas of the signal of three protons of acrylate groups of PEG before and after reaction between AA-PEG-AA and 1,6-hexanedithiol for the specific time interval. $I_{3.6}$ is the integral area of the protons of the PEG backbone. The intensity of the signal at 3.6 remained unaltered before and after the reaction.

Evaluation of effect of Au NPs on the rate of amine-acrylate Michael addition reaction at the macromolecular chain-ends

Michael addition reaction between AA-PEG-AA and H_2N-PEG-NH_2 was performed in the presence and absence of Au-(PEG-SH)$_n$ NPs. The solution of AA-PEG-AA (0.01 g, 2.66×10^{-5} mol of acrylate group in 1 mL of D_2O) was prepared and then Au-(PEG-SH)$_n$ NPs were dispersed in it. Next, H_2N-PEG-NH_2 (0.02 g, 2.66×10^{-5} mol of amine groups, in 2 mL of D_2O) was added. The concentration of individual AA-PEG-AA and H_2N-PEG-NH_2 was 0.008 M.

The ^{1}H NMR spectra were recorded after a specific time interval. The conversion of acrylate groups was calculated by the following formula:

$$Conversion\ of\ acrylate\ functional\ groups = \frac{I_{5.5-6.5(0)}/I_{3.6} - I_{5.5-6.5(t)}/I_{3.6}}{I_{5.5-6.5(0)}/I_{3.6}} \times 100\ \ (\%, {}^{mol}/_{mol}) \quad (2)$$

where $I_{5.5\text{-}6(0)}$ is the integral area of the signal of three protons of acrylate bond of PEG and $I_{3.6}$ is the integral area of the proton backbone acrylate terminated PEG and amine-terminated PEG at the initial time, i.e. just after mixing the two reactants. Symbol t at the bracket [(t)] indicates corresponding intensity values of the signal at specific time interval of reaction.

Characterizations of hydrogels in terms of porosity

For the determination of porosity, square shaped hydrogel was prepared. The hydrogel was allowed to swell in water to attain equilibrium water uptake. The surface water was then removed, and the weight of the swollen hydrogel was recorded. The hydrogel was then dried, and the weight of the dried hydrogel was recoded. The porosity of the hydrogel was determined by the following equation:

$$\varepsilon = (w_l - w_d)/Ald_w \qquad (3)$$

where d_w is the density (0.998 g cm^3) of water; A is the effective area of the hydrogel sample (cm^2), and l is the thickness (cm) of the sample.

SEM Analysis

As obtained water-swollen hydrogels were used for the analysis. The samples were frozen by liquid nitrogen and were broken. The samples were freeze-dried and stored in silica gel filled desiccator. The freeze-dried samples were then coated with gold using sputter coater (LEICA

EM ACE 200 gold coater). The gold-coated samples were analyzed by SEM (FESEM, JSM-7100F).

Haemolysis

Haemolytic activity of Au-$(PEG\text{-}SH)_n$ NPs, was evaluated according to the reported protocol.[1,2] The heparinised human blood (2 mL) was taken from the blood bank and centrifuge for 5 min at 2000 rpm. The pallet was collected and washed thrice with the PBS (pH 7.4). The collected RBC pallet was dispersed and diluted up to 20 mL with PBS pH 7.4. The sample of Au-$(PEG\text{-}SH)_n$ NPs (0.01% w/v), was added into the 1 mL of the RBC solution separately and allowed to incubate for 24 h at 37 ^oC. The PBS (50 µL) and Triton X 100 (10 µL of 0.1%) was used as negative control and positive control respectively. After 24 h of incubation, tubes were centrifuged at 3000 rpm for 5 min, and the supernatant was taken into the 96 well plates, and then UV-Visible analysis was performed by spectrophotometer (Spectramax plus 384, Molecular Devices, USA) at 540 nm. The amount of haemolysis was calculated by the following formula:

$$Hemolysis\ (\%) = \frac{OD\ 540_{sample} - OD\ 540_{negative\ control}}{OD\ 540_{positive\ control} - OD\ 540_{negative\ control}} X\ 100 \quad (4)$$

where OD sample, OD negative control, and OD positive control are the absorbance values of the samples, and negative and positive controls respectively. All haemolysis experiments were performed in triplicates.

Furthermore, RBC samples treated with negative control, positive control and Au-$(PEG\text{-}SH)_n$ NPs were prepared for the SEM analysis by fixing with glutaraldehyde followed by dehydration by a series of washing with ethanol. The sample were placed over the clean silicon wafers and subjected to SEM analysis.[4]

^{1}H NMR Spectrum of N_3-PEG-N_3

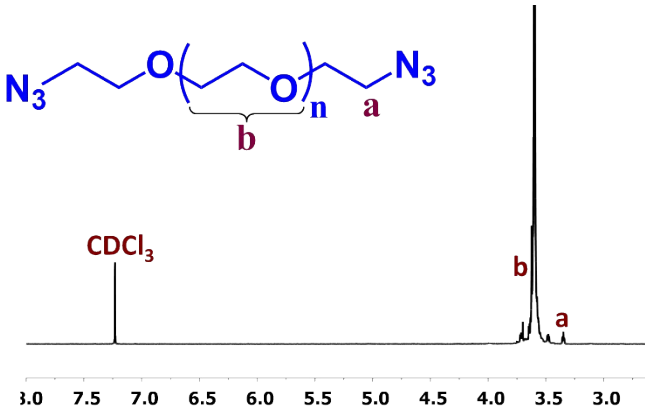


Fig. S1. ^{1}H NMR (600 MHz, $CDCl_3$) spectrum of N_3-PEG-N_3.

^{1}H NMR Spectrum of PVA-alkyne

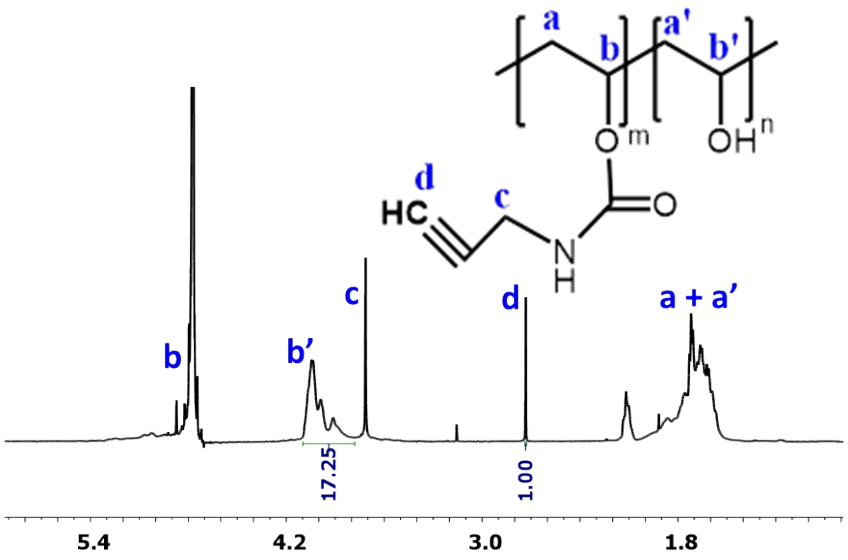


Fig. S2. ^{1}H NMR (D_2O, 600 MHz) spectrum of PVA-alkyne. The degree of alkyl-substituted (DS) was calculated to be 0.03.

^{1}H NMR spectra and GPC analysis of $(PEG\text{-}SH)_n$, (HS-PEG-SH) and (HS-PEG-SH') stabilizers

^{1}H NMR (Fig. S3A) and GPC (Fig. S3D) analysis showed the formation of well-defined $(PEG\text{-}SH)_n$ by the reaction between Cl-PEG-Cl and 2-aminoethanethiol. The GPC trace of $(PEG\text{-}SH)_n$ is shifted to the lower elusion volume than that of starting Cl-PEG-Cl. This confirms the formation of segmented $(PEG\text{-}SH)_n$. The ^{1}H NMR spectra of HS-PEG-SH and HS-PEG-SH' showed di-thiol functional nature of these stabilizers (Fig. S3B and S3C). Furthermore, GPC

traces of starting PEG and prepared HS-PEG-SH and HS-PEG-SH' showed no change in M_n (Fig. S3E and S3F).

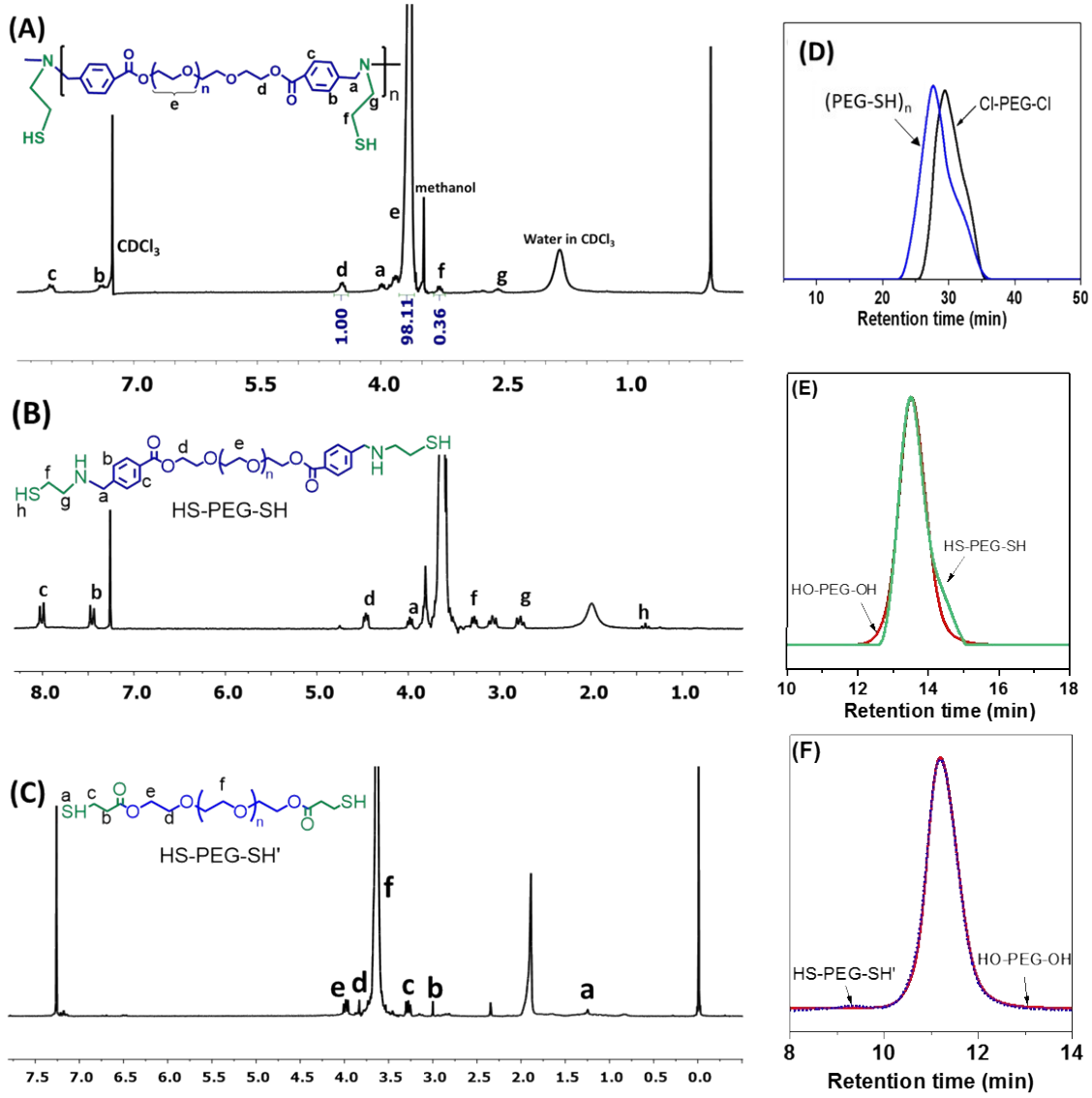


Fig. S3. ^{1}H NMR spectra ($CDCl_3$, 600 MHz) of (A) $(PEG\text{-}SH)_n$, (B) (HS-PEG-SH), and (C) (HS-PEG-SH') stabilizers. (D) GPC traces of starting Cl-PEG-Cl, and prepared $(PEG\text{-}SH)_n$. The GPC was performed using DMF (with 0.1 %w/v LiBr) as eluent at a flow rate of 8 mL/min. (E) GPC traces of starting HO-PEG-OH (4000 g/mol), and prepared HS-PEG-SH. (F) GPC traces of starting HO-PEG-OH (35000 g/mol) and prepared HS-PEG-SH' (Graph F). The GPC (E and F) was performed using water as eluent at a flow rate of 1 mL/min. PEG standard samples were used for the calibration.

Stability of Au-(PEG-SH)$_n$ NPs in xylene

The separated Au-(PEG-SH)$_n$ NPs were re-dispersed in xylene and incubated for 48 h at 25 ^{0}C and 100 ^{o}C separately. The plasmon resonance band of the NPs at this temperature range remained unchanged (546 nm). The plasmon resonance band of as-prepared NPs in aqueous medium appeared at 514 nm. Thus, a red shift was observed in xylene. Furthermore, TEM analysis showed dispersed NPs in xylene with reduced distance between them (Fig. S6, vide infra). This indicates that the red shift may be due to vibrational coupling and due to solvent effect. Thus in xylene, the NPs remained stable.

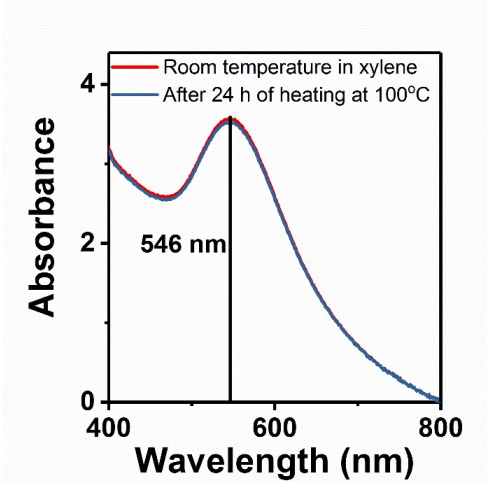
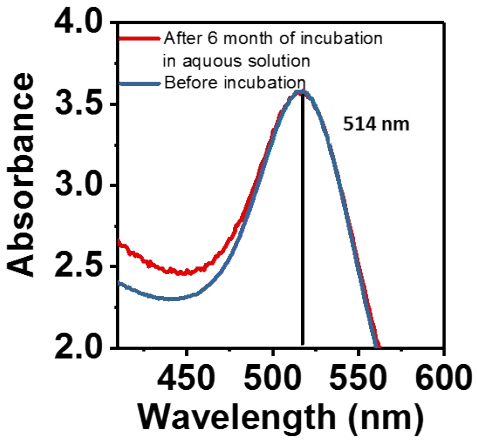


Fig. S4. UV-Visible spectra of Au-(PEG-SH)$_n$ NPs dispersed in xylene. The NPs were incubated in xylene for 48 h at 25 ^{o}C and 100 ^{o}C separately.



Stability of the Au-(PEG-SH)$_n$ NPs upon storage

Fig. S5. UV-Visible spectra of Au-(PEG-SH)$_n$ NPs dispersed in deionised water. The spectra were taken before (as prepared) and after storage of NPs for six months at 25 ^{o}C.

Stability of the Au-(PEG-SH)$_n$ NPs by TEM analysis

TEM images showed that the NPs remained stable after treatment with different extraneous reagents. The NPs were incubated in the presence of BSA protein and xylene separately. The size and shape of the NPs remained unchanged upon treatment with these extraneous reagents (Fig. S6). The stability of the NPs is comparable with that of as-prepared NPs (compare Fig. S6 and Fig. 1B main text). However, in xylene (Fig. S6B), the distance between the NPs decreases than that of as-prepared NPs. This may lead to vibrational coupling and causes a red shift in plasmon resonance band of the NPs in presence of xylene (Fig. 1D, main text).

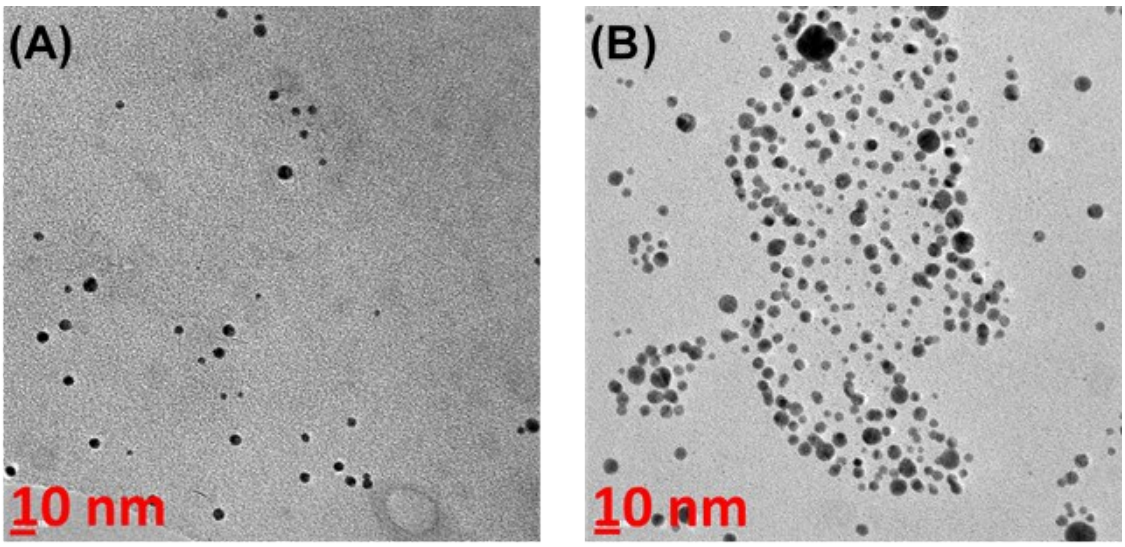


Fig. S6. TEM images of the Au-(PEG-SH)$_n$ NPs recorded after subjected to incubation in presence of (A) BSA protein, and (B) in xylene for 48 h at 25 ^{o}C.

Hemocompatibilty of the Au-(PEG-SH)$_n$ NPs

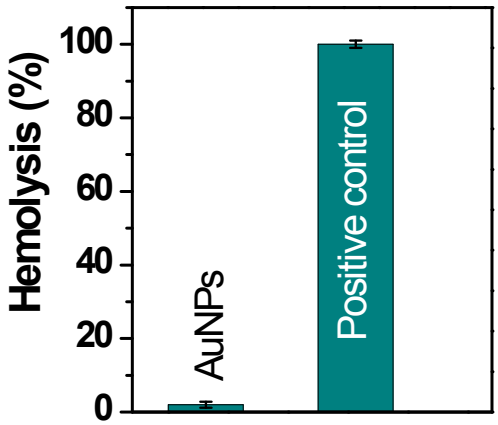


Fig. S7. Viability of red blood cell (RBC) in the presence of Au-(PEG-SH)$_n$ NPs. The red blood cells were incubated in the presence of Au-(PEG-SH)$_n$ (0.06% w/v) NPs at 37 ^{o}C for 24 h.

^{1}H NMR Spectra of the in situ formed adducts through Au-(PEG-SH)$_n$ promoted AAC reactions between NaN_3 and PVA-alkyne : Effect of (PEG-SH)$_n$ stabilizer on the reaction Rate

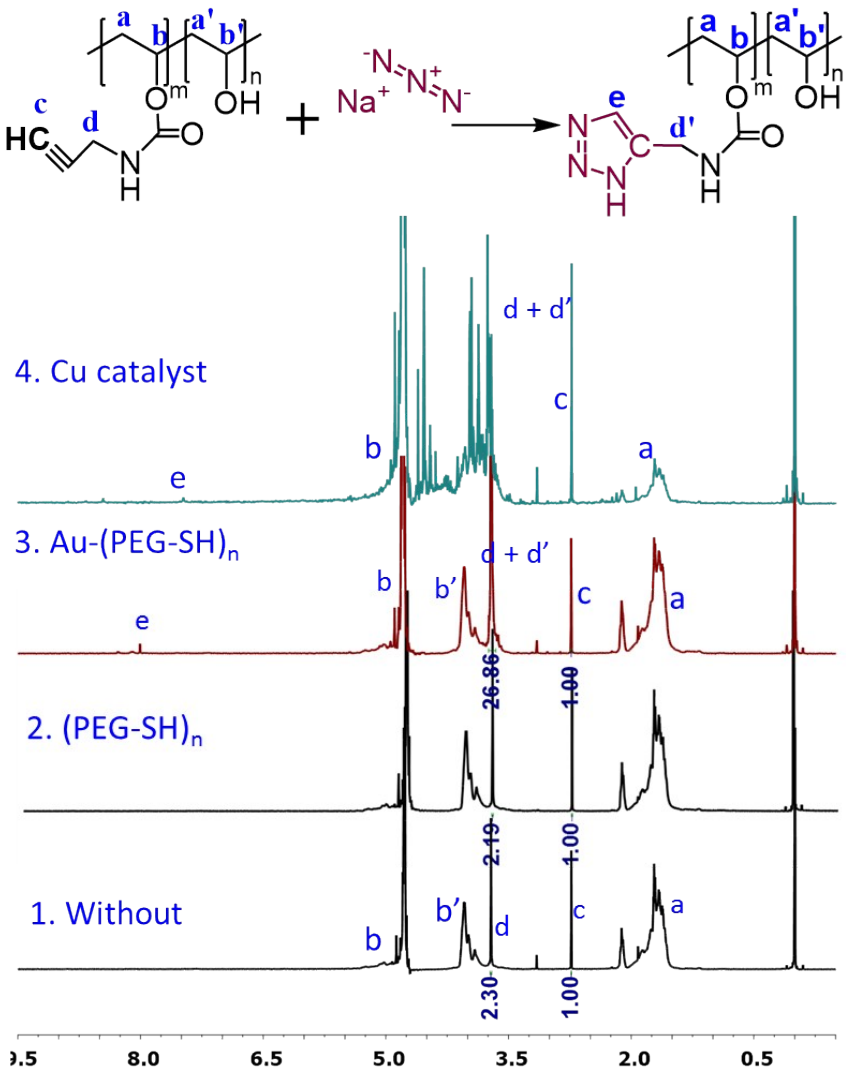


Fig. S8A. ^{1}H NMR spectra of adducts formed by AAC reaction between sodium azide (1.7x 10^{-4} mol), and PVA-alkyne (4.8×10^{-6} mol) in D_2O. The reactions were performed at 25 ^oC in presence of Cu catalyst (1.4% w/v, 95 mM), or Au-(PEG-SH)$_n$ NPs (0.01% w/v). No cycloaddition reaction took place in the absence of any catalyst or in presence of (PEG-SH)$_n$

(0.01% w/v). The conversion of alkyne was determined by comparing intensity ratio of NMR signals of d and c (staring reaction mixture), and after reaction (c and d+d').

^{1}H NMR spectrum of the in situ formed adduct by Au-(PEG-SH)$_n$ promoted AAC reaction between N_3-PEG-N_3 and 1-hexyne

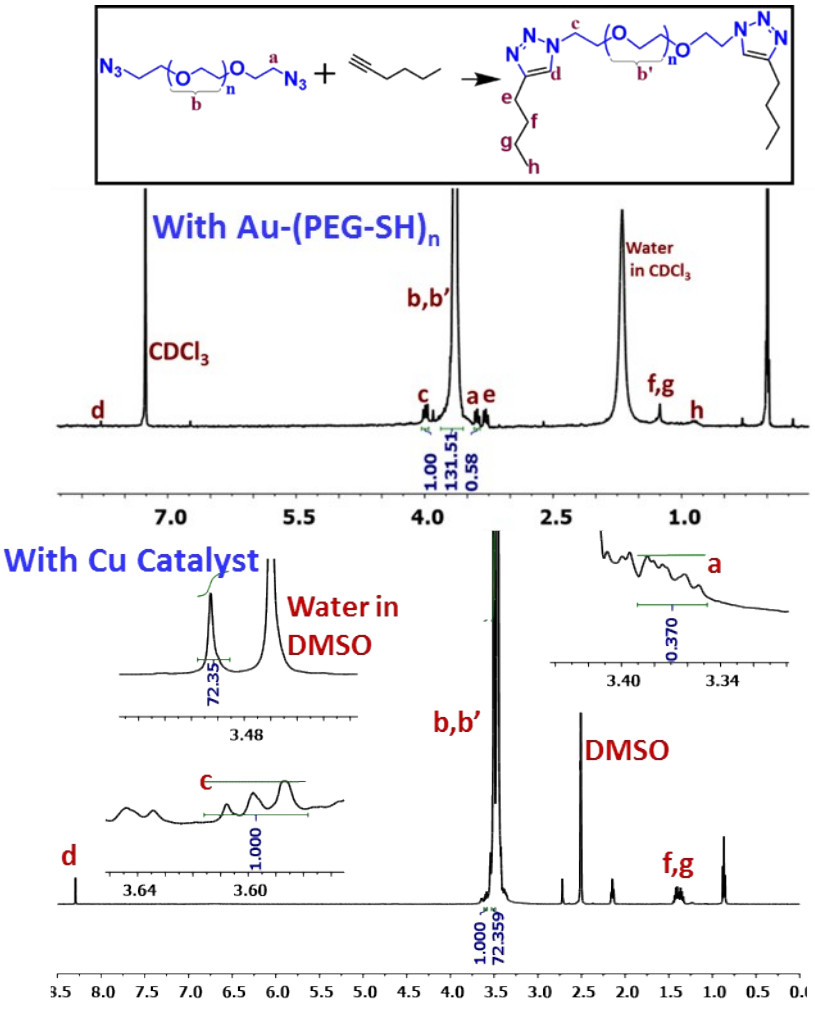


Fig. S8B. ^{1}H NMR (600 Hz) spectrum of the purified adduct formed by the AAC reaction between N_3-PEG-N_3 (0.0075 M) and 1-hexyne (0.0157 M) in the presence of Au-(PEG-SH)$_n$ NPs ($CDCl_3$) and Cu catalyst (DMSO-d6). The conversion of the alkyne group was about 70% and 74% in presence of Au-(PEG-SH)$_n$ NPs ($CDCl_3$) and Cu catalyst (DMSO-d6) respectively after 24 h at 25 ^{o}C.

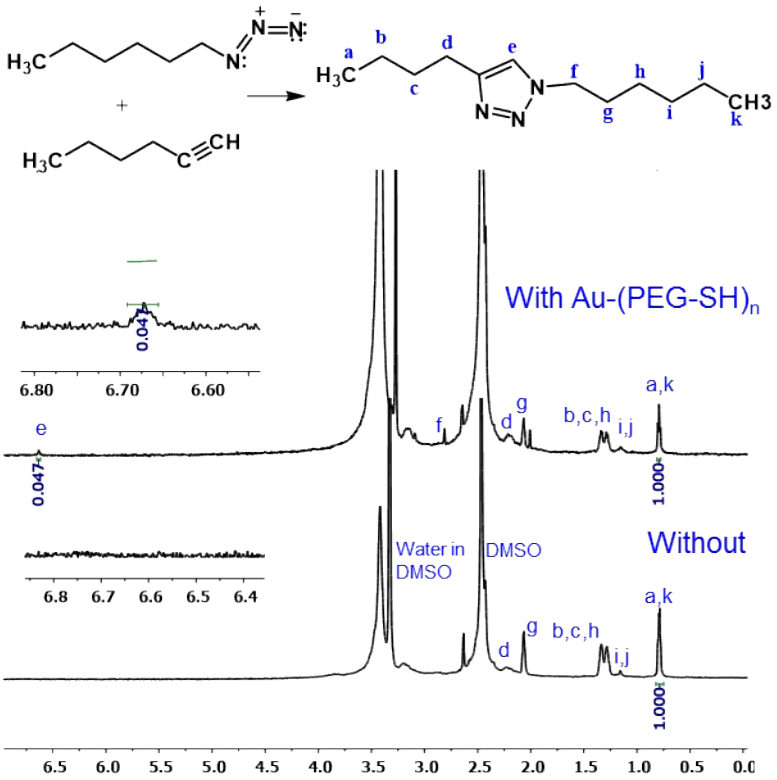


Figure 8C. ^{1}H NMR (DMSO-d6, 600 Hz) spectrum of the in situ adducts formed by the AAC reaction between 1-azidohexane (0.000586 M) and 1-hexyne (0.000586 M) in the presence and absence of Au-(PEG-SH)$_n$ NPs. The conversion of the alkyne group was about 30% after 24 h at 25 ^{o}C.

Determination of conversion of acrylate functional groups by ^{1}H NMR analysis: effect of Au-(PEG-SH)$_n$ NPs

The thiol-acrylate Michael addition reaction between AA-PEG-AA and 1,6-hexanedithiol is extremely slow in the absence of NPs. Conversion of about 15% acrylate groups took place after 5 h of reaction (Fig. S9A). The conversion reached to about 91% in the presence of Au-(PEG-SH)$_n$ NPs after 5 h of reaction (Fig. S9B). This indicates the efficient activation of the

acrylate functional group by the Au-$(PEG\text{-}SH)_n$ NPs. The Au-$(PEG\text{-}SH)_n$ NPs also promote amine-acrylate Michael addition reaction (Fig. S9C and S9D) as demonstrated by the ^1H NMR analysis.

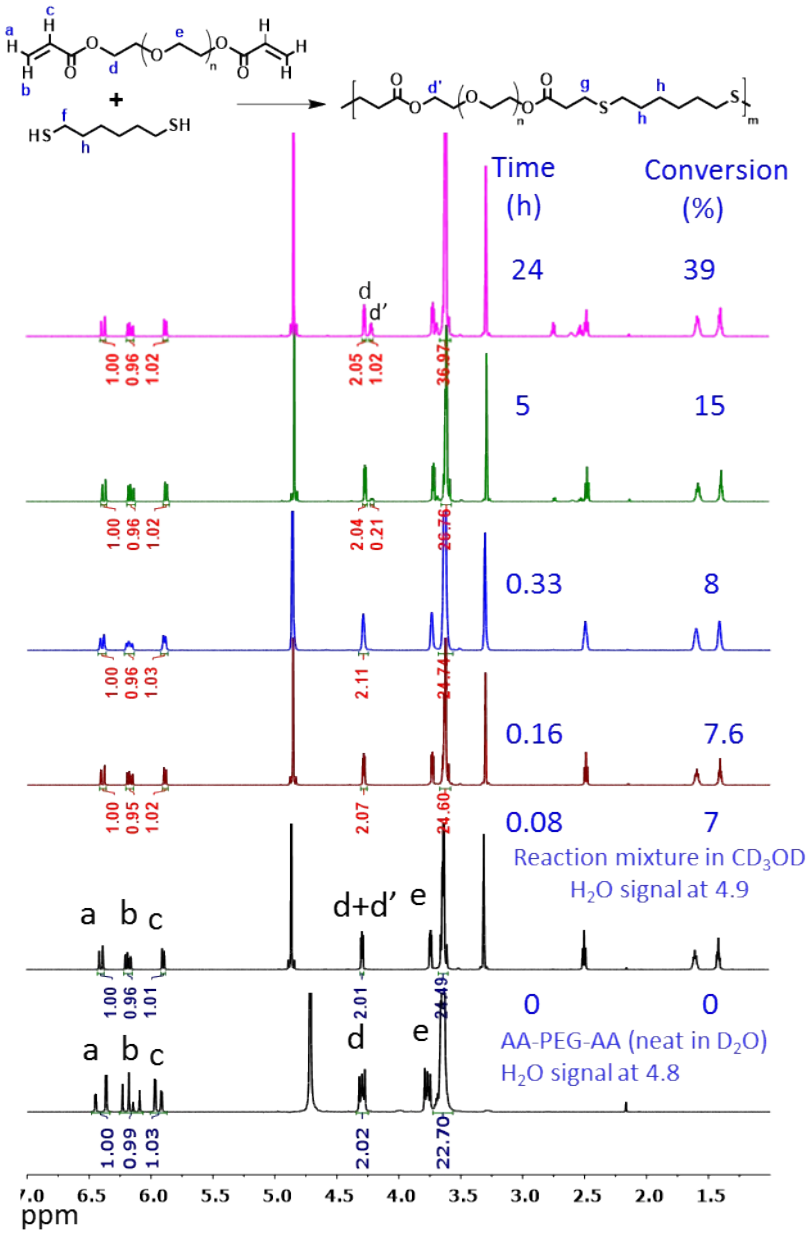


Fig. S9A. Real-time change in the ^1H NMR spectra (methanol-d4, 600 MHz) of reaction mixture consisting of AA-PEG-AA ($5.3x10^{-5}$ mol of acrylate groups) and 1,6-hexanedithiol ($5.3x10^{-5}$ mol of thiol groups) in the absence of Au-$(PEG\text{-}SH)_n$ NPs (representative thiol-

Michael addition reaction) at temperature 25 °C. The intensity ratio ($I_{5.5\text{-}6.6}/I_{3.6}$) of the signals at δ value 5.5-6.5 ppm to the signal at δ value 3.6 was considered for the calculation (experimental part in ESI). A spectrum (D_2O) of AA-PEG-AA is included for the purpose of comparison.

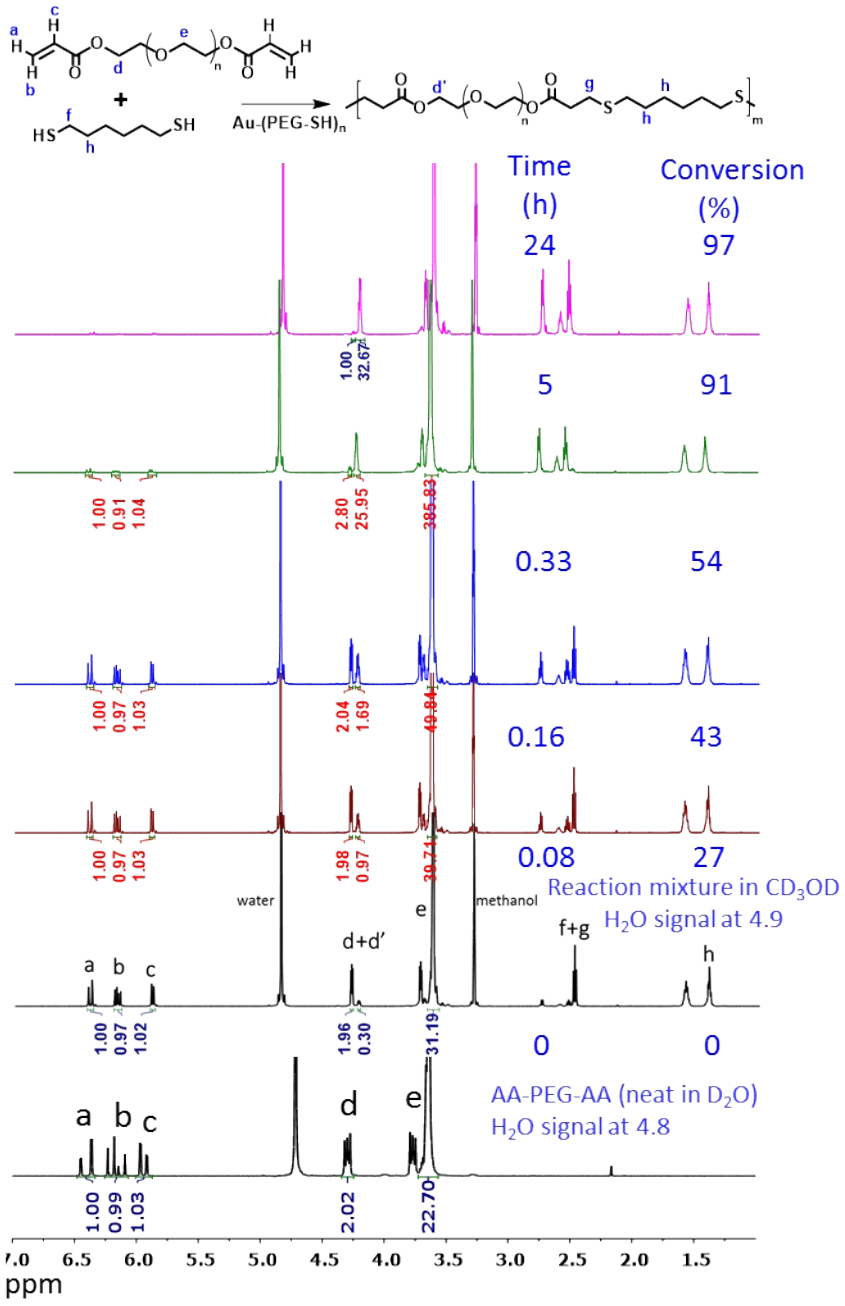


Fig. S9B. Real-time change in the ^{1}H NMR (methanol-d4, 600 MHz) spectrum of reaction mixture consisting of AA-PEG-AA ($5.3\text{x}10^{-5}$ mol of acrylate) and 1,6-hexanedithiol ($5.3\text{x}10^{-5}$

mol of thiol) in the presence of Au-$(PEG\text{-}SH)_n$ NPs (representative thiol-Michael addition reaction) at temperature 25 ^oC. The intensity ratio ($I_{5.5\text{-}6.6}/I_{3.6}$) of signals (due to 6H of acrylate moieties) at δ value 5.5-6.5 ppm to the signal (due to methylene protons of PEG of the backbone) at δ value 3.6 ppm was considered for the calculation (experimental part in ESI). A spectrum of AA-PEG-AA is included for the purpose of comparison.

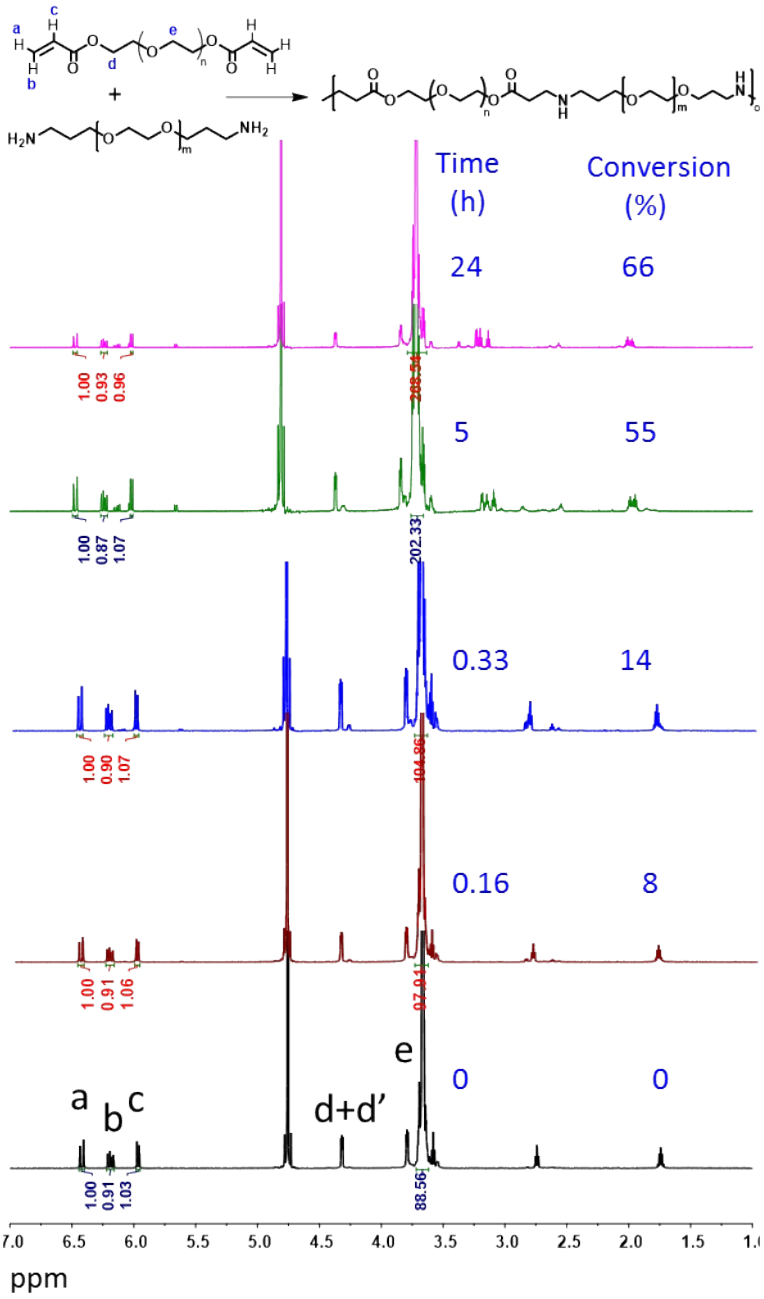


Fig. S9C. Real-time change in the ^{1}H NMR (D_2O, 600 MHz) spectrum of the reaction mixture consisting of AA-PEG-AA (2.66×10^{-5} mol) and H_2N-PEG-NH_2 (2.66×10^{-5} mol) in the absence of Au-$(PEG\text{-}SH)_n$ NPs (representative amine-acrylate Michael addition reaction) at temperature 25 °C. The intensity ratio ($I_{5.5\text{-}6.6}/I_{3.6}$) of the signals (due to 6H of acrylate moieties) at δ value 5.5-6.5 ppm to signals (due to ether methylene protons of PEG) at δ value 3.6 ppm was considered for calculation (experimental part in ESI).

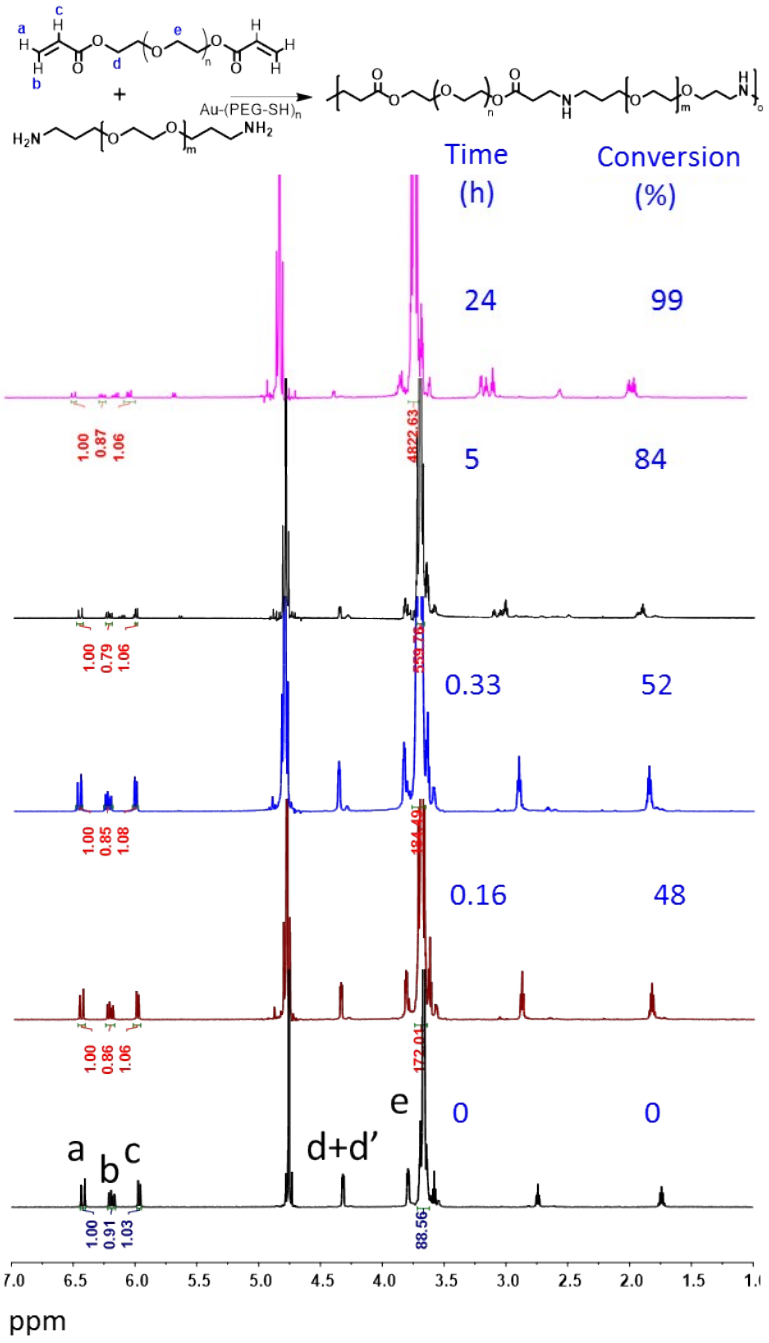
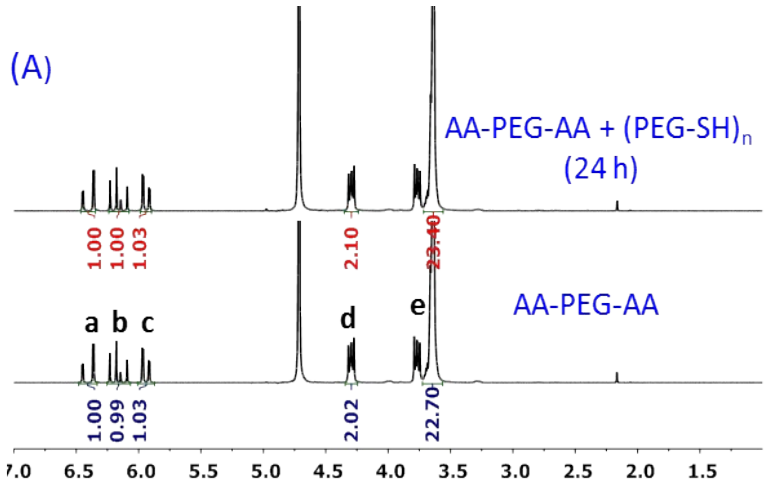
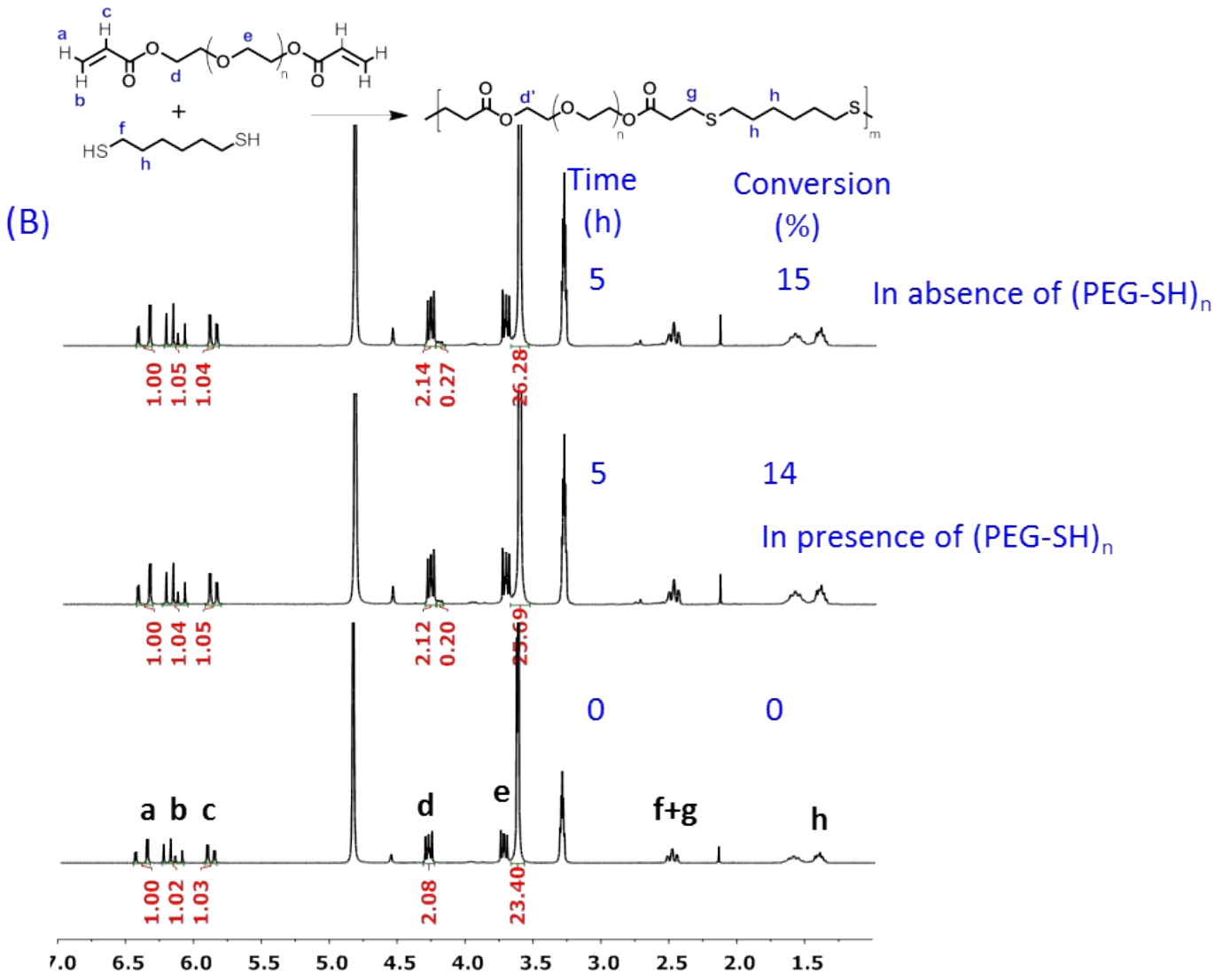


Fig. S9D. Real-time change of ^{1}H NMR (D_2O, 600 MHz) spectra of reaction mixture consisting of AA-PEG-AA (2.66×10^{-5} mol) and H_2N-PEG-NH_2 (2.66×10^{-5} mol) in the presence of Au-$(PEG\text{-}SH)_n$ NPs (representative amine-acrylate Michael addition reaction) at temperature 25 ^oC. The intensity ratio ($I_{5.5\text{-}6.6}/I_{3.6}$) of signals (due to 6 H of acrylate moieties) at δ value 5.5-6.5 ppm to signal (due to ether methylene protons of PEG) at δ value 3.6 ppm was considered for the calculation (experimental part in ESI).

Effect of $(PEG\text{-}SH)_n$ on the conversion of acrylate groups of AA-PEG-AA by thiol-acrylate (between AA-PEG-AA and 1,6-hexanedithiol) and amine-acrylate (AA-PEG-AA and H_2N-PEG-NH_2) Michael addition reactions

No conversion of acrylate moieties of AA-PEG-AA was detected in presence of $(PEG\text{-}SH)_n$ stabilizer (Fig. S10A). This stabilizer could not accelerate the rate of reaction between AA-PEG-AA and 1,6-hexanedithiol or H_2N-PEG-NH_2 as confirmed by the ^{1}H NMR spectroscopy (Fig. S10B and S10C).




(B)
Time (h)
Conversion (%)
5
15
In absence of (PEG-SH)n
5
14
In presence of (PEG-SH)n
0
0
a b c
d
e
f+g
h
1.00 1.05 1.04
2.14 0.27
26.28
1.00 1.04 1.05
2.12 0.20
25.69
1.00 1.02 1.03
2.08
23.40
7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5

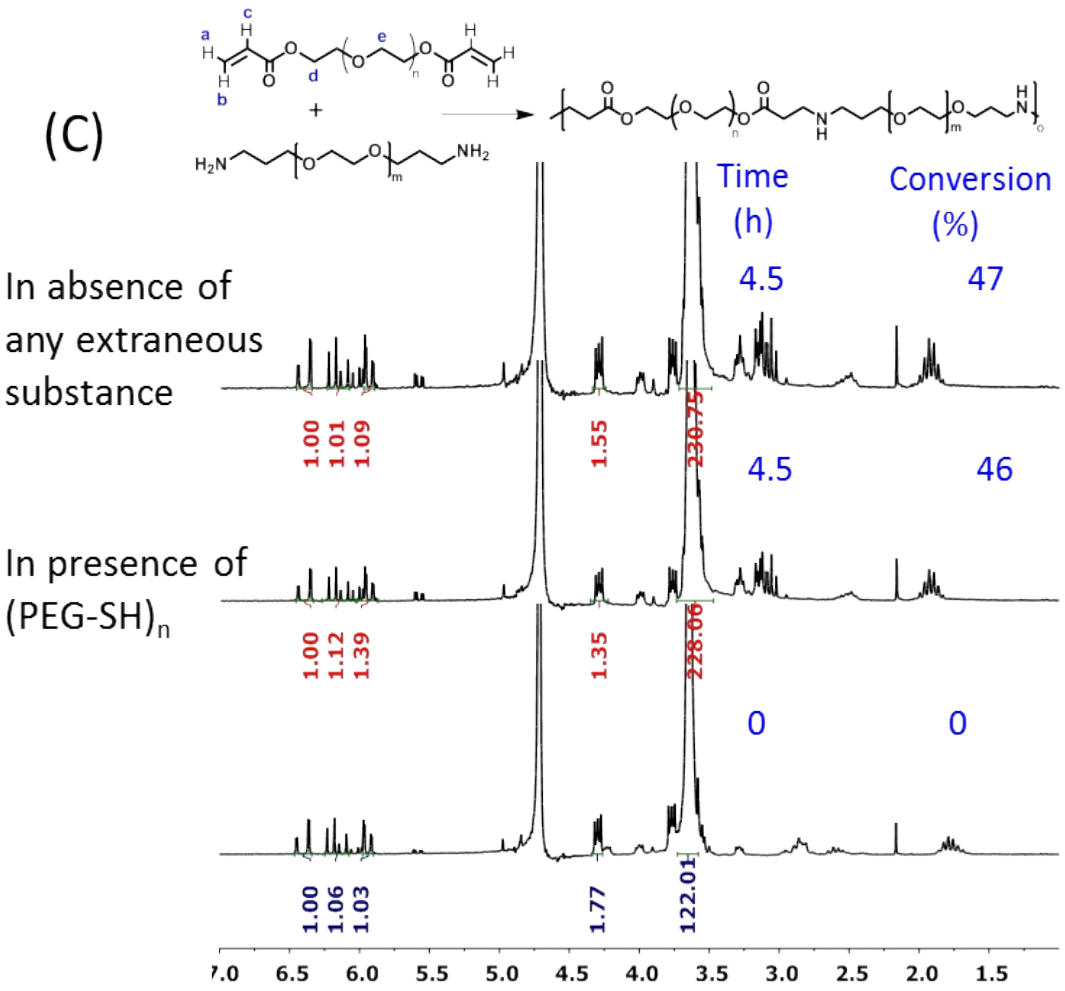


Fig. S10. (A) ^{1}H NMR (D_2O, 600 MHz) spectra of neat AA-PEGAA, and AA-PEGAA+$(PEG\text{-}SH)_n$ mixture respectively. The ^{1}H NMR spectrum of the mixture was taken after 24 h. (B) ^{1}H NMR spectra (methanol-d4, 600 MHz) of reaction mixture consisting of AA-PEG-AA (5.3×10^{-5} mol of acrylate) and 1,6-hexanedithiol (5.3×10^{-5} mol of thiol group) in absence or presence of $(PEG\text{-}SH)_n$ (0.01% w/v). (C) ^{1}H NMR spectra (D_2O, 600 MHz) of reaction mixture

consisting of AA-PEG-AA (2.66×10^{-5} mol) and H_2N-PEG-NH_2 (2.66×10^{-5} mol) in presence or absence of $(PEG\text{-}SH)_n$.

Effect of Au-$(PEG\text{-}SH)_n$ NPs on modulus of the hydrogels

No gelation of PVA-alkyne and N_3-PEG-N_3 took place in the absence of Cu catalyst or Au-$(PEG\text{-}SH)_n$ NPs at similar experimental condition (Fig. S11). On the other hand, the value of G' became higher than that of G" and remained unaltered in the frequency range of 0.1 to 100 Hz for the formed hydrogel in the presence of Au-$(PEG\text{-}SH)_n$ NPs. The G">>G', indicates the formation of stable hydrogel in presence of Au-$(PEG\text{-}SH)_n$ NPs.

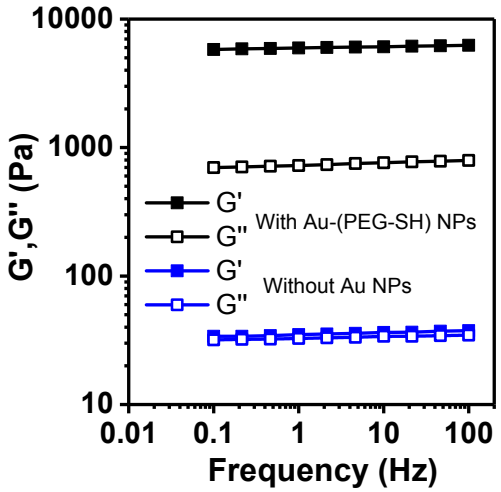


Fig. S11. Effect of Au-$(PEG\text{-}SH)_n$ NPs on the modulus of the hydrogels formed by AAC reaction between N_3-PEG-N_3 and PVA-alkyne. The frequency sweep experiment was performed by scanning in the range 0.1 to 100 Hz at 1% strain (viscoelastic region) and at temperature 37 ^oC after the time sweep experiments.

Stability of Au-(PEG-SH)$_n$ NPs on the hydrogel matrix by UV-Visible analysis

The Au NPs remained stable in the hydrogel matrix. The hydrogel was formed by the Michael addition reaction between poly(lysine) and AA-PEG-AA (Fig. S13). The plasmon resonance band of NPs dispersed in the PBS, prepolymer solution, and hydrogel matrix remained unaltered. This indicates no agglomeration of NPs during gelation or in the hydrogel matrix.

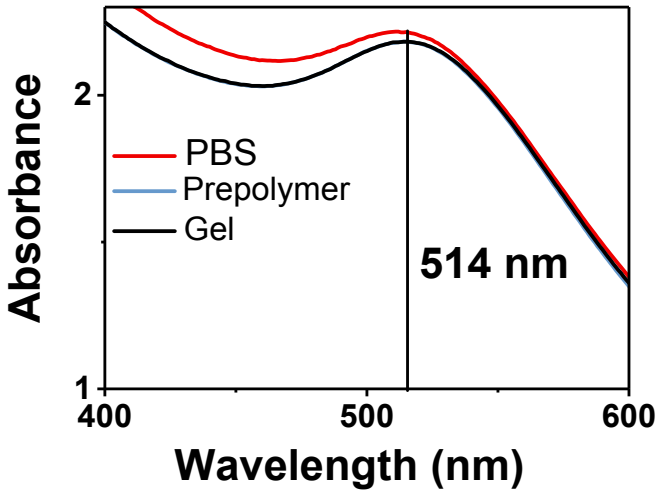


Fig. S12. UV-Visible spectra of Au-(PEG-SH)$_n$ NPs dispersed in PBS, prepolymer solution, and hydrogel matrix. The hydrogel was prepared by the Michael addition reaction between AA-PEG-AA and poly(lysine) (Fig. 4C, main text).

Effect of Au-(PEG-SH)$_n$ NPs on the modulus of the hydrogels formed by amine-acrylate Michael addition reaction

The formation of hydrogel through Michael addition reaction was not only accelerated in the presence of Au-(PEG-SH)$_n$ NPs (Fig. 4D, main text), the modulus also increased (Fig. S13, ESI, and Fig. 4 in the main text). The reason of enhancement of hydrogel modulus has been discussed in the main text. Stable hydrogels formed in both presence and the absence of NPs. However, in presence of NPs, the value of G' was higher. The differences between gelation by AAC and amine acrylate Michael addition reactions are in former system no gelation occurred in absence of NPs, while the later system proceeds without NPs but in a relatively slower rate in the absence of NPs.

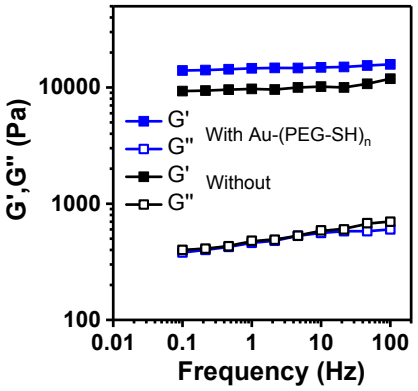


Fig. S13. Effect of the presence of Au-(PEG-SH)$_n$ NPs on the modulus of the hydrogel. The hydrogel was prepared by the Michael addition reaction between AA-PEG-AA (0.25 g) and

amine groups of poly(lysine) (0.5 g, 20% w/v in PBS). The frequency sweep experiment was performed by scanning in the range 0.1 to 100 Hz at 1% strain at temperature 37 ^{o}C after the time sweep experiments.

Release of Au-(PEG-SH)$_n$ NPs from a hydrogel of AA-PEG-AA and 1,6-hexanedithiol

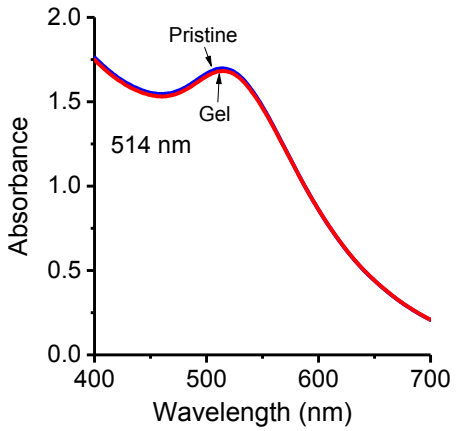


Fig. S14. UV-Visible spectra of as prepared Au-(PEG-SH)$_n$ NPs (pristine) and NPs leached out from the hydrogel of AA-PEG-AA and 1,6-hexanedithiol. The Plasmon resonance band of the leached NPs remained unaltered (514 nm) to that of as prepared NPs.

^{1}H NMR spectra of degraded masses of the hydrogels to probe the presence of unreacted acrylate groups: Effect of Au NPs

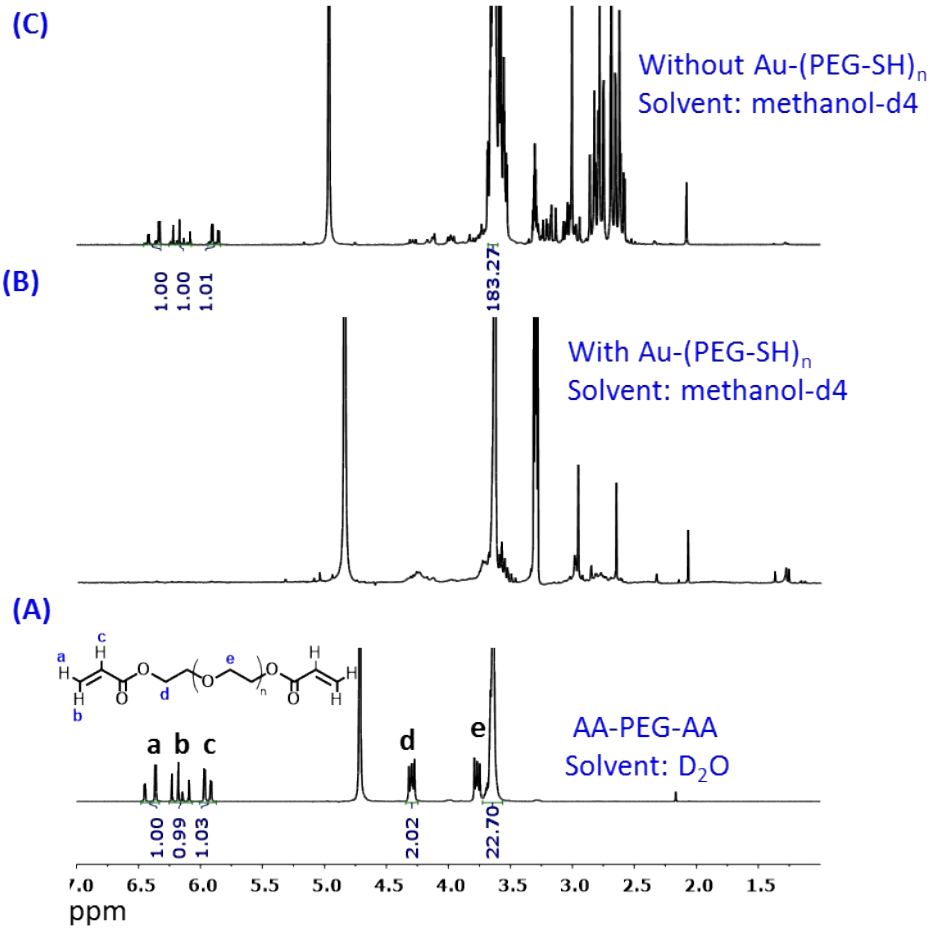


Fig. S15. ^{1}H NMR spectra of (A) neat AA-PEG-AA, and (B and C) masses recovered after degradation of hydrogels of AA-PEG-AA and tetra-thiol. The hydrogels were prepared in (B)

presence, and (C) absence of Au-(PEG-SH)$_n$ NPs. The degradation was performed by incubating the hydrogel samples at pH 6 and at temperature 37 ^oC. After degradation, the soluble masses were freeze dried. The ^1H NMR experiments were performed by dissolving the degraded masses in methanol-d4.

Cross-sectional SEM images of the hydrogels: Effect of Au NPs

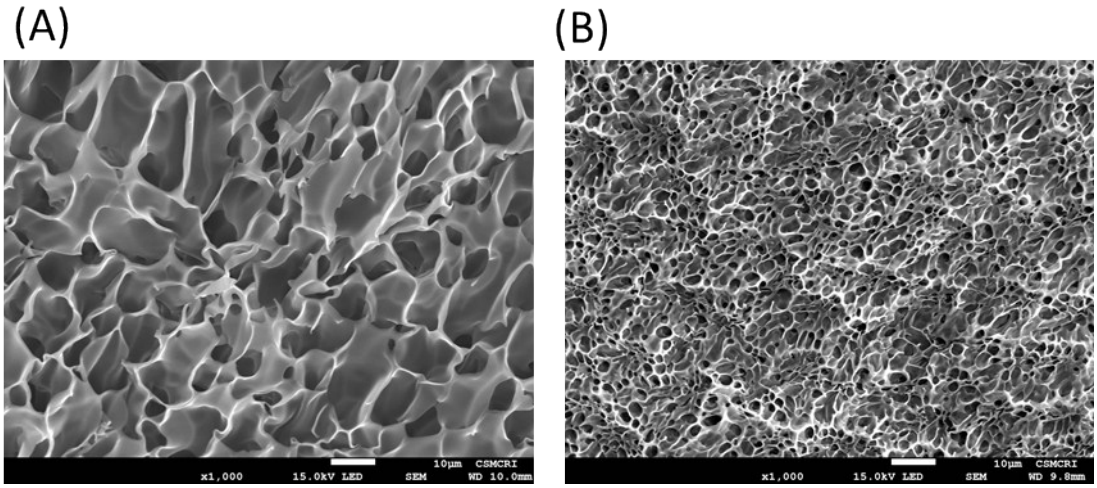
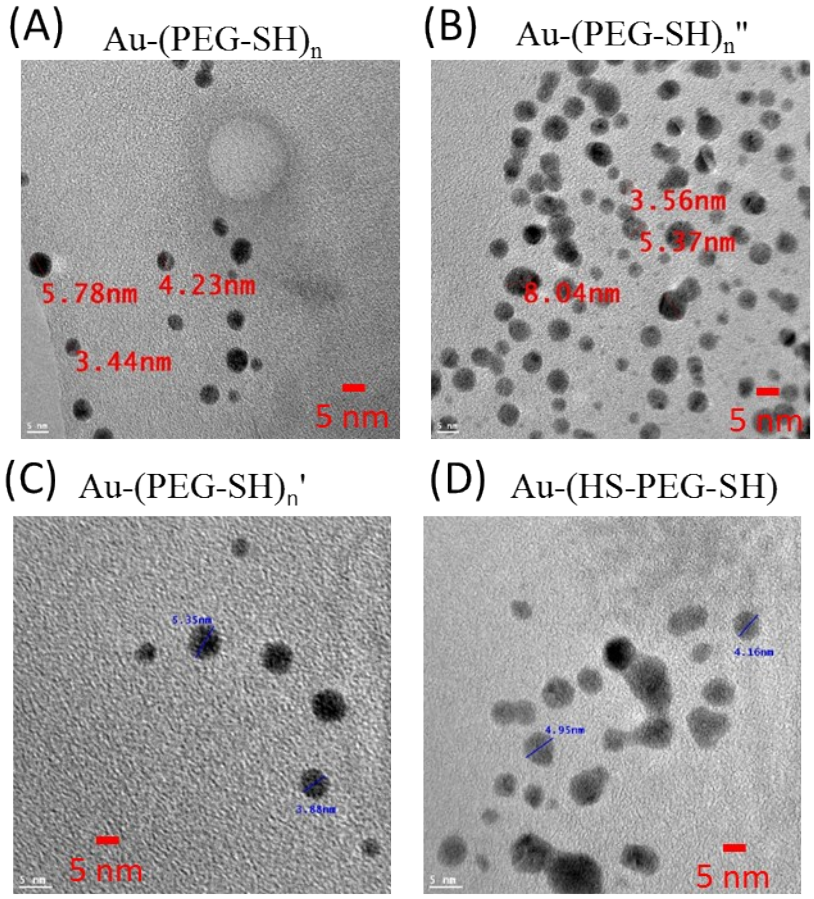


Fig. S16. Cross-sectional SEM Images of (A) Au-(PEG-SH)$_n$ nanocomposite hydrogel, and (B) pristine hydrogel. The hydrogels were prepared by the thiol-acrylate Michael addition reaction in presence and absence of the NPs.



Determination of core diameter (D_{core}) of different Au NPs by TEM analysis

Fig. S17. TEM images of (A) Au-(PEG-SH)$_n$, (B) Au-(PEG-SH)$_n$", (C) Au-(PEG-SH)$_n$', and (D) Au-(HS-PEG-SH) NPs. Spherical NPs were considered for the determination of average

core diameter (D_{core}). The D_{core} was calculated from the different TEM images of each type of NPs. Averages of 40 spherical NPs were considered.

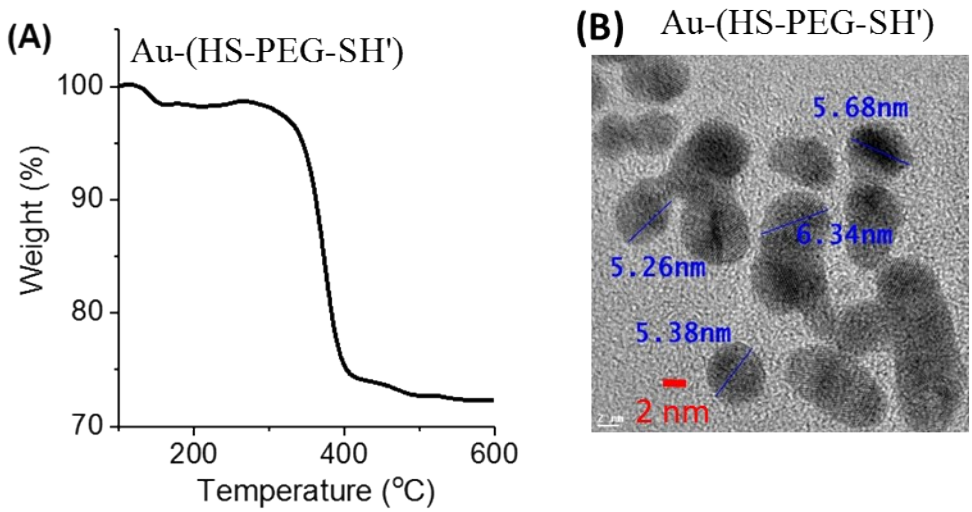


Fig. S18. (A) TGA curve, and (B) TEM image of Au-(HS-PEG-SH') NPs. The D_{core} was calculated from the different TEM images of NPs. Averages of about 40 spherical NPs were considered for the calculation.

Determination of activating effect of Au-(HS-PEG-SH') NPs by ^{1}H NMR spectroscopy and by rheological time sweep experiment.

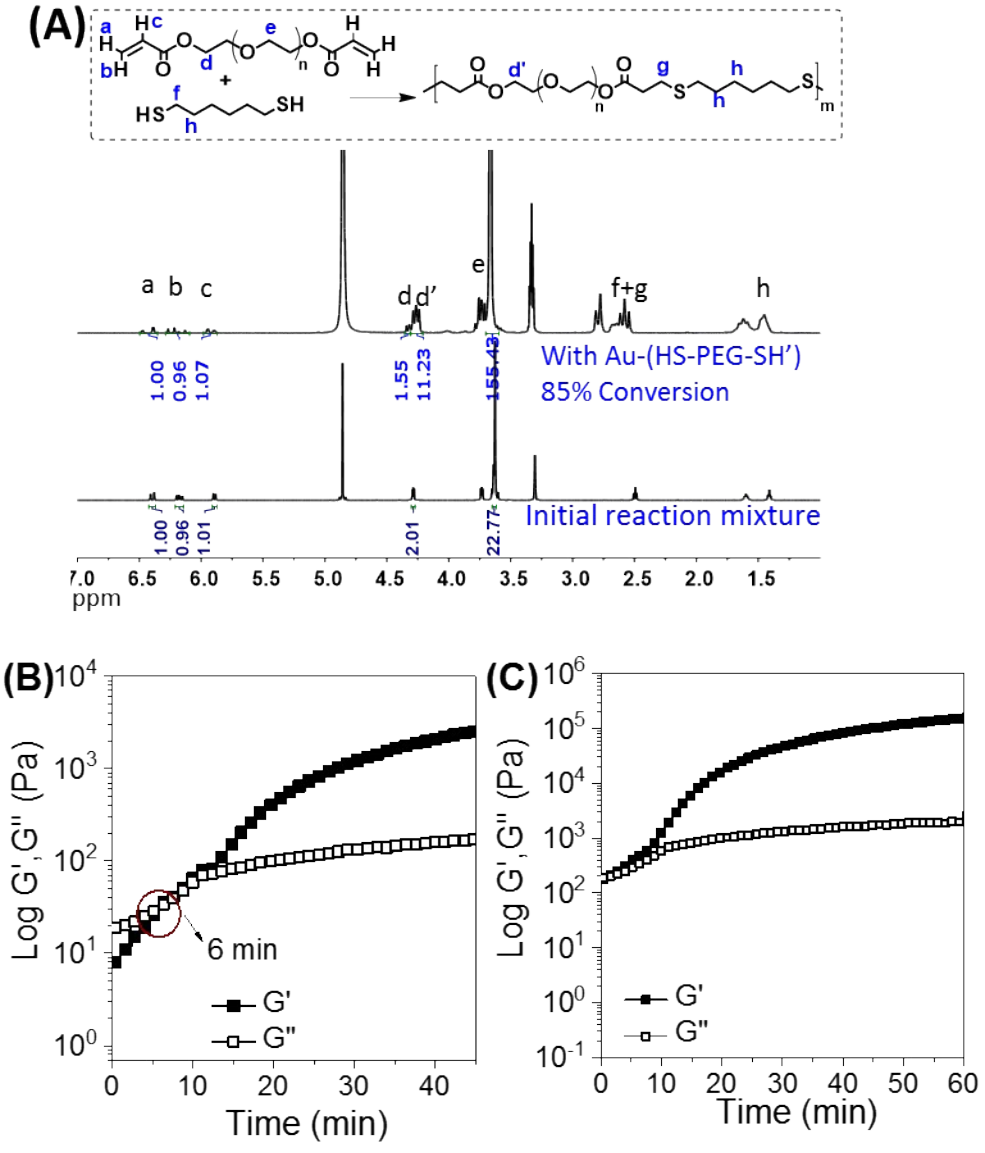


Fig. S19. (A) ^{1}H NMR (methanol-d4, 600 MHz) spectrum of reaction mixture of AA-PEG-AA and 1,6-hexanedithiol in presence of Au-(HS-PEG-SH') NPs (initial). The ^{1}H NMR spectrum of the reaction mixture was taken after 5 h of reactions. Rheological time sweep experiments with prepolymers containing (B) PVA-alkyne (16 % w/v in PBS) and N_3-PEG-N_3(16 % w/v in PBS), and (C) AA-PEG-AA and pentaerythritol tetra(3-mercaptopropionate) in DMSO. The gelation was conducted in presence of Au-(HS-PEG-SH') NPs.

Determination of stability of Au-(HS-PEG-SH') NPs after gelation by UV-Visible spectroscopy

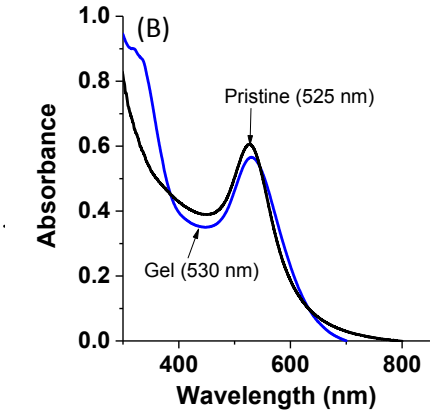


Fig. S20. UV-Visible spectra of as prepared Au-(HS-PEG-SH') NPs and extracted NPs from hydrogel of AA-PEG-AA and 1,6-hexanedithiol. The Plasmon resonance band of the extracted NPs red shifted to about 5 nm than that of as prepared NPs.

Reference

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5. A. K. S. Chandel, D. Kannan, B. Nutan, S. Singh, S. K. Jewrajka, *J. Mater. Chem. B,* 2017, **5**, 4955-4965.