Supporting Information for:

# Thiol-Disulfide Exchange as a Route for Endosomal Escape of Polymeric Nanoparticles

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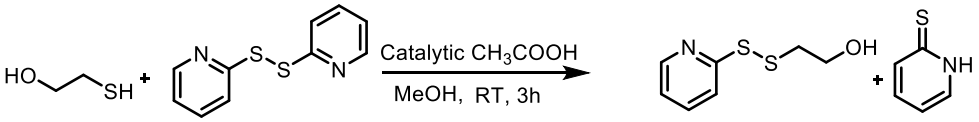
## 1. Materials

Methacryloyl chloride, acryloyl chloride, 2-mercaptoethanol, di-*tert*-butyldicarbonate, ethanolamine, pentafluorophenol, polyethyleneglycol monomethylether methacrylate (PEGMA; MW 500), polyethylene glycol monomethylether acrylate (PEGA; MW 480), D, L-dithiothreitol (DTT), tetraethyleneglycol diamine, 4-cyano-4-[(dodecylsulfanylthiocarbonyl)sulfanyl]pentatonic acid, were bought from Sigma-Aldrich and used as is without any further purification. 2,2'-Azobis-(2-methylpropionitrile) (AIBN) was obtained from Sigma-Aldrich and recrystallized prior to use. Thiol-peg-alcohol was purchased from BroadPharm. Ellman's reagent (DTNB) was purchased from ThermoFisher.

## 2. Synthesis of monomers

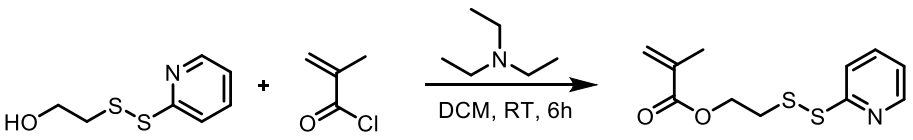
### 2.1 Synthesis of pyridyl disulfide ethyl methacrylate (PDSMMA) monomer:

**Step 1:**




15 g (68 mmol, 2 equiv) of 2,2'-dipyridylsulfide was taken in a 250 mL round bottom flask and was dissolved in 75 mL of methanol. Then 1 mL of acetic acid was added slowly to the solution and allowed to stir for 15 minutes. Afterwards, a 25 mL solution of 2.65 g (33 mmol, 1 equiv) of 2-mercaptoethanol in methanol was added dropwise to the reaction mixture. After 3 h of stirring, methanol was evaporated to obtain a yellow viscous liquid, to which diethylether was added and swirled vigorously. The crystals were filtered out and diethylether was evaporated to obtain the product mixture. Finally, the product was purified using flash column chromatography using hexane/ethyl acetate as an eluent. Yield: 58%.$^{1}$H NMR (400 MHz, $CDCl_3$) (δ ppm):8.52-8.53 (d, 1H), 7.58-7.62 (t, 1 H), 7.41-7.43 (d, 1H), 7.15-7.18 (t, 1H), 5.72-5.75 (t, 1H), 3.80-3.84 (dd, 2H), 2.96-2.98 (t, 2H). $^{13}$C NMR (100 MHz, $CDCl_3$) (δ ppm): 159.09, 149.75, 137.01, 122.04, 121.58, 58.25, 42.77.

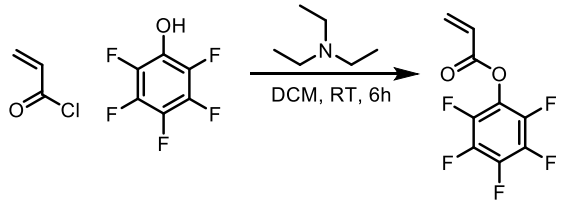
**Step 2:**




3 g (16 mmol, 1 equiv) of 2-(pyridine-2-yldisulfanyl)ethanol was dissolved in 12 mL of DCM and cooled in an ice bath. Then, 1.94 g (19 mmol, 1.2 equiv) of triethylamine was added dropwise to this solution. After 30 minutes of stirring, 1.67 g (16 mmol, 1 equiv) of methacryloyl chloride in 10 mL of DCM was added to the reaction dropwise and allowed to stir for 6 h. The product was first isolated by adding 50 mL of DCM and then washing the crude mixture with distilled water (3x50 mL), and saturated brine solution (1x50 mL). Finally, it was dried over sodium sulfate anhydrous, and the organic layer was purified via flash column chromatography using hexane/ethyl acetate. Yield: 89 %. $^{1}$H NMR (400 MHz, $CDCl_3$) (δ ppm): 8.48-8.49 (d, 1H), 7.70-7.72 (d, 1H), 7.62-7.66 (t, 1H), 7.09-7.13 (t, 1H), 6.14 (s, 1H), 5.60 (s, 1H), 4.40-4.43 (t, 2 H), 3.09-3.13 (t, 2H), 1.96 (s, 1H). $^{13}$C NMR (100 MHz, $CDCl_3$) (δ

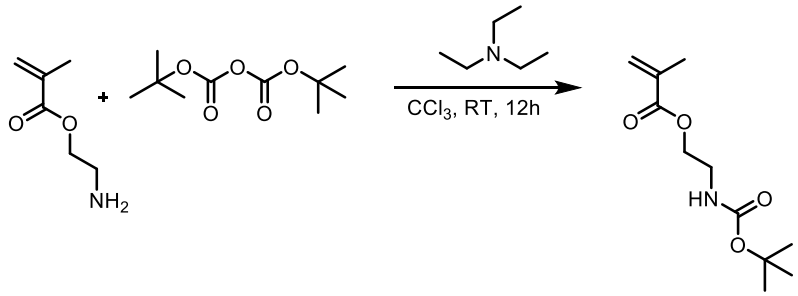
ppm): 167.05, 159.75, 149.69, 137.09, 135.96, 126.04, 129.85, 119.80, 62.4, 37.45, 18.28. The characterization was in correlation to the previously reported pyridyl disulfide monomer.[1]

**2.2 Synthesis of pentafluoro phenol acrylate (PFPA) monomer**:



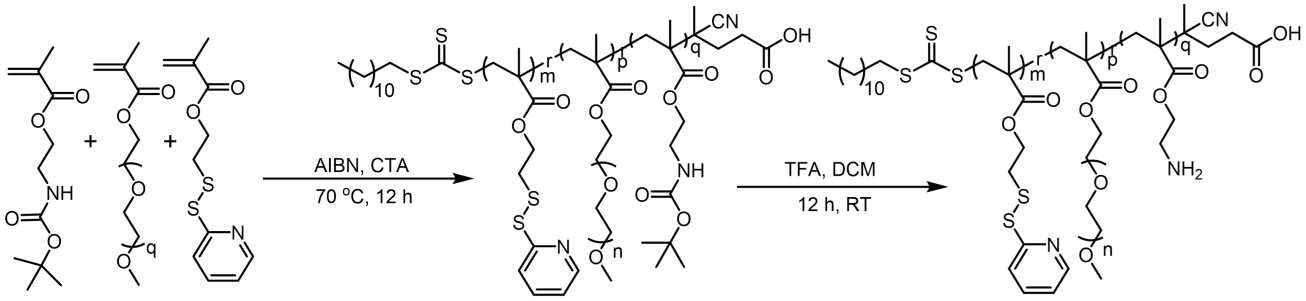

5.08 g (27 mmol, 1 equiv) of pentafluorophenol was dissolved in 24 mL of DCM and cooled in an ice bath. Then, triethylamine was added to the reaction mixture. After 30 minutes of stirring, 2.5 g (27 mmol, 1 equiv) of acryloyl chloride in 10 mL of DCM was added dropwise to the reaction mixture and allowed to react for 6 h. Afterwards, the reaction mixture was washed with distilled water (3x50 mL), saturated brine solution (1x50 mL) and finally the organic layer was dried over sodium sulfate anhydrous. The product was purified by flash column chromatography using hexane/ethyl acetate as eluent. Yield: 63%. $^{1}$H NMR (400 MHz, $CDCl_3$) (δ ppm): 6.72-6.76 (d, 1H), 6.35-6.42 (dd, 1H), 6.18-6.21 (d, 1H). $^{19}$F NMR (300 MHz, $CDCl_3$) (δ ppm): -152.60 to -152.52 (md, 2F), -157.99 to -157.87 (t, 1F), -162.37 to -162.27 (dd, 2F). $^{13}$C NMR (100 MHz, $CDCl_3$) (δ ppm): 171.18, 161.71, 135.54, 125.39, 60.43, 21.07, 14.22. The characterization of PFPA was in correlation to previously reported synthesis of pentafluorophenyl acrylate PFPA.[2]

**2.3 Synthesis of amino ethyl methyl methacrylate (AEMMA) monomer:**

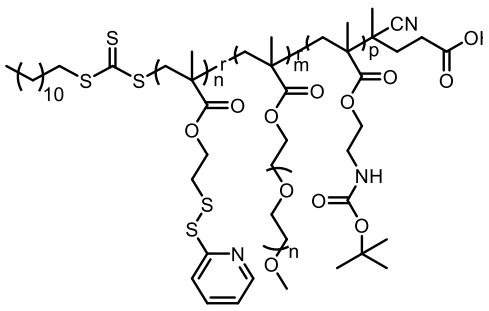



500 mg (4 mmol, 1 equiv) of 2-aminoethyl methacrylate hydrochloride was taken in 100 mL round bottom flask and dissolved chloroform. Then 593.98 mg (5 mmol, 1.2 equiv) of triethylamine was added to the reaction media. After 15 minutes of stirring, 1.28 g (5 mmol, 1.2 equiv) of di-tert-butyl decarbonate in chloroform was added slowly to the solution. After 12 h of stirring, the chloroform was evaporated, the crude mixture was redissolved in ethyl acetate and washed in saturated sodium bicarbonate solution (1x50 mL) followed by saturated brine solution (2x50 mL). The organic layer was collected, dried over sodium sulfate anhydrous, concentrated, and purified by flash column chromatography using hexane/ethyl acetate. Yield: 89%. $^{1}$H NMR (400 MHz, $CDCl_3$) (δ ppm): 6.14 (s, 1H), 5.61 (s, 1H), 4.78 (s, 1H), 4.21-4.24 (t, 2H), 3.46-3.47 (d, 2H), 1.97 (s, 3H), 1.46 (s, 9H).

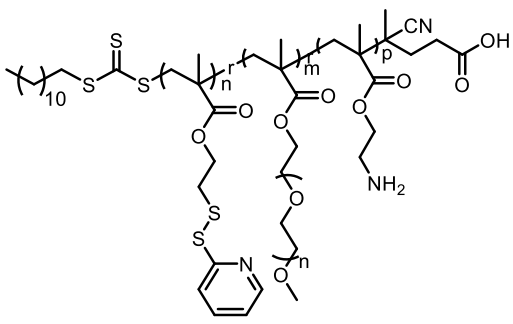
## 3. Synthesis of polymers



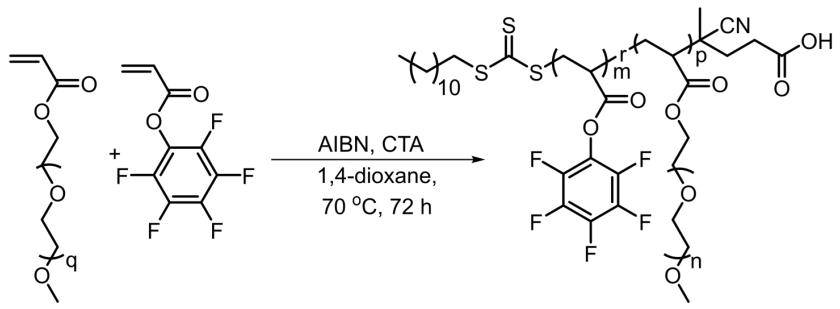

**Scheme S1:** Synthetic scheme of $\mathbf{P_{DS\text{-}NGs}}$ polymer.



**3.1 Synthesis of p(PDSMMA-co-PEGMA-co-AEMMA) polymer, $\mathbf{P_{DS\text{-}NGs}}$:** The p(PDSMA-co-PEGMA-co-AEMA) polymer was synthesized using RAFT polymerization technique. 250 mg (0.98 mmol) of PDSMA, 226.14 mg (0.452 mmol) of PEGMA, 17.28 mg (0.075 mmol) AEMA, and 13.27 mg (0.032 mmol) 4-cyano-4-[(dodecylsulfanylthiocarbonyl)sulfanyl] pentatonic acid was taken in a 7 mL glass vial and were dissolved in 1000 µL dry THF. Then, 1.08 mg (0.0065 mmol) of AIBN in dry THF was added to the solution and reaction vial was sealed tightly with septum and tape. The solution was degassed by performing four freeze-pump-thaw cycles with the help a needle. Afterwards, the reaction mixture was placed in a preheated oil bath of 70 °C for 12 h. The reaction mixture was quenched, dissolved in minimum amount of DCM and precipitated in diethyl ether for three times. Finally, the resultant polymer was dissolved in DCM, transferred in a preweighed 25 mL glass vial, and dried in high vacuum for overnight. Yield: 86%. GPC (DMF), $M_n$:9334, Đ: 1.4. $^1$H NMR (400 MHz, $CDCl_3$) (δ ppm): 8.48, 7.69, 7.12, 4.24, 4.09, 3.67, 3.56, 3.55, 3.39, 3.04, 1.92, 1.86, 1.61, 1.45, 1.27, 1.08, 0.91. The relative ratios of monomers were calculated considering the pyridyl proton, methoxy proton and tertiary butyl proton which was found to be n:m:p = 63:32:5.



The final polymer was obtained by deprotecting the t-Boc functionality in the polymer chain. 100 mg (0.305 mmol) of p(PDSMA-co-PEGMA-co-AEMA) polymer was dissolved in DCM and 696 mg (6.11 mmol) trifluoroacetic acid (TFA) was added to the solution. After 12 h, the crude mixture was dried using rotary evaporator, dissolved in minimum amount of DCM, reprecipitated in diethyl ether for three times. Finally, the polymer was transferred to a glass vial, concentrated in vacuo, and dried under high vacuum to obtain a yellow viscous polymer. Yield: 95%. $^1$H NMR (400 MHz, $CDCl_3$) (δ ppm): 8.50, 7.74, 7.14, 5.40, 4.23, 4.09, 3.56, 3.39, 3.05, 1.92, 1.85, 1.27, 1.07, 0.91. $^{13}$C NMR (100 MHz, $CDCl_3$) (δ ppm): 159.29, 149.35, 137.74, 121.16, 120.18, 71.95, 70.62, 70.58, 70.53, 68.48, 59.05, 44.90, 36.90, 31.91, 29.63, 29.48, 29.35.




**Scheme S2:** Synthetic scheme of $\mathbf{P_{NonDS\text{-}NGs}}$ polymer.

**3.2 Synthesis of p(PFPA-co-PEGA) polymer, $\mathbf{P_{NonDS\text{-}NGs}}$:** Pentafluoro phenyl acrylate was synthesized by RAFT polymerization with pentafluoro phenol acrylate (PFPA) and PEGA (commercially available) monomers following our previously established protocol[2]. 200 mg (0.8399 mmol) of PFPA, 268.83 mg (0.560 mmol) of PEGA and 12.61 mg (0.0312 mmol) 4-cyano-4-[(dodecylsulfanylthiocarbonyl)sulfanyl]pentanoic acid were weighed in a 7 ml glass vial and were dissolved in 900 µL of dry 1,4-dioxane. Then, 1.026 mg (0.00625 mmol) of AIBN in dry 1,4-dioxane was added to the final solution and the vial was sealed tightly with rubber septum and tape. Afterwards, it was degassed by three cycle of freeze-pump-thaw and set the reaction in a preheated oil bath at 70 °C for 72 h. Next, it was dissolved in a minimum amount of DCM and precipitated in hexane for three times. Finally, the polymer was transferred to a pre-weighed glass vial and dried under high vacuum for overnight. Yield: 71%. $^{1}$H NMR (400 MHz, $CDCl_3$) (δ ppm): 3.78, 3.76, 3.75, 3.66, 3.57, 3.39. $^{19}$F NMR (300 MHz, $CDCl_3$) (δ ppm): -149.79, -151.26, -156.88, -161.79. The relative ratios of monomers were considered based on the feed ratios which is m:p = 70:30.

## 4. Molecular weight measurement of polymers

Gel permeation chromatography (GPC) was used to estimate the molecular weight of polymers using THF as the eluent with poly(methyl methacrylate) (PMMA) as the internal standard.

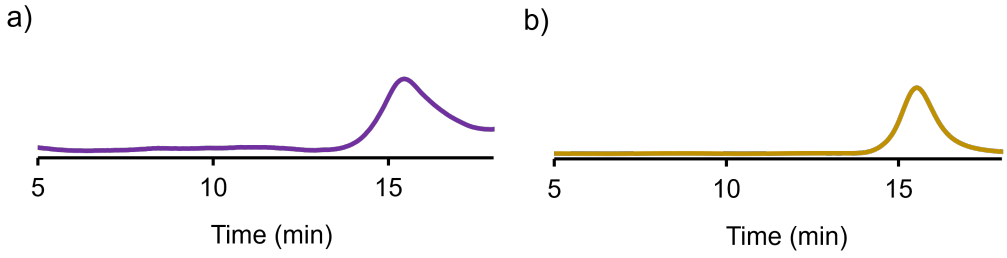



**Figure S1:** GPC for polymers (a) $\mathbf{P_{DS\text{-}NGs}}$ and (b) $\mathbf{P_{NonDS\text{-}NGs}}$

## 5. Dye conjugation to the polymers

Sulfonated cy3 (Sulf cy3) dye was conjugated to both $\mathbf{P_{DS\text{-}NGs}}$ and $\mathbf{P_{NonDS\text{-}NGs}}$ polymers to carry out the endosomal escape evaluation. Sulf cy3-NHS and Sulf cy3-$NH_2$ dyes were used for conjugation to $\mathbf{P_{DS\text{-}NGs}}$ and $\mathbf{P_{NonDS\text{-}NGs}}$ polymers respectively. For $\mathbf{P_{DS\text{-}NGs}}$ polymer, 50 mg polymer was dissolved in 2 mL of DCM and triethylamine was added to the solution. After 15 minutes, 3.35 mg of Sulf cy3-NHS dye was added to the solution and let it stir for 12 h. Then the solution was dialyzed by 3.5K MWCO snakeskin dialysis membrane against DCM. The solvent was changed at regular interval for 48 h till the solution became clear. Afterwards, the DCM was evaporated, and the polymer was dried in high vacuum overnight. For $\mathbf{P_{NonDS\text{-}NGs}}$ polymer, 50 mg polymer was 2 mL of THF and DIPEA was added to the solution. Then 3.356 mg of Sulf cy3-$NH_2$ was added to the mixture and let it stir at 50 °C. The solvent was adjusted according to the solubility. After 4 h, the polymer was dialyzed using 3.5K MWCO snakeskin dialysis membrane against acetone for 48 h to get rid of unreacted dyes. The solvent was changed at a regular interval until the solution was clear. Finally, the solvent was evaporated, and the polymer was dried in high vacuum overnight.

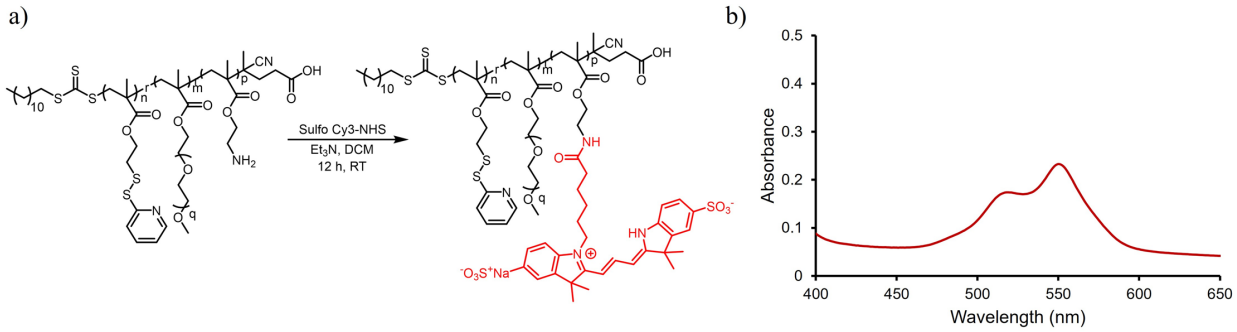



**Figure S2:** (a) Reaction scheme of sulf cy3-NHS dye conjugation to $\mathbf{P_{DS\text{-}NGs}}$ polymer. (b) UV-Vis spectrum of dye conjugated DS-NGs.

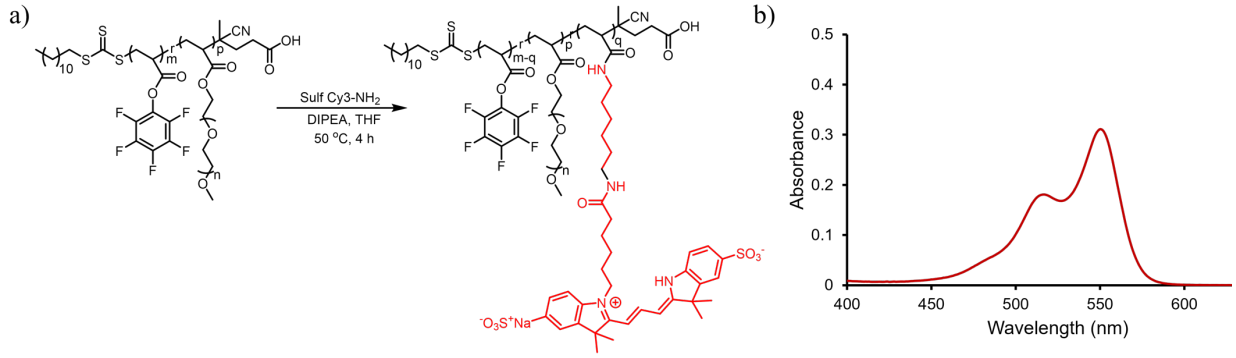



**Figure S3:** (a) Reaction scheme of sulf cy3-NHS dye conjugation to $\mathbf{P_{NonDS\text{-}NGs}}$ polymer. (b) UV-Vis spectrum of dye conjugated NonDS-NGs.

## 6. Crosslinking reaction to synthesize polymeric nanogels

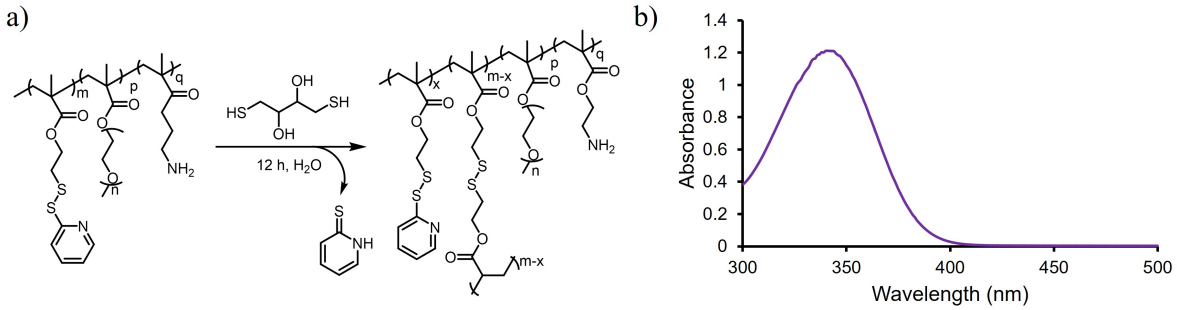



**Figure S4:** (a) DS-NGs nanogel formation by crosslinking reaction of $\mathbf{P_{DS\text{-}NGs}}$ polymer with DTT. (b) UV-vis spectrum for the reporter molecules (pyridithione) of crosslinking reactions.

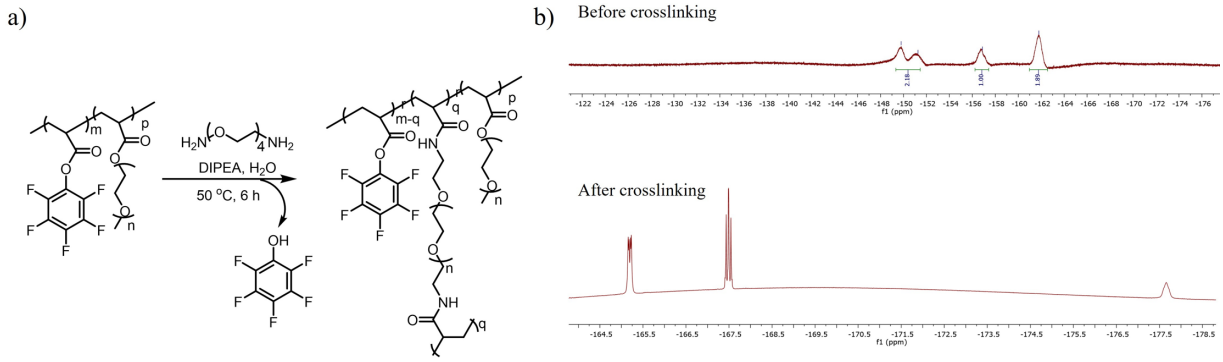



**Figure S5:** (a) Reaction scheme of $\mathbf{P_{NonDS\text{-}NGs}}$ polymer crosslinking to form NonDS-NGs nanogel. (b) $^{19}$F NMR spectra of NonDS-NGs nanogel before and after the crosslinking.

DS-NGs nanogel was synthesized following previously established procedure in the group (Scheme S5).[1] 2 mg $\mathbf{P_{DS\text{-}NGs}}$ polymer was dissolved in 0.1% acetone and followed by addition of 1 mL water with constant stirring. The vial was left open for 3 hours to evaporate acetone and afterwards, 0.32 mg of DTT was added to the solution to attain 100% crosslinking. The extent of crosslinking reaction was monitored by measuring the absorbance of 2-mercaptopyridine as the reporter molecule (Scheme 1b). Similarly, for NonDS-NGs Nanogel, 2 mg of $\mathbf{P_{NonDS\text{-}NGs}}$ polymer was dissolved in 1 mL of water and to this, 1 equivalent of DIPEA and 0.2491 mg of tetraethyleneglycol diamine were added. Afterwards, the solution was heated at 50 °C for 6 h to obtain desired nanogel and reaction was monitored by the appearance of pentafluorophenol (PFP) using $^{19}$F NMR spectroscopy (Scheme 1b). Both nanogels were dialyzed against water for 48 h to remove the respective byproducts and afterwards, the size profile and zeta potentials were evaluated. The percentage dye conjugation in each nanogel was evaluated using the Beer Lambert law and the feed ratios were determined according to the conjugation efficiency.

## 7. Measurement of nanogel sizes and zeta-potentials

The sizes and zeta potential measurements of DS-NGs and NonDS-NGs were performed in Malvern Nanozetasizer-ZS instrument.

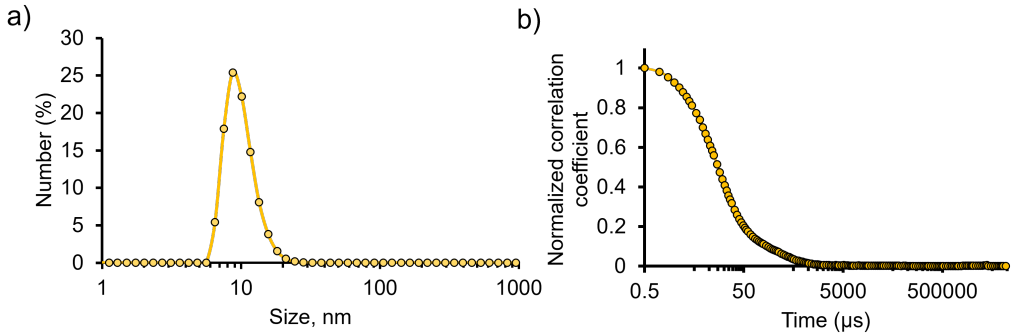



**Figure S6:** The size profile (a) and correlation coefficient (b) of NonDS-NGs

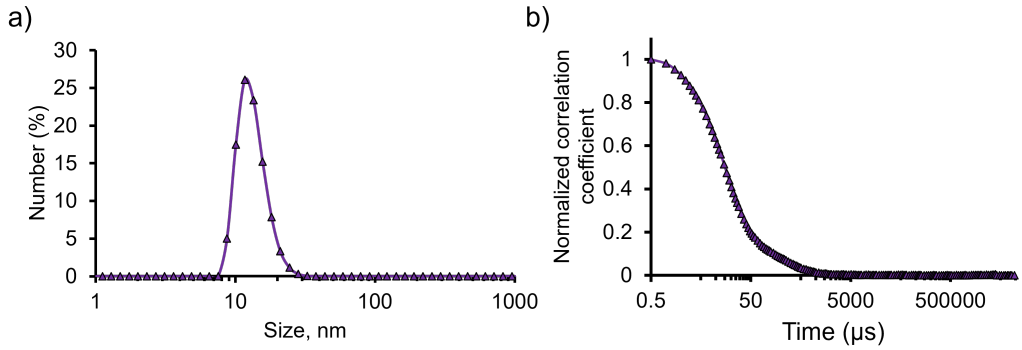



**Figure S7:** The size profile (a) and correlation coefficient (b) of DS-NGs nanogel

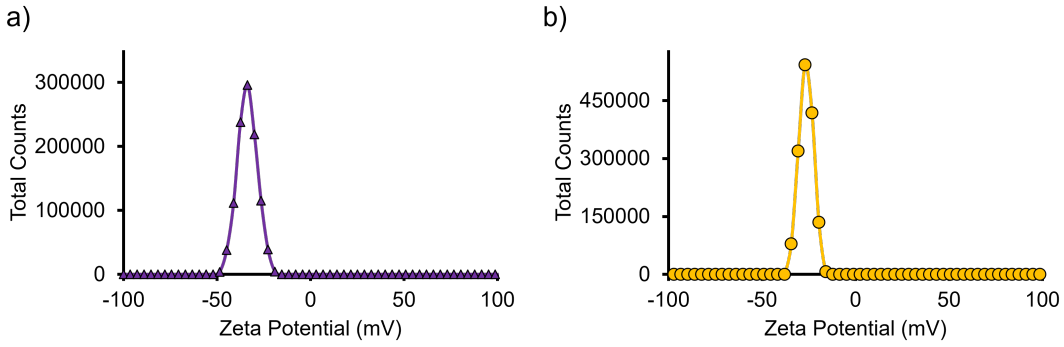



**Figure S8:** ζ poteintial profiles of (a) DS-NGs and (b) NonDS-NGs nanogels as measured

## 8. Cell culture studies

EMT6 (Mammary carcinoma) cells were cultured in 100 mm cell culture petri dish using Dulbecco's modified Eagle's medium/nutrient mixture F-12 (DMEM/F-12). Cells were cultured in a humified incubator maintaining 5% $CO_2$ and 37 °C. Culture media were prepared by mixing 10% fetal bovine

serum (FBS), 1% L-glutamine, 1% antibiotic-antimycotic (100 units/mL penicillin, 100 µg/mL streptomycin, and 0.25 µg/mL amphotericin B).

**8.1 Cellular uptake quantification studies:**

$1*10^5$ EMT6 cells were seeded in 24 well-plate and incubated for overnight at 37 °C and 5% $CO_2$. Next, cells were washed with PBS buffer and replaced with new media containing required amount of dye labelled DS-NGs (Sulf-Cy3 NHS) and NonDS-NGs (Sulf-Cy3 $NH_2$) nanogel. After 4 hours of incubation, the media was removed again, washed cells two times with PBS and detached the cells by treating with 500 µL of 0.25% of trypsin-EDTA at 37 °C for three minutes. The cells were collected and pelleted using centrifugation at 3000 rpm for 5 minutes. The supernatant was removed, the palette was washed with 500 µL PBS and centrifuged again at the same set of conditions. After repeating the same procedure one more time, the cells were resuspended in 200 µL of cold PBS and flowcytometry analysis was done in BD LSRFortessa™ instrument. Data analysis was done using FlowJo software to calculate the mean fluorescence intensities.

**8.2 Exofacial surface-thiol blocking:**

$1*10^5$ EMT6 cells were seeded in 24 well-plates incubated overnight. After 24 h of incubation, the media was replaced with 1.2 mM solution of DTNB (5,5'-dithiobis-2-nitrobenzoic acid) or sodium iodoacetate in Dulbecco's modified eagle medium (DMEM) for 30 min. Then the solution was removed, cells were washed with PBS (3x500 µL) and then, cells were treated with 100 nM nanogel solutions in DMEM for 4 h (400 µL media). Finally, the cells were harvested following the same procedure as discussed in section 8.1 and analyzed accordingly.

**8.3 Cell cytotoxicity evaluation:**

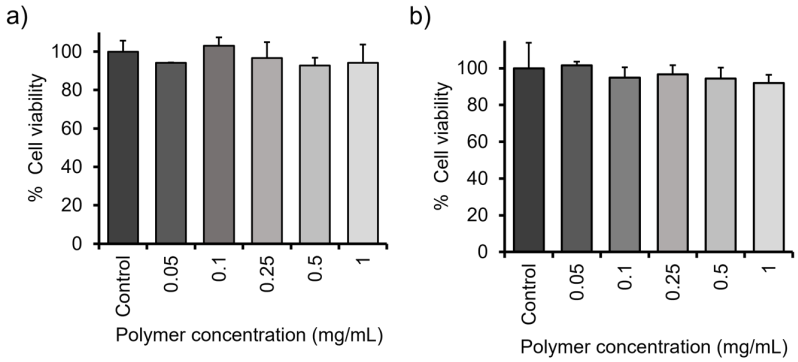



**Figure S9:** Cytotoxicity evaluation of (a) DS-NGs and (b) NonDS-NGs nanogels in EMT6 cell line. Both nanogels were found to be nontoxic in to the cell.

AlamarBlue assay was used to measure the cellular toxicity of polymeric nanogel used. $10^4$ EMT6 cells in 100µL of DMEM per well were plated in 96-well tissue culture plate and incubated at 37 °C maintaining 5% $CO_2$. After 24 h, the media was replaced with fresh media containing different concentrations of each nanogels (0.05, 0.1, 0.25, 0.5, 1 mg/mL). The media was replaced after 24 h of incubation, and cells were washed with PBS (2x100 µL). Then each well was treated with 10% 200µL of amalarBlue solution and incubated at 37 °C for 1 h (depending on the time taken to change color of media). Finally, solutions were transferred to a black 96-well flat-bottomed plate for

fluorescence measurement with SpectraMax M5 microplate reader (excitation, 560 nm: emission, 590 nm).

**8.4 Quantification of disulfide on nanogel:**

To quantify the surface exposed disulfide on nanogel, first we drawn a calibration curve using different concentrations of thiol fluorophore (Z-Rhodamine-SH). Then we prepared both DS-NGs and NonDS-NGs nanogel by mixing 1 mg polymer in water. Afterwards we added 5 µL of 1.054 µM fluorophore solution to both nanogel and let it react at RT. After 24 h they were dialyzed against water for 48 h followed the UV-vis absorbance to quantify the particle disulfide. As a control, same volume of fluorophore was added to 1 mL of water, stirred for 24 h and dialyzed parallelly. DS-NGs showed the absorbance peak at 562 nm which confirms the presence of disulfide whereas, NonDS-NGs didn't show reactivity and the absorbance spectra show similar nature to that of control.

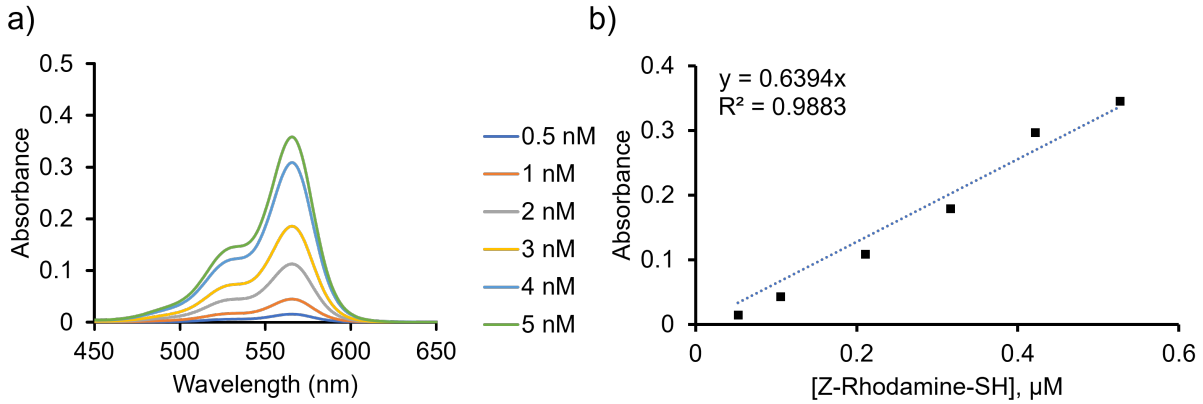



**Figure S10:** (a) The absorbance peak of Z-Rhodamine-SH at different concentrations. (b) The calibration curve of thiol fluorophore.

**8.5 Endocytosis inhibition studies:**

$1*10^5$ EMT6 cells were seeded in 24 well-plates incubated overnight. Next, the cells were washed with cold PBS (2x500 µL) and preincubated with Hyper-osmolar sucrose (45 mM, clathrine mediated pathways), EIPA (100 µM, macro-pinocytosis pathways), Nystatin (30 µM, caveolin pathways), Dyngo (30 µM, clathrin-independent/dynamine-dependent pathways), 7-keto-cholesterol (30 µM, clathrin-independent/dynamine-independent pathways), Cytochalasin D (5 µM, phagocytosis and macropinocytosis pathways), $NaN_3$/2-deoxy-D-glucose (affects all pathways) in serum free media for 1 h at 37 °C. After that, solution was removed, and cells were co-incubated with 100 nM nanogel solution and above-mentioned concentration of inhibitors for different time points. Finally, the media was removed again, washed cells for two times with PBS and detached the cells by treating with 500 µL of 0.25% of trypsin-EDTA at 37 °C for three minutes. Afterwards same procedure was followed to prepare sample for flow cytometry and analyzed data accordingly.

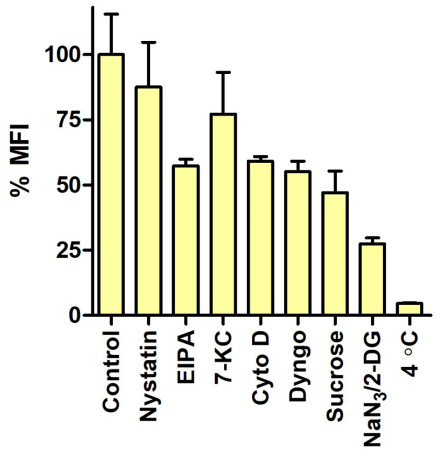



**Figure S11:** Mechanisms of cellular uptake of NonDS-NGs nanogels in presence and absence of inhibitors.

### 8.6 Nanogel coincubation studies:

The dye (AF488) conjugation to $P_{DS\text{-}NGs}$ polymer was done following the previously described procedure and the percentage conjugation was evaluated using the UV-vis spectroscopy. The confocal studies were performed following the procedure mentioned in 'endosomal escape studies using confocal microscopy' section.

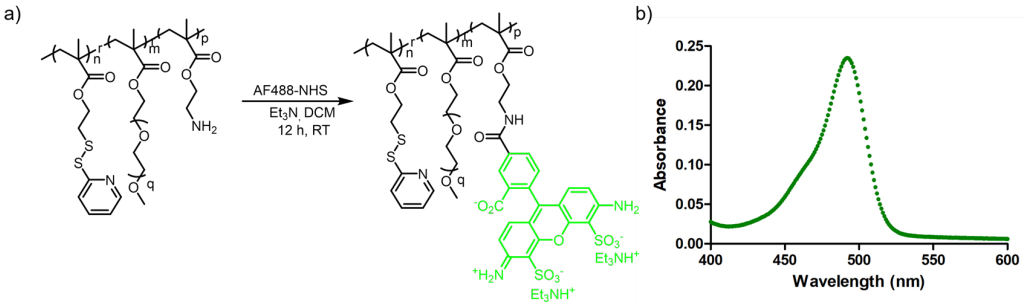



**Figure S12:** (a)The reaction scheme showing the dye conjugation to the $P_{DS\text{-}NGs}$ polymer, (b) UV-vis absorbance spectra of dye conjugated DS-NGs.

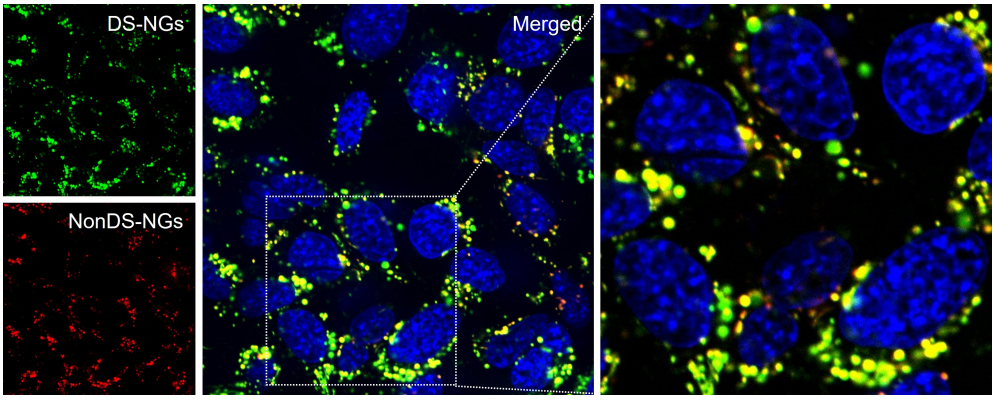



**Figure S13:** Confocal microscopy images shows the coincubation studies of DS-NGs and NonDS-NGs at 4 h of incubation.

### 8.7 Endosomal escape studies using confocal microscopy:

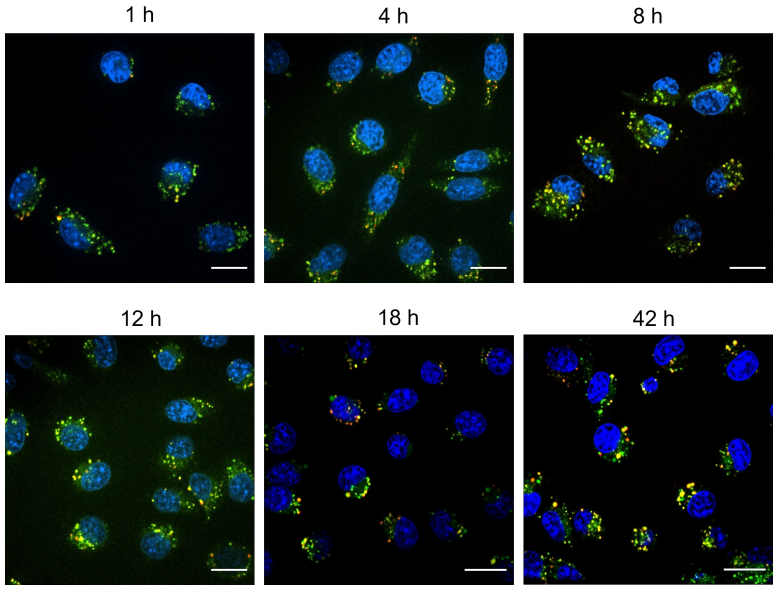



**Figure S14:** Time dependent monitoring of endosomal escape evaluation of NonDS-NGs nanogel using confocal microscopy. Scale bar 20 μm.

EMT6 cells were plated at $10^5$ density (in 1 mL) in 35 mm glass-bottom petri-dishes and incubated at 37 °C maintaining 5% $CO_2$ for 24 h. To study the endosomal escape, the media was replaced, and cells were washed with PBS (2x1mL). After that, cells were incubated with fresh media containing 0.2 mg/mL nanogel solution (1mL of media) for different hours depending on the experiments. Before subjecting to confocal imaging, the cells were washed again PBS (2x1mL) and replaced with 1 mL new media. Lysotracker green was used to satin endosome/lysosome as per the protocol and

NucBlue™ Live ReadyProbes™ reagent was used for the nucleus. Cells were imaged with Nikon Spinning Disk Confocal microscope.

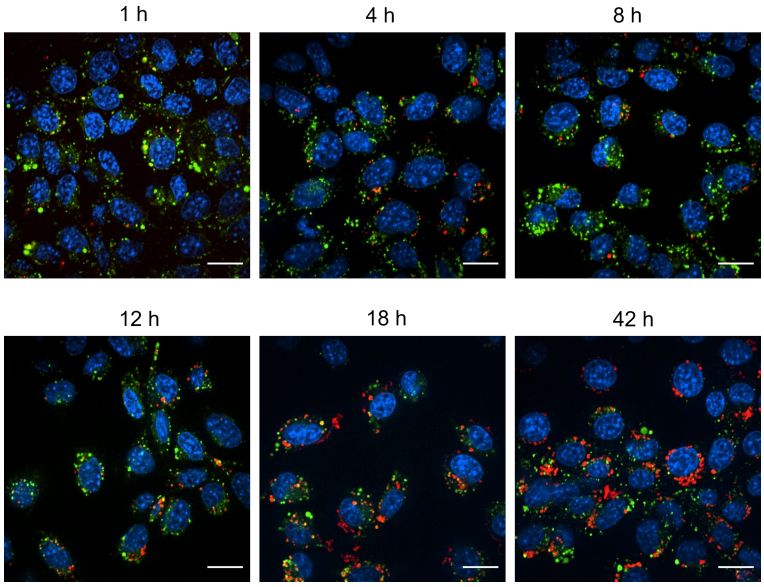



**Figure S15:** Time dependent studies for the endosomal escape of DS-NGs nanogel using confocal microscopy. Lower colocalization of Sulf-cy3 tagged DS-NGs and lysotracker green were observed with time which indicate endosomal escape. Scale bar 20 μm.

**8.8 Nile red uptake studies by confocal microscopy:**

The punctate nature of cytosolic fluorescence usually indicates endosomal entrapment, and a similar fluorescence for DS-NGs was observed. Since the dye is conjugated to the polymer, it is reasonable that after escaping from endosome the nanogel forms nanoaggregates in the cytosol owing to its amphiphilic nature. To test this, Nile red was encapsulated in the DS-NGs nanogel and performed the confocal imaging following same procedure as described in section 7.4.

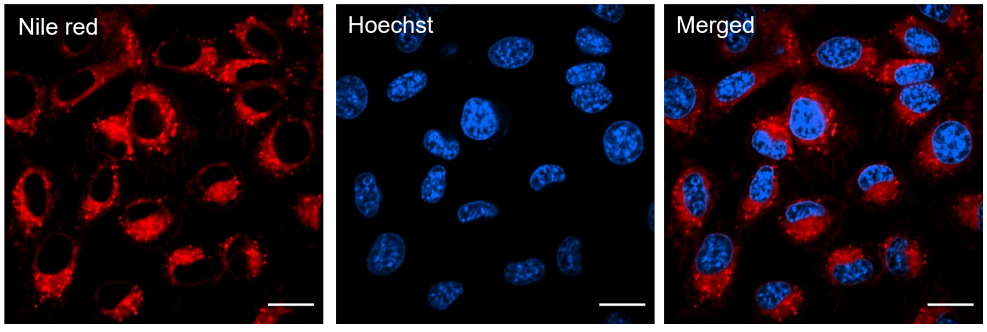



**Figure S16:** Confocal microscopy images of Nile red encapsulated DS-NGs. Diffused cytosolic fluorescence indicates the succesful endosomal escape and dye release from nanogel. Scale bar 20 μm.

**8.8 Uptake evaluation upon chloroquine treatment:**

EMT6 cells were plated at $10^5$ density (in 1 mL) in 35 mm glass-bottom petri-dishes and incubated at 37 °C maintaining 5% $CO_2$ for 24 h. After 18 h of incubation with the respective nanogels, media was removed, and cell were washed with PBS (2x1mL). Next, cells were treated with 100 μM chloroquine in fresh media and incubated for 2 h. Then cells were images using confocal microscopy following same procedure as described in section 7.5.

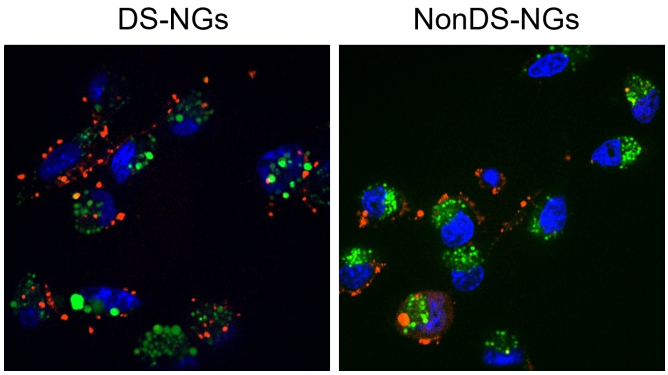



**Figure S17:** Confocal microscopy images showing the endosomal escape of both DS-NGs and NonDS-NGs upon treatment with chloroquine. The successful escape of NonDS-NGs upon chloroquine treatement shows the inherent inability to get out of endosome. Scale bar 20 μm.

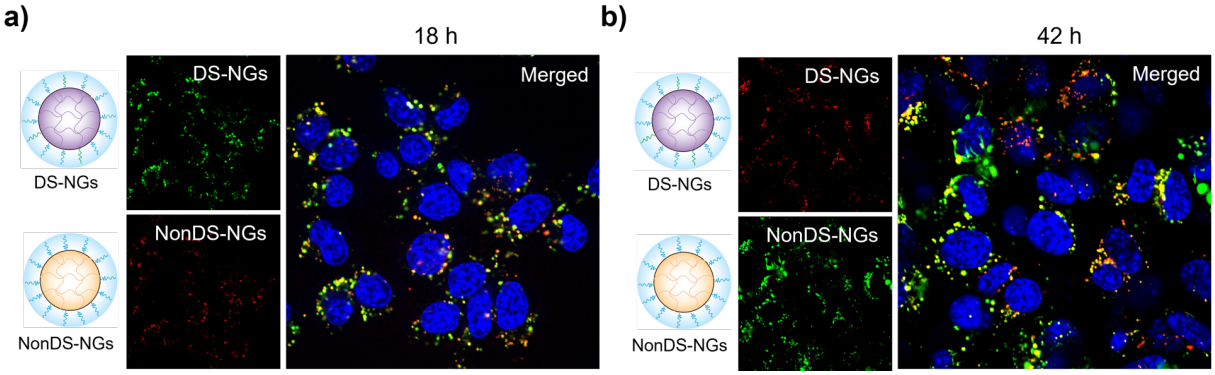



**Figure S18:** Confocal microscopy images showing the seperation of DS-NGs (AF488) and NonDS-NGs (sulfo-cy3) nanogels at different time points.

**8.9 Calcein assay:**

EMT6 cells were plated at $10^5$ density (in 1 mL) in 35 mm glass-bottom petri-dishes and incubated at 37 °C maintaining 5% $CO_2$ for 24 h. Then cells replaced with new media and were coincubated with 100 μM calcein in serum-free media with/without (control) for different time points. Then it was replaced with new media after washing the cells with PBS for three times. The nucleus was stained for with NucBlue™ Live ReadyProbes™ reagent and cells were imaged with Nikon Spinning Disk Confocal microscope.

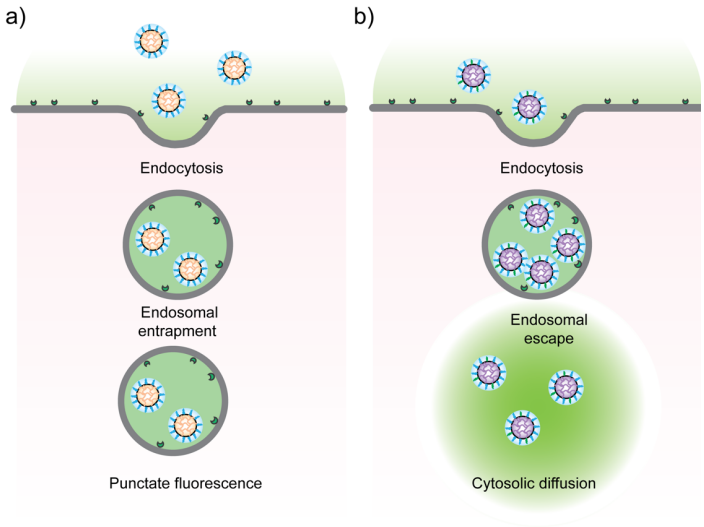



**Figure S19:** Schematic represnetation of the porposed evaluation of calcein assay. We hypothesized that (a) the calcein will remain entrapped in endosome along with NonDS-NGs nanogel and it will show puncate fluorescence . Whereas, (b) it will show a diffused fluorescence upon coincubation with disulfide containing nanogels as it able to ble escape the endosome.

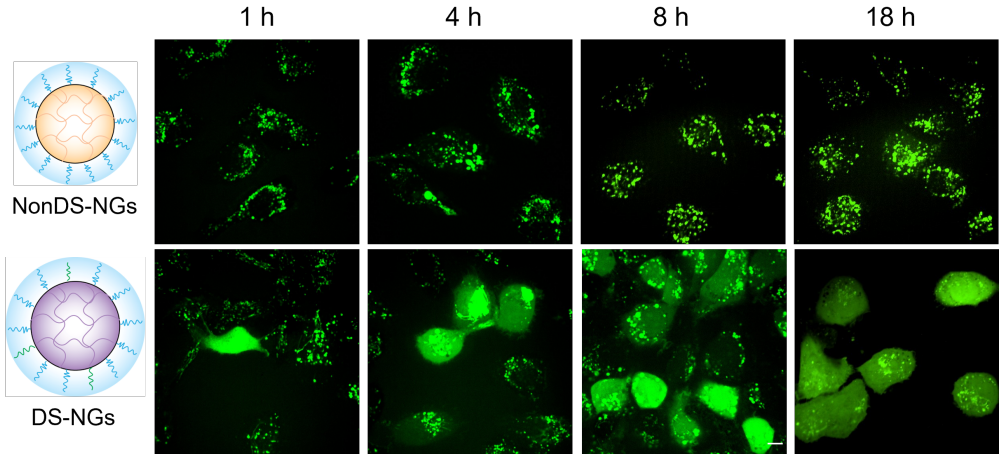
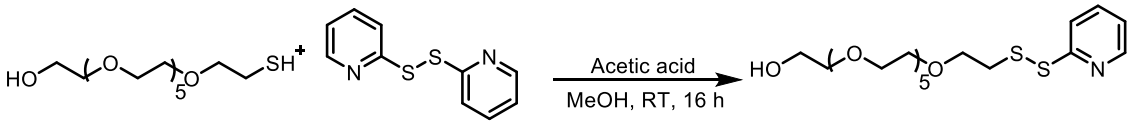



**Figure S20:** The calcein release studies for NonDS-NGs and DS-NGs at different time points.

## 9. Synthesis of PeriDS-NGs nanogel

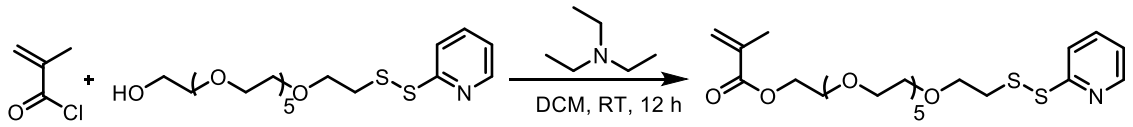
### 9.1 Synthesis of pyridyl disulfide penta-ethylene glycol methyl methacrylate (PDEGMMA) monomer:

**Step 1:**



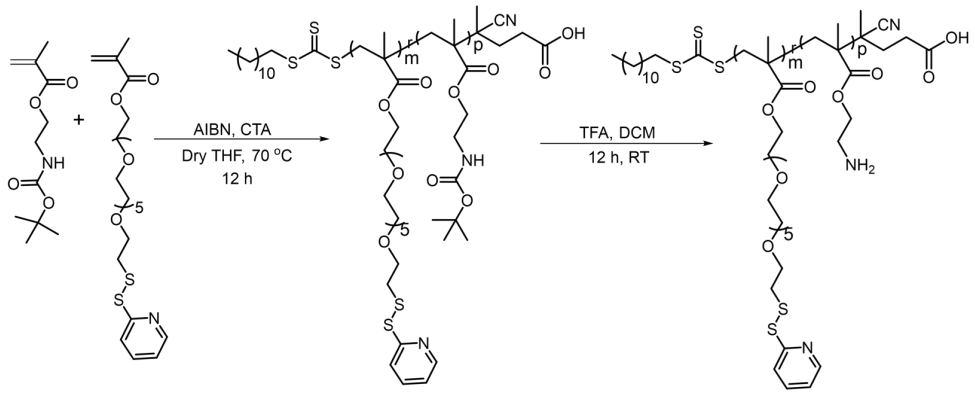

295.31 mg (1.34 mmol, 2 equiv) 2,2'-dipyridylsulfide was dissolved in methanol and 75 µL of acetic acid was added to the reaction mixture. After 30 minutes, 200 mg (06 mmol, 1 equiv) of thiol-PEG5-alcohol in methanol was added to the solution dropwise and allowed to react for 3 h. Afterwards, the methanol was evaporated, and diethyl ether was added for the precipitation of reaction byproduct. No crystals were observed. Then the final product was purified by flash chromatography using DCM/methanol as eluent. Yield:53%. $^{1}$H NMR (400 MHz, $CDCl_3$) (δ ppm): 8.46-8.48 (d, 1H), 7.79-7.81 (d, 1 H), 7.66-7.70 (t, 1H), 7.09-7.12 (t, 1H), 3.61-3.76 (m), 3.00-3.03 (t, 2 H). $^{13}$C NMR (100 MHz, $CDCl_3$) (δ ppm): 157.34, 148.76, 138.20, 137.93, 136.89, 122.94, 120.04, 72.55, 70.57, 68.72, 61.71, 53.44, 38.65.

**Step 2:**

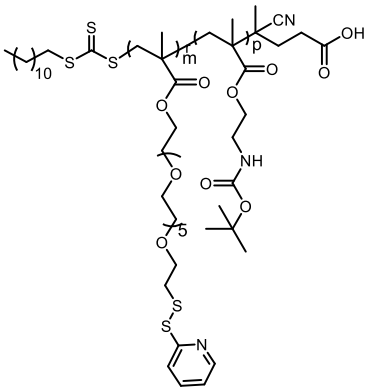



300 mg (0.736 mmol, 1 equiv) of (2-pyridyldithio)-PEG5-alcohol was dissolved in DCM in an ice bath. Triethylamine was then added to this solution. After 30 minutes of stirring, 76.94 mg (0.736 mmol, 1 equiv) methacryloyl chloride in DCM was added dropwise to the reaction mixture and allowed to react for 6 h. After, the crude mixture was washed with distilled water (2x50 mL), saturated brine solution (1x50 mL) and dried over sodium sulfate anhydrous solution. The final product was purified by flash chromatography using DCM/methanol as eluent. Yield: 81%. $^{1}$H NMR (400 MHz, $CDCl_3$) (δ ppm): 8.47-8.48 (d, 1H), 7.78-7.80 (d, 1H), 7.66-7.70 (t, 1H), 7.08-7.12 (d, 1H), 6.14 (s, 1H), 5.58-5.59 (d, 1H), 4.30-4.33 (t, 2H), 3.59-3.77 (m), 3.00-3.03 (t, 2H), 1.98 (s, 3H). $^{13}$C NMR (100 MHz, $CDCl_3$) (δ ppm): 167.37, 160.45, 149.53, 137.11, 136.17, 125.74, 120.60, 119.63, 68.97-70.68, 63.89, 38.46, 18.33.

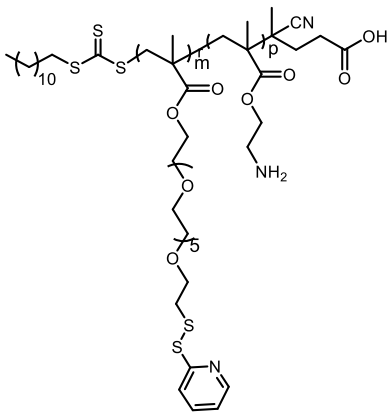
### 9.2 Synthesis of p(PDEGMMA-co-AEMA) polymer, $P_{PeriDS\text{-}NGs}$:




**Scheme S7:** Synthetic scheme of $\mathbf{P_{PeriDS\text{-}NGs}}$ polymer



The p(PDEGMMA-co-AEMMA) polymer was also synthesized using RAFT polymerization. 100 mg (0.29 mmol) of PDSEGMA monomer, 3.5 mg (0.015 mmol) of AEMA monomer and 4.17 mg (0.0103 mmol) 4-Cyano-4-[(dodecylsulfanylthiocarbonyl)sulfanyl]pentanoic acid were taken in a 7 mL glass vial and dissolved in 400 µL of dry THF. After that, 0.33 mg (0.002 mmol) of AIBN in dry THF was added to the reaction mixture. The vial was sealed tightly with rubber septum and duct tape and degassed the vial freeze-pump-thaw cycles for three times. Then the polymerization was set in preheated oil bath at 70 °C for 12 h. The crude was dissolved in minimum amount of DCM and then precipitated in diethyl ether for three times. The polymer was transferred to a glass vial and dried under high vacuum for overnight. Yield: 78% $^{1}$H NMR (400 MHz, $CDCl_3$) (δ ppm): 8.47, 8.46, 7.81, 7.79, 7.71, 7.69, 7.67, 7.13, 7.11, 7.10, 4.09, 3.75, 3.71, 3.60, 3.59, 3.02, 3.01, 2.99, 1.64, 1.46, 1.26, 1.04, 0.89, 0.87. $^{13}$C NMR (100 MHz, $CDCl_3$) (δ ppm): 206.95, 160.43, 149.52, 137.20, 120.67, 119.64, 70.58, 68.94, 38.49, 30.94. The relative ratios of monomers were calculated considering the pyridyl proton and tertiary butyl proton which was found to be m: p = 93:07.



The final polymer was obtained by deprotecting the amine functionality in the polymer chain. 80 mg (0.236 mmol) of p(PDSEGMA-co-AEMA) polymer was dissolved in DCM and 540.13 mg (4.73 mmol) trifluoroacetic acid (TFA) was added to the solution. After 12 h, the crude mixture was dried using rotary evaporator, dissolved in minimum amount of DCM, reprecipitated in diethyl ether for three times. Finally, the polymer was transferred to a glass vial, evaporate the solvent, and dried under high vacuum to obtain a yellow viscous polymer. Yield: 91% $^{1}$H NMR (400 MHz, $CDCl_3$) (δ ppm): 8.53, 8.52, 7.91, 7.89, 7.80, 7.28, 7.20, 5.45, 4.09, 3.74, 3.72, 3.67, 3.59, 3.58, 3.04, 3.02, 3.00, 1.26, 1.04, 1.03, 0.91, 0.87, 0.86.

**9.3 Dye Conjugation to PeriDS-NGs polymer:**
50 mg polymer was dissolved in 2 mL of DCM and triethylamine was added to the solution. After 15 minutes of stirring, the sulf cy3-NHS dye was added to the mixture and let it stir for 12 h (scheme S8). Then the solution was dialyzed in 3.5K MWCO snakeskin dialysis membrane against DCM. The solvent was changed at a regular interval for 48 h depending on the color change. Afterwards, the DCM was evaporated, and polymer was dried under high vacuum overnight.

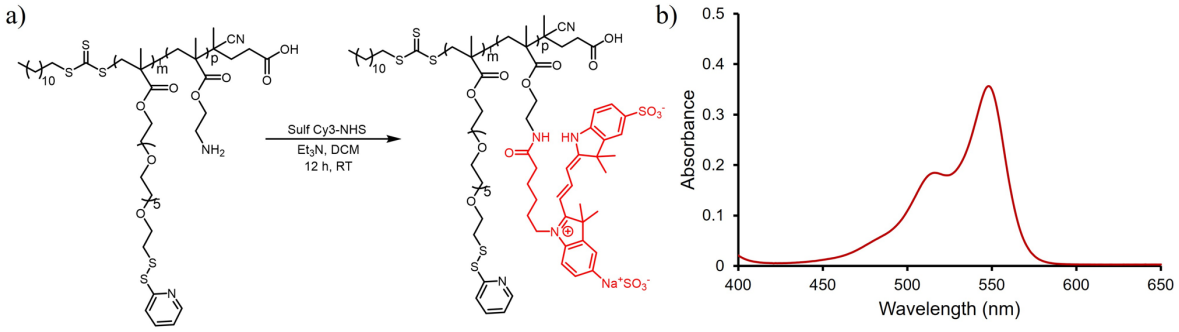



**Figure S21:** (a) Reaction scheme of sulf cy3-NHS dye conjugation to $\mathbf{P_{PeriDS\text{-}NGs}}$ polymer. (b) UV-Vis spectrum of dye conjugated PeriDS-NGs.

**9.4 PeriDS-NGs nanogel preparation:** 2 mg $\mathbf{P_{PeriDS\text{-}NG}}$ polymer was dissolved in 0.1% acetone first and then 1 mL water was added to it. The solution was stirred with open cap for 2 h and then DTT solution was added to attain 100% crosslinking. The extent of crosslinking reaction was monitored by measuring the absorbance of 2-mercaptopyridine as the reporter molecule (Figure 4a). The nanogel was dialyzed in a 2 mL mini dialysis kit for 48 h.

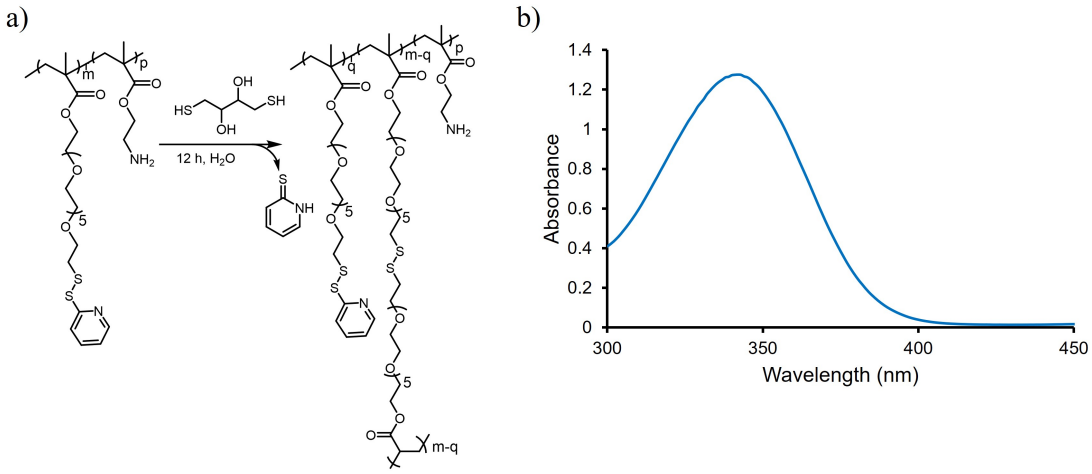



**Figure 22:** (a) PeriDS-NGs nanogel formation by crosslinking reaction of $\mathbf{P_{PeriDS\text{-}NGs}}$ polymer with DTT. (b) UV-vis spectrum for the reporter molecules of crosslinking reactions.

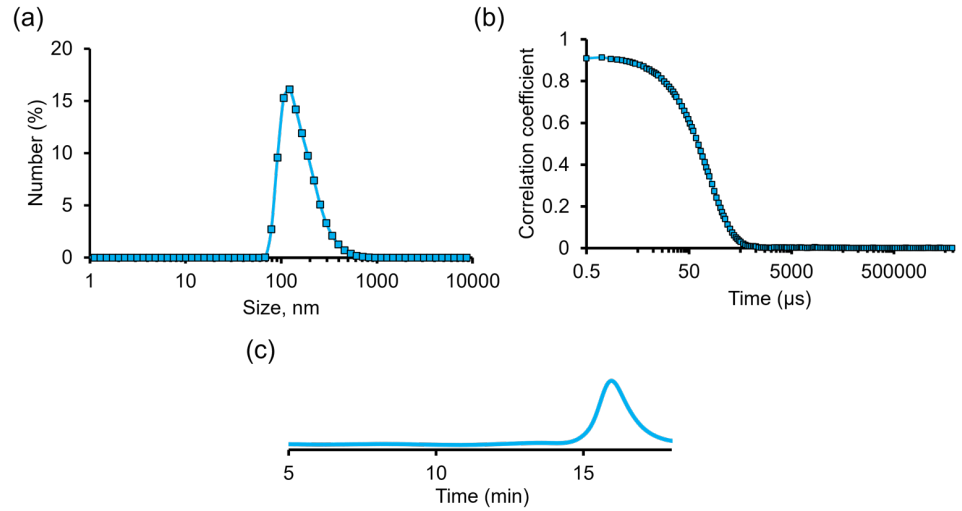



**Figure S23:** The DLS (a) size profile and (b) correlation function of PeriDS-NGs. (c) GPC (THF) of **$P_{PeriDS\text{-}NGs}$.**

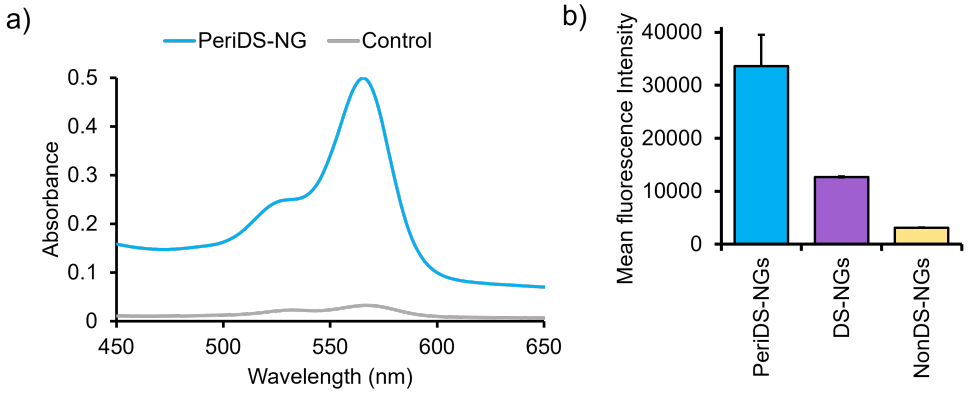



**Figure 24:** (a) UV-vis spectra representing the PeriDS-NGs nanogel reacting with thiol fluorophores. (b) Particle internalization of different sized DS-NGs nanogels quantified by the flow cytometry.

**9.5 DS-NGs nanogel size variation:** The size variation of DS-NGs nanogel was done following the previously established procedure in our group.[3] 10 mg DS-NGs polymer was taken in three different vials and were dissolved in different salt concentrations. For ~10 nm nanogel, the 10 mg polymer was dissolved in 1 mL of water, sonicated for 2 h and let it stir with required amount of DTT (depending on the % crosslinking) for 3 h at RT. For ~30 nm particles, 10 mg polymer was dissolved in 15 mM $Na_2SO_4$ buffer, sonicated for 2 h and let it stir with DTT at RT for 3 h. For ~100 nm particles size, polymer was dissolved in 20 mM $Na_2CO_3$ buffer, sonicated for 2 h and afterwards, let it stir with DTT at 70 °C for 3 h. After 3 h, all particles were dialyzed against water for 24 h to get rid of the crosslinking byproduct (pyridithione).

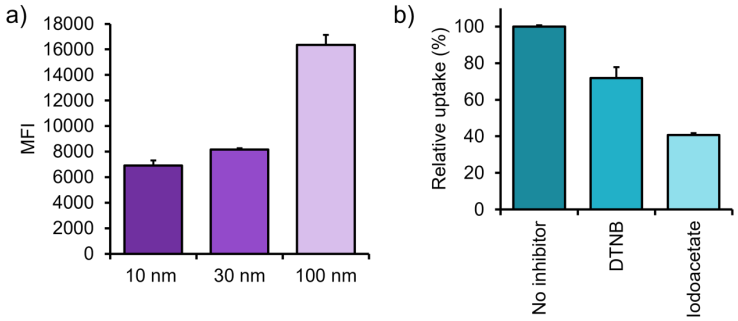



**Figure 25:** (a) Mechanisms of cellular uptake of PeriDS-NGs nanogels in presence and absence of inhibitors. (b) Particle internalization of different sized DS-NGs nanogels quantified by the flow cytometry.

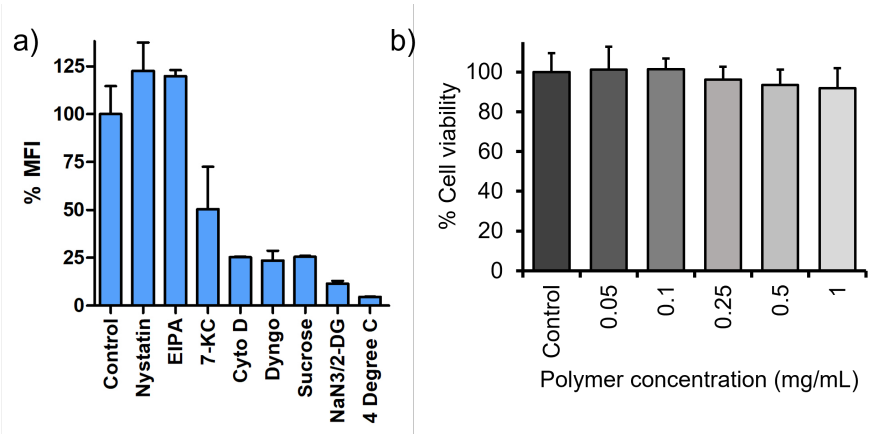



**Figure S26:** (a) Endocytosis inhibition study shows the predominant route of uptake is clathrin-mediated endocytosis. (b) EMT6 cells were treated with PeriDS-NGs at different concentrations and cells were found to be viable.

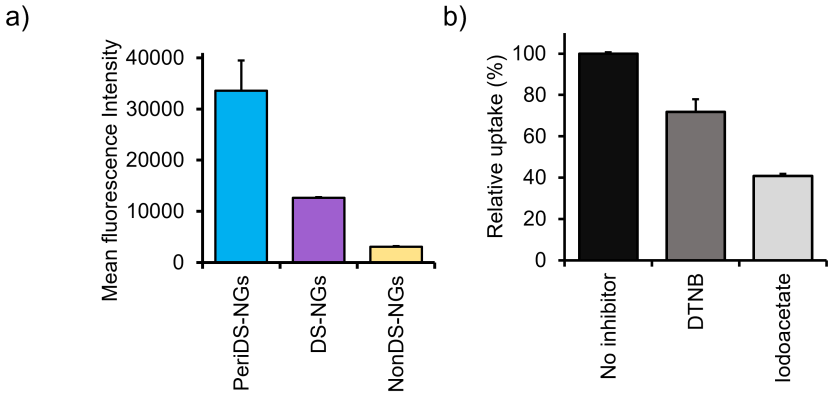



**Figure S27:** (a) Comparision uptake study of PeriDS-NGs, DS-NGs and NonDS-NGs using flow cytometry. Exposing the disulfide bond twords periphery have improved the uptake of PeriDS-NGs by ~3 fold in comapre the DS-NGs. (b) Pretreating the cells with DTNB reduced ~30% cellular uptake shows the role of disulfide-cell surface thiol interactions in cellular internalizations.

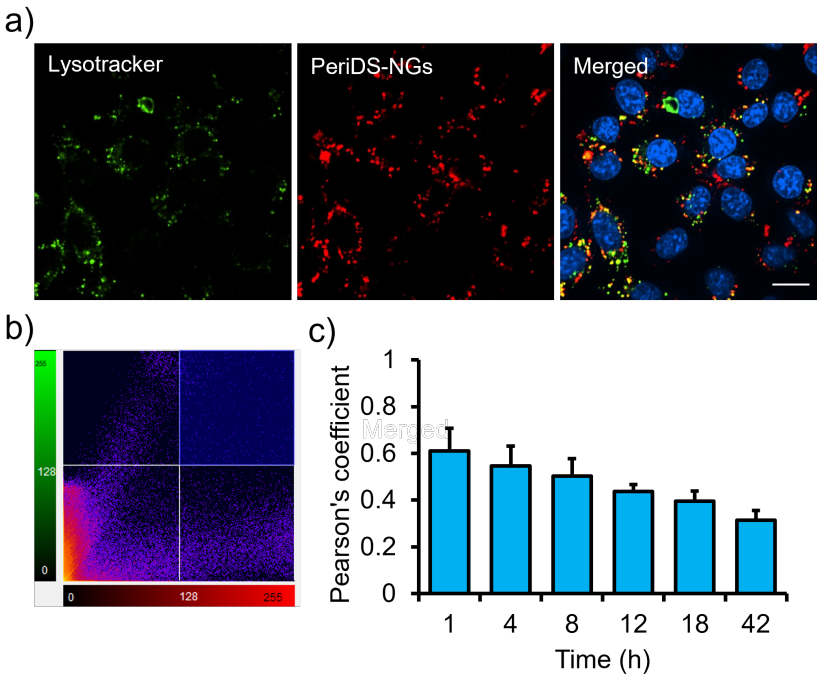



**Figure S28:** (a) Confocal microscopy image showing the endosomal escape profile of PeriDS-NGs at 18 h in EMT6 cell. (b) Pearson's correlation coefficient was calculated at 42 h. It shows a bifurcation between the Sulf-cy3 and lysotrackers green indicate minimal colocalization. (c) The graph showing the time dependent lowering of Pearson's coefficients from 1 h to 42 h shows the gradual endsosomal escape profile of PeriDS-NGs.

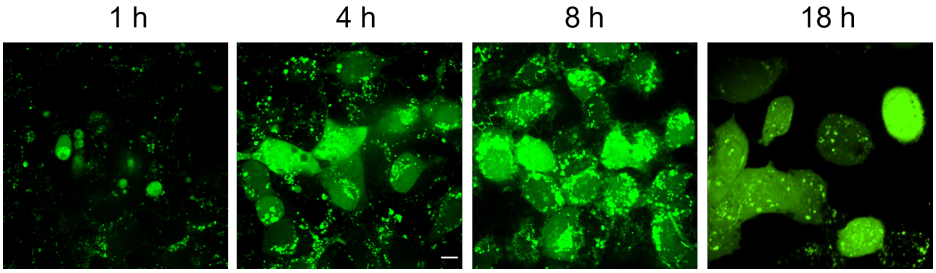



**Figure S29:** The calcein release studies for PeriDS-NGs at different time points.

## 10. NMR spectra

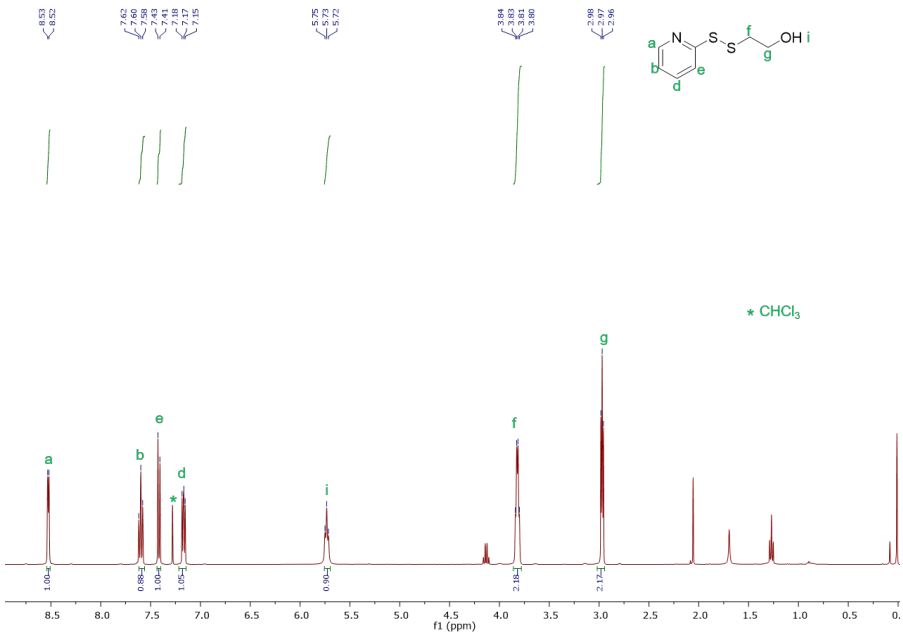



**Figure S30:** $^{1}$H NMR (400 MHz) of 2-(pyridine-2-yldisulfanyl) ethanol in $CDCl_3$.

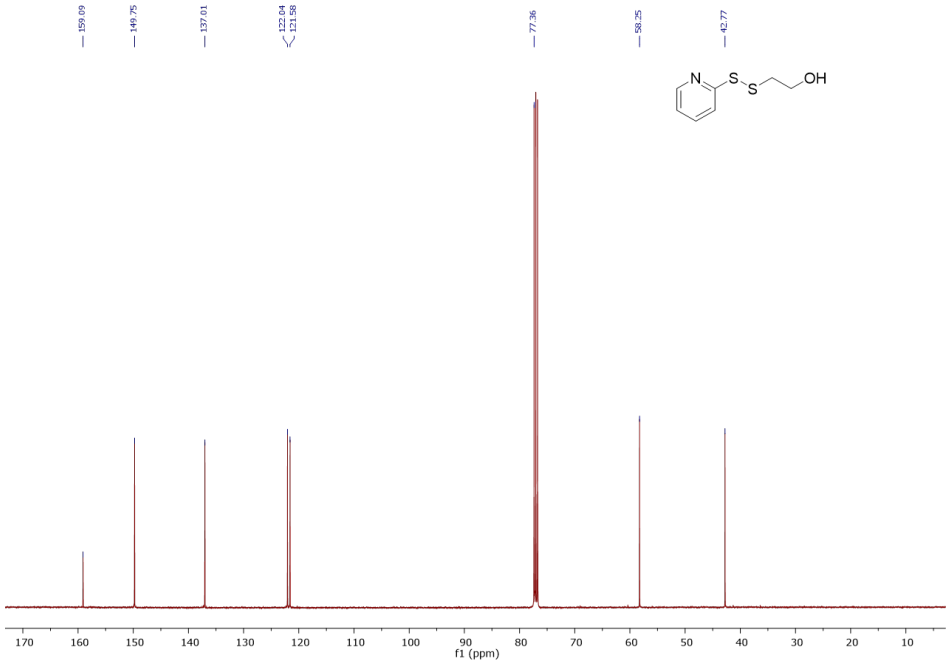



**Figure S31:** $^{13}$C NMR (100 MHz) spectra of 2-(pyridine-2-yldisulfanyl) ethanol in $CDCl_3$.

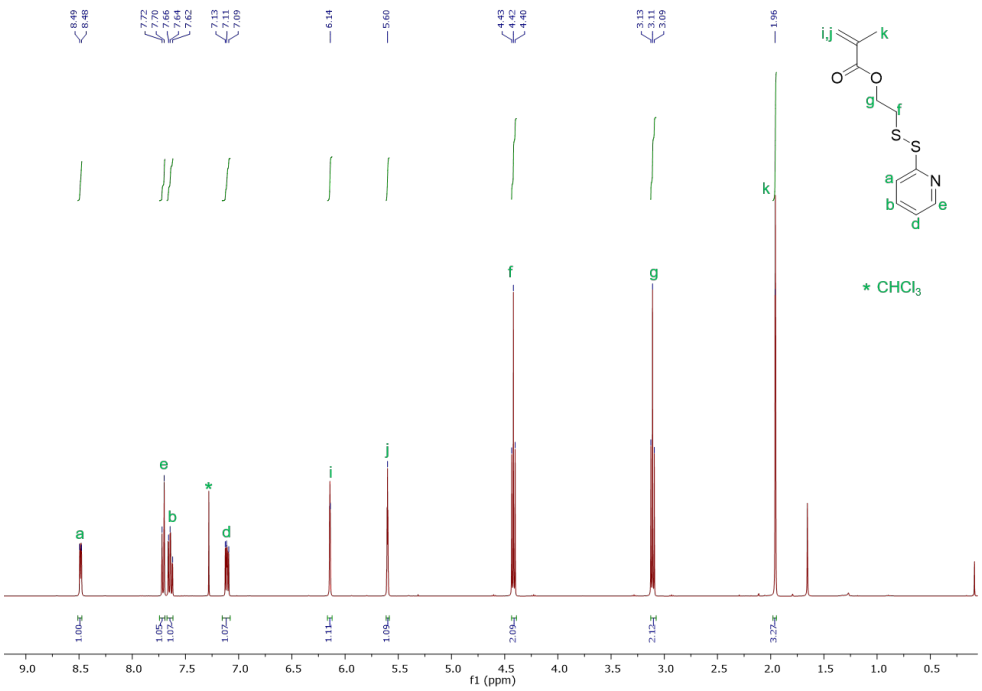



**Figure S32:** $^{1}$H NMR (400 MHz) of pyridyl disulfide ethyl methacrylate (PDSMMA) monomer in $CDCl_3$.

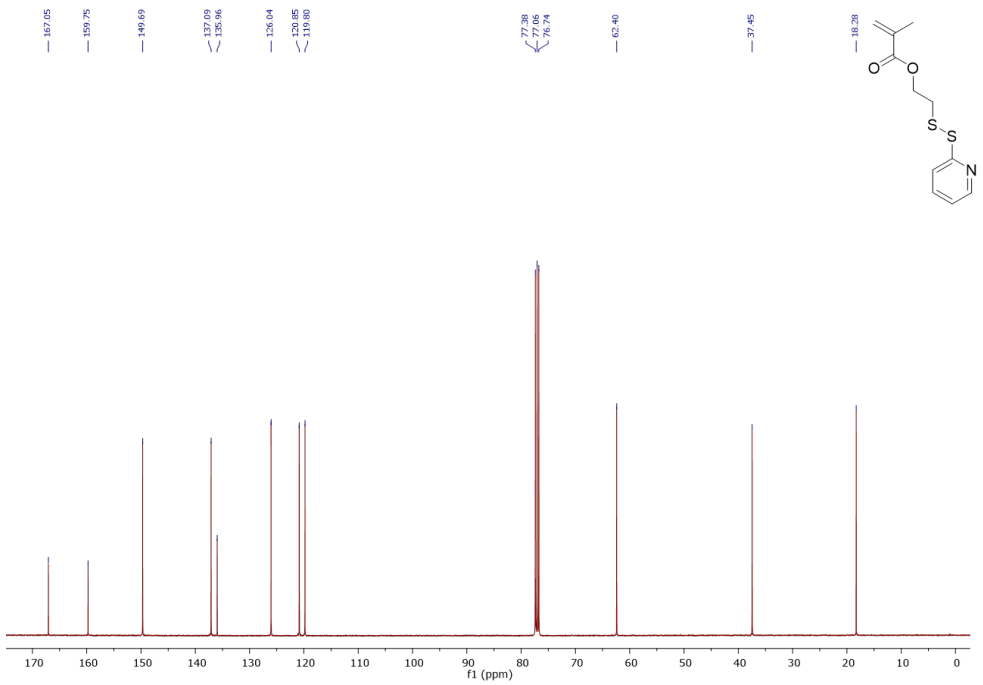



**Figure S33:** $^{13}$C NMR (100 MHz) spectra of pyridyl disulfide ethyl methacrylate (PDSMMA) monomer in $CDCl_3$.

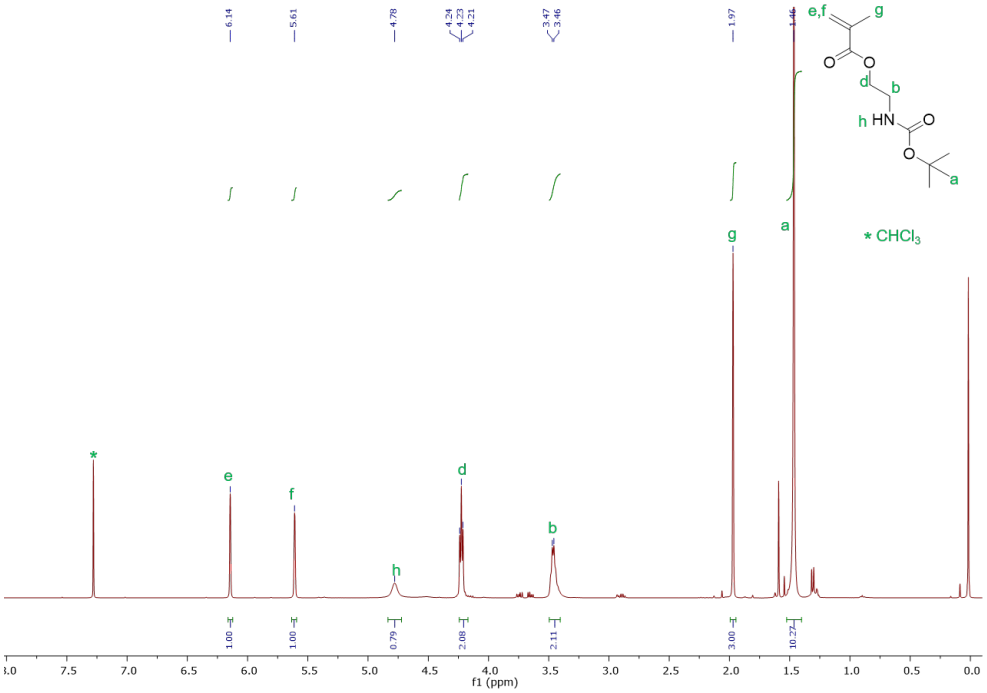



**Figure S34:** $^{1}$H NMR (400 MHz) of amino ethyl methyl methacrylate (AEMMA) monomer in $CDCl_3$.

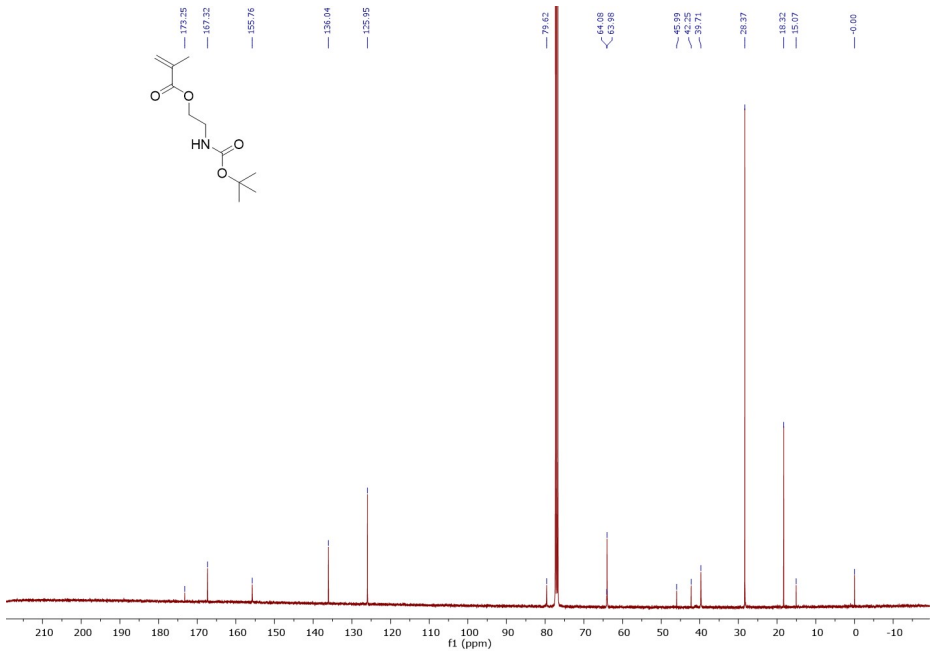



**Figure S35:** $^{13}$C NMR (100 MHz) of amino ethyl methyl methacrylate (AEMMA) monomer in $CDCl_3$.

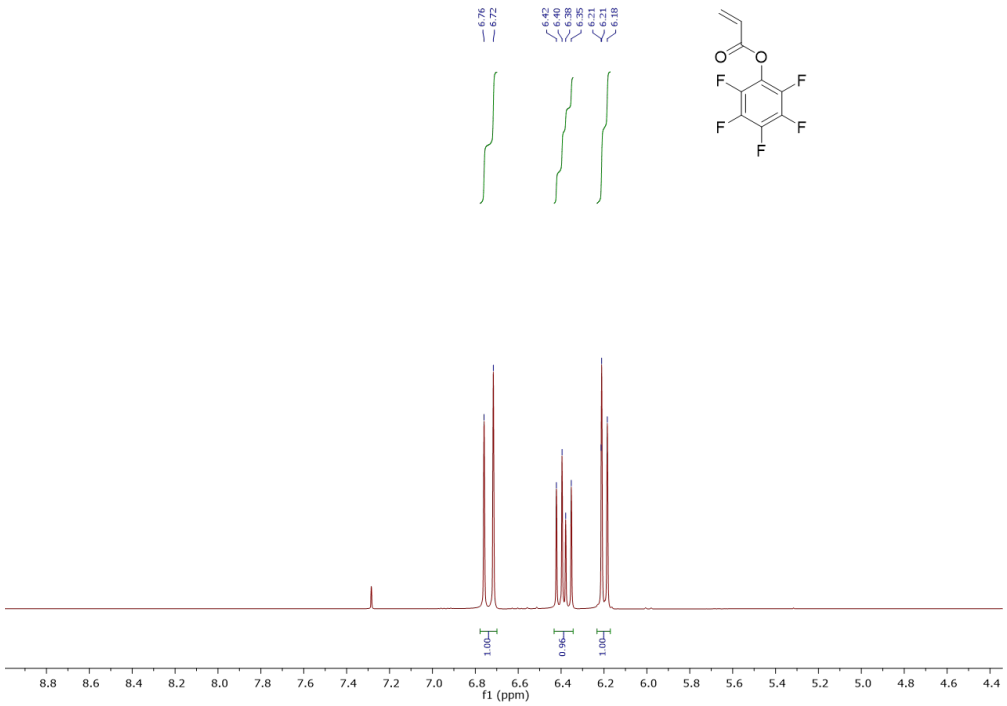



**Figure S36:** $^{1}$H NMR (400 MHz) of pentafluoro phenol acrylate (PFPA) monomer in $CDCl_3$.

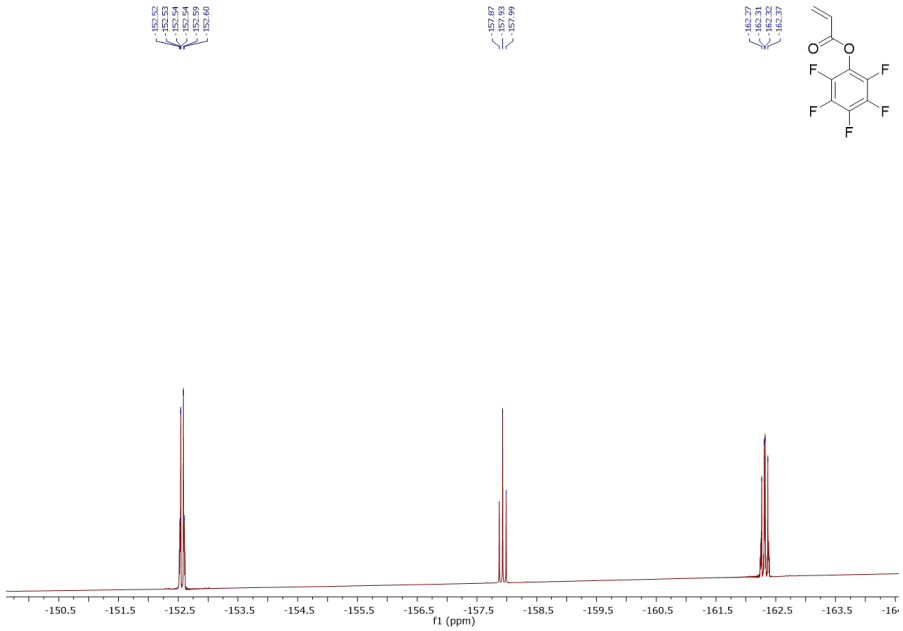



**Figure S37:** $^{19}$F NMR spectrum (376 MHz) of PFPA in CDCl3.

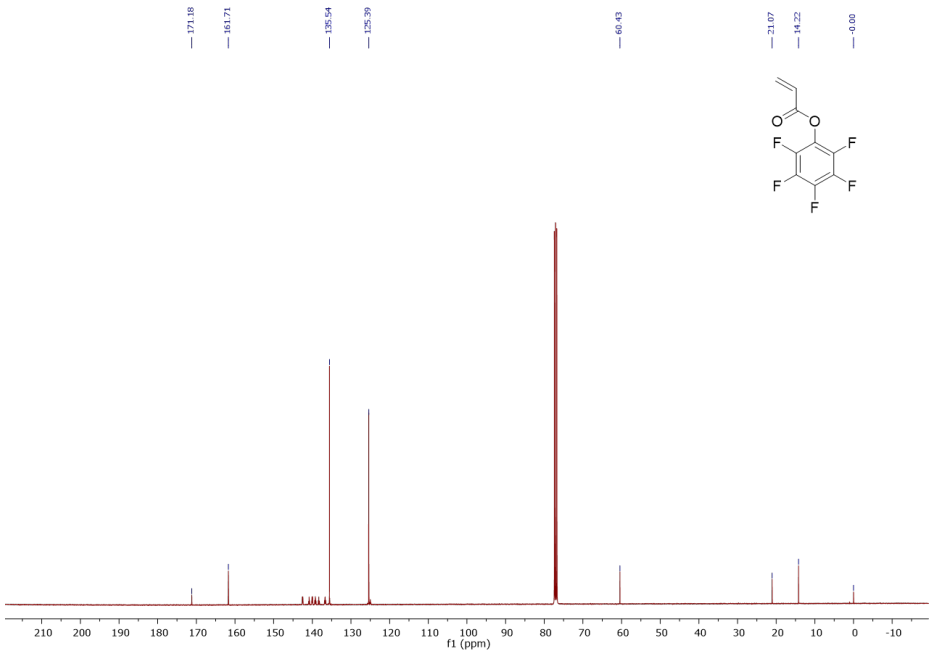



**Figure S38:** $^{13}C$ NMR spectrum (100 MHz) of PFPA in CDCl3.

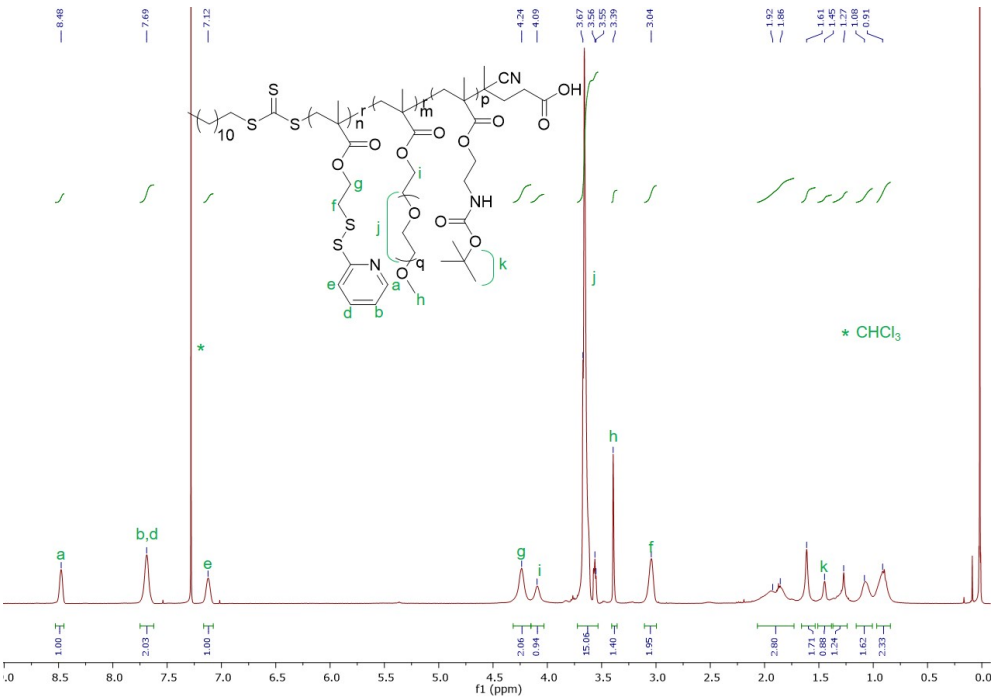



**Figure S39:** $^{1}H$ NMR (400 MHz) of p(PDSMMA-co-PEGMA-co-AEMMA) polymer (Boc-Protected) in $CDCl_3$.

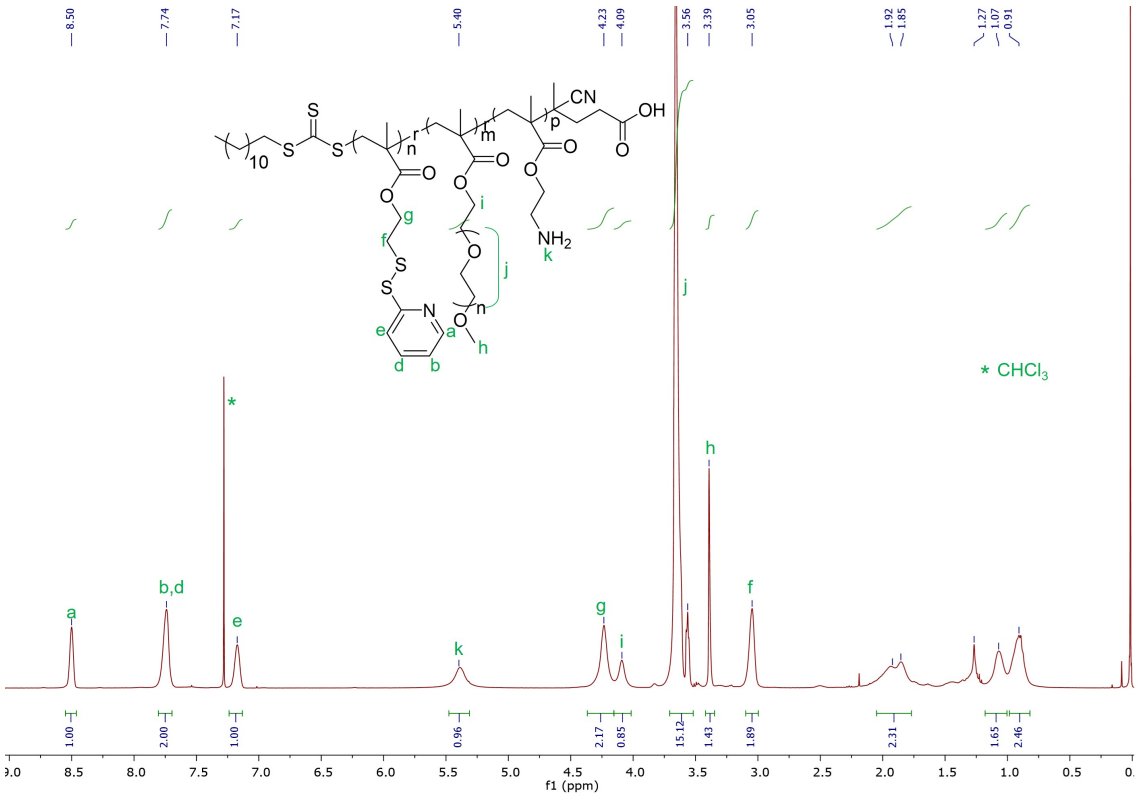



**Figure S40:** $^{1}$H NMR (400 MHz) of p(PDSMMA-co-PEGMA-co-AEMMA), $P_{DS\text{-}NGs}$ polymer in $CDCl_3$.

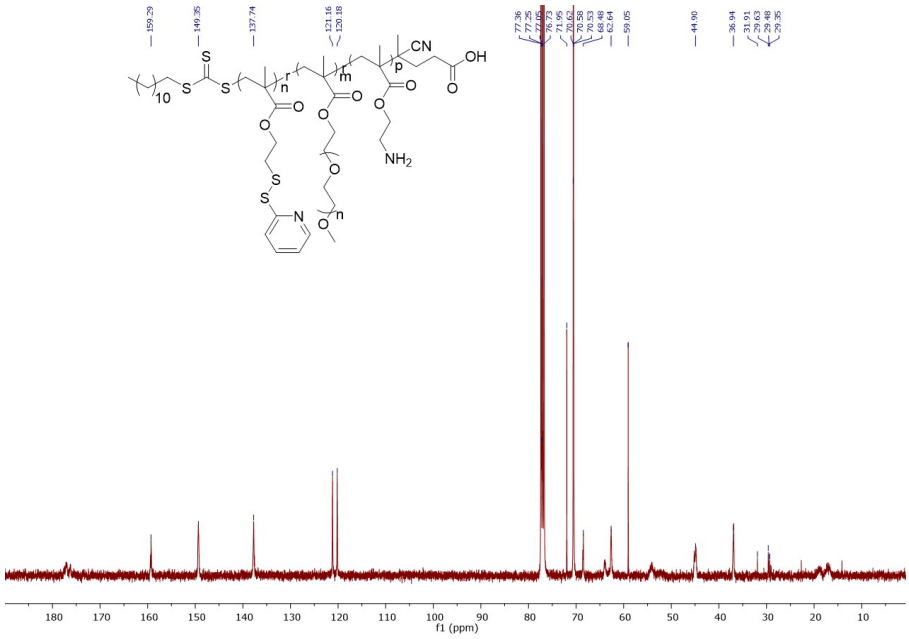



**Figure S41:** $^{13}$C NMR (100 MHz) of p(PDSMMA-co-PEGMA-co-AEMMA), $P_{DS\text{-}NGs}$ polymer in $CDCl_3$.

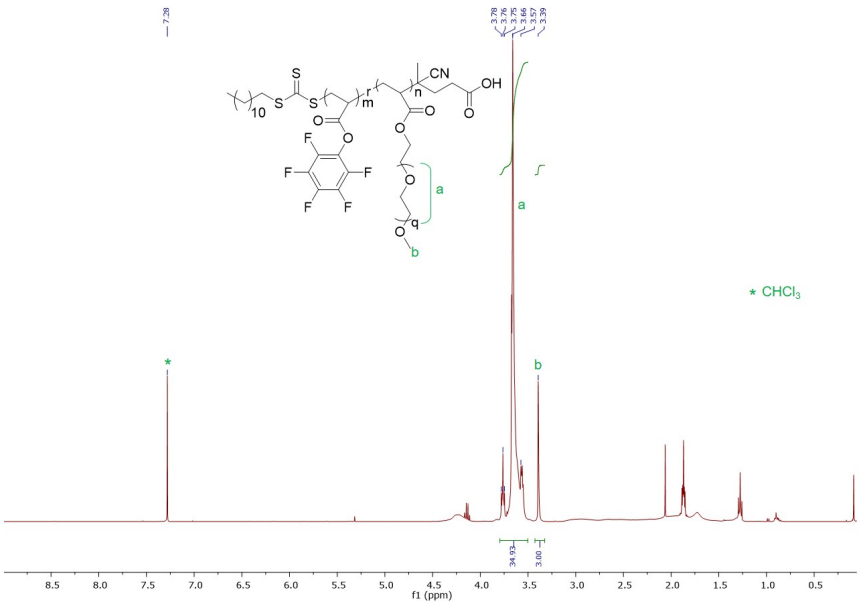



**Figure S42:** $^{1}$H NMR (400 MHz) of p(PFPA-co-PEGA), $P_{NonDS\text{-}NGs}$ polymer in $CDCl_3$.

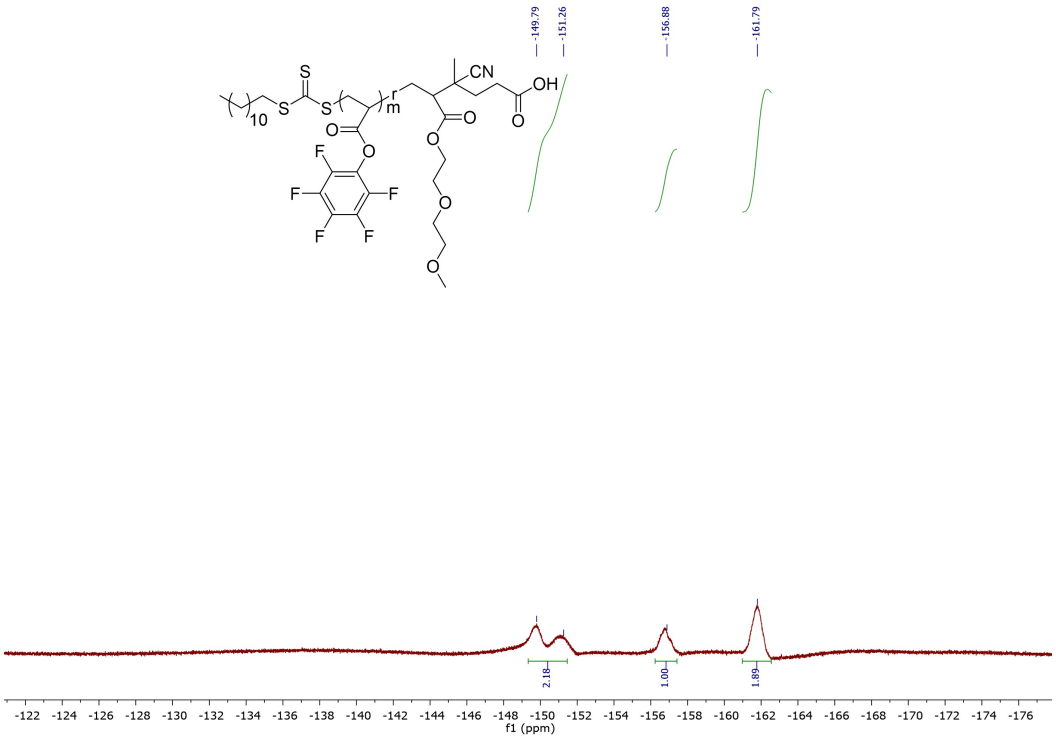



**Figure S43:** $^{19}$F NMR spectrum (376 MHz) of p(PFPA-co-PEGA), $P_{NonDS\text{-}NGs}$ polymer in $CDCl_3$.

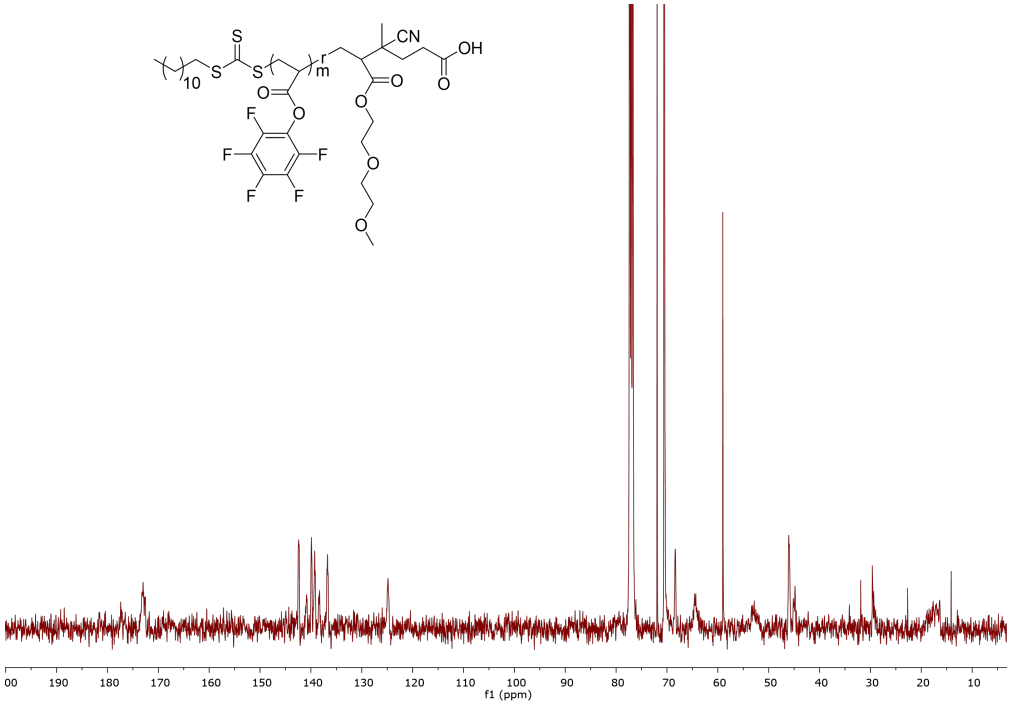



**Figure S44:** $^{13}C$ NMR (100 MHz) of p(PFPA-co-PEGA), $P_{NonDS\text{-}NGs}$ polymer in $CDCl_3$.

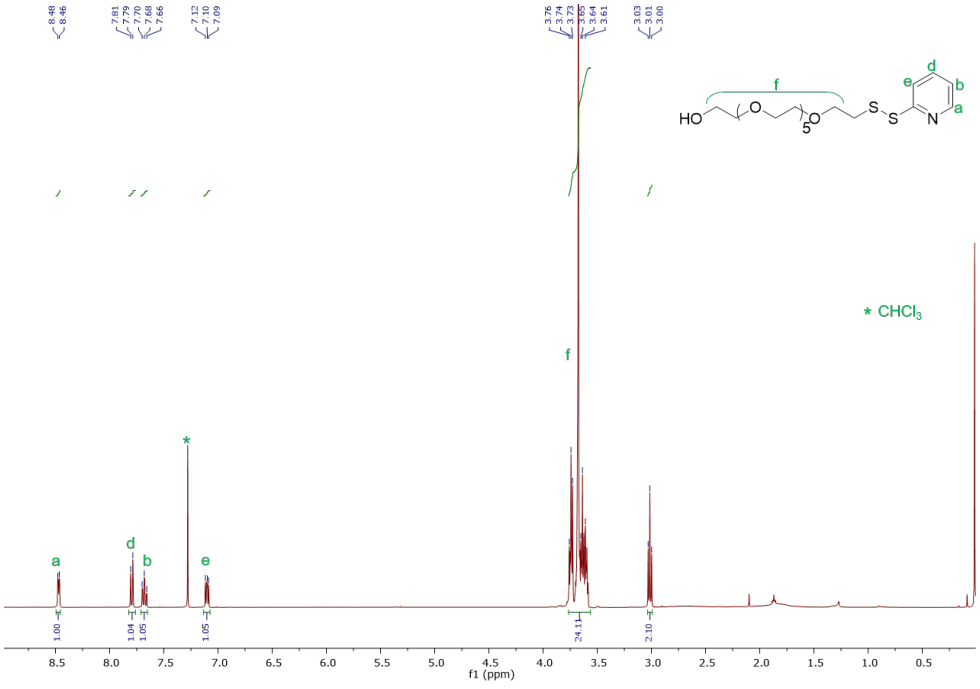



**Figure S45:** $^{1}H$ NMR (400 MHz) of 2-pyridyldithio-PEG5-alcohol in $CDCl_3$.

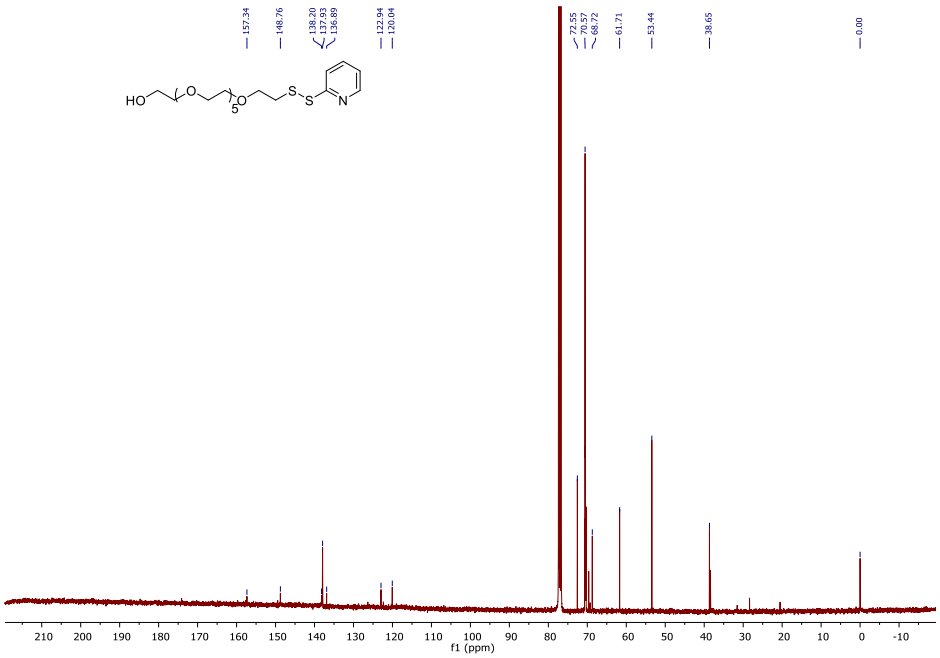



**Figure S46:** $^{13}C$ NMR (100 MHz) of 2-pyridyldithio-PEG5-alcohol in $CDCl_3$.

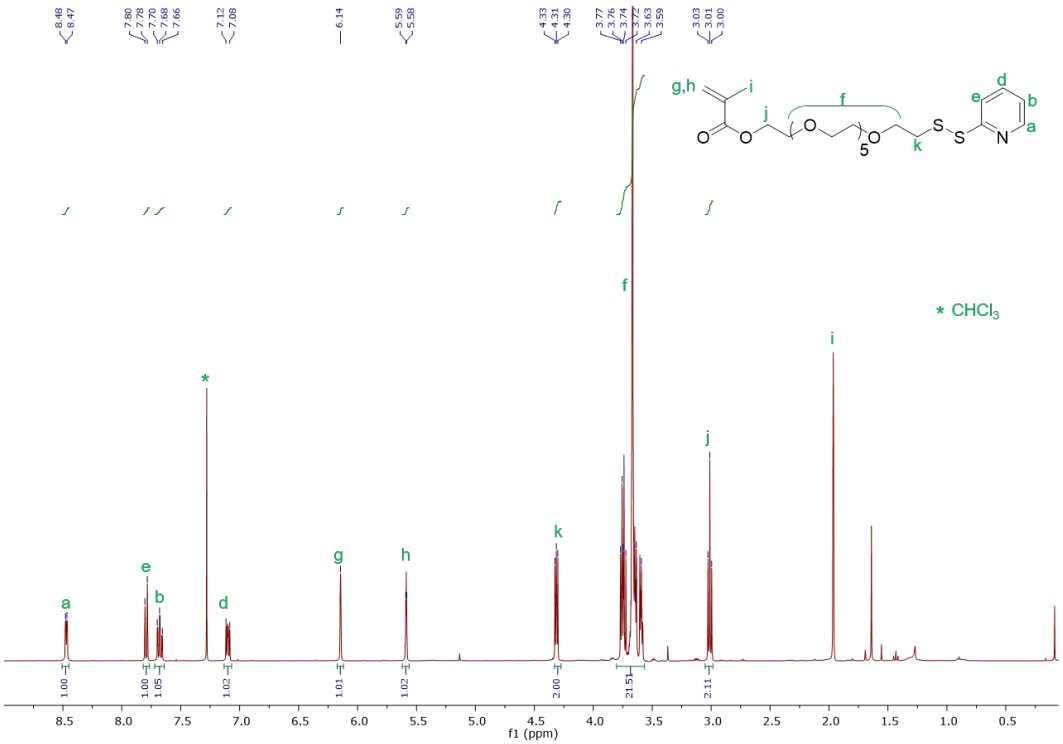



**Figure S47:** $^{1}H$ NMR (400 MHz) of pyridyl disulfide penta-ethylene glycol methyl methacrylate (PDEGMMA) in $CDCl_3$.

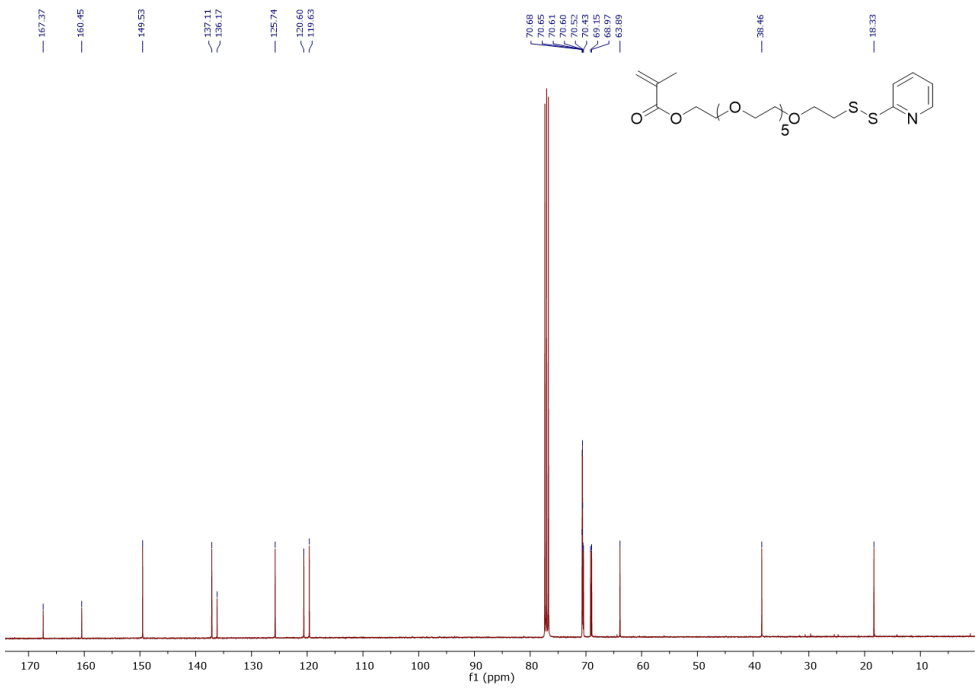


**Figure S48:** $^{13}$C NMR (100 MHz) of pyridyl disulfide penta-ethylene glycol methyl methacrylate (PDEGMMA) in $CDCl_3$.

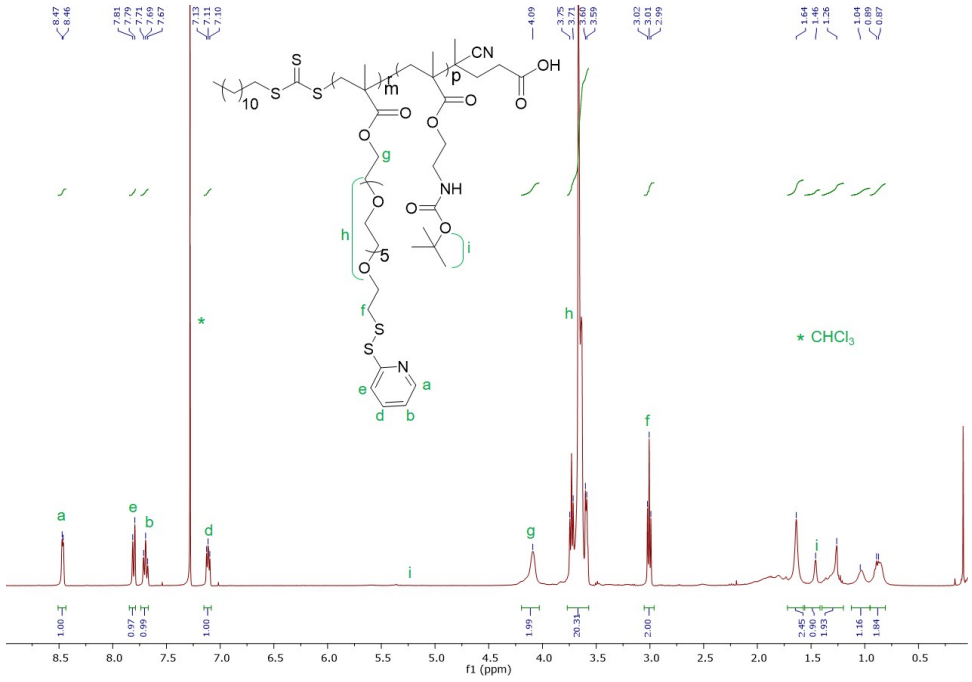


**Figure S49:** $^{1}$H NMR (400 MHz) p(PDEGMMA-co-AEMA) polymer (Boc-Protected) in $CDCl_3$.

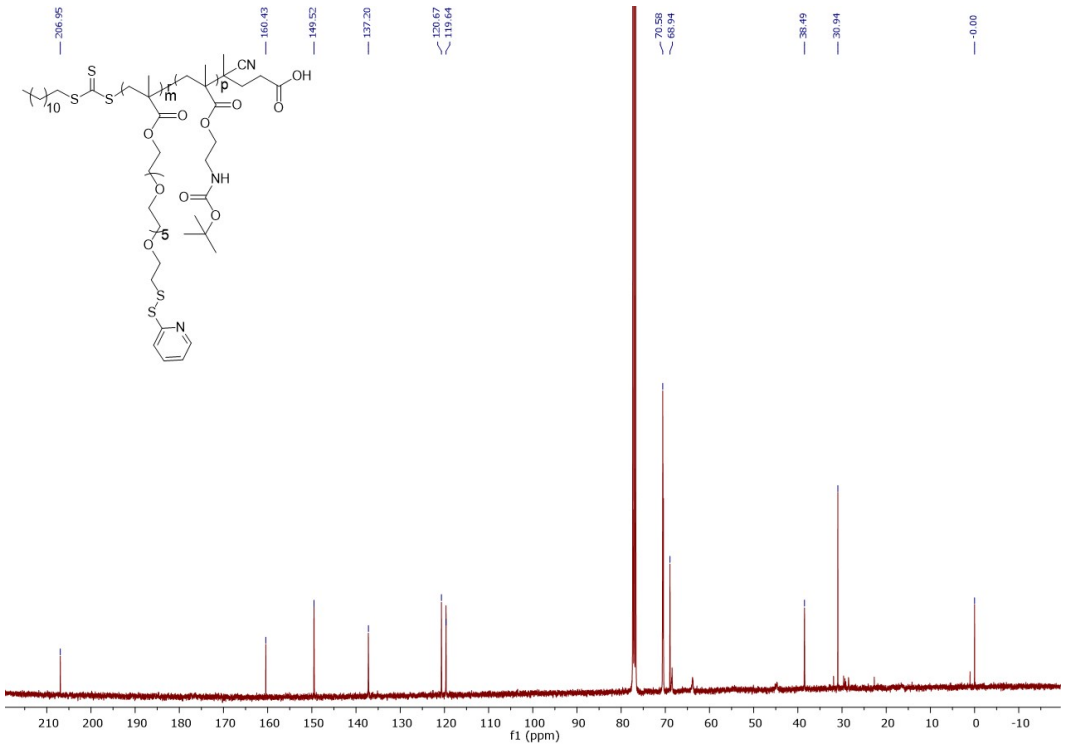



**Figure S450:** $^{13}C$ NMR (100 MHz) p(PDEGMMA-co-AEMA) polymer (Boc-Protected) in $CDCl_3$.

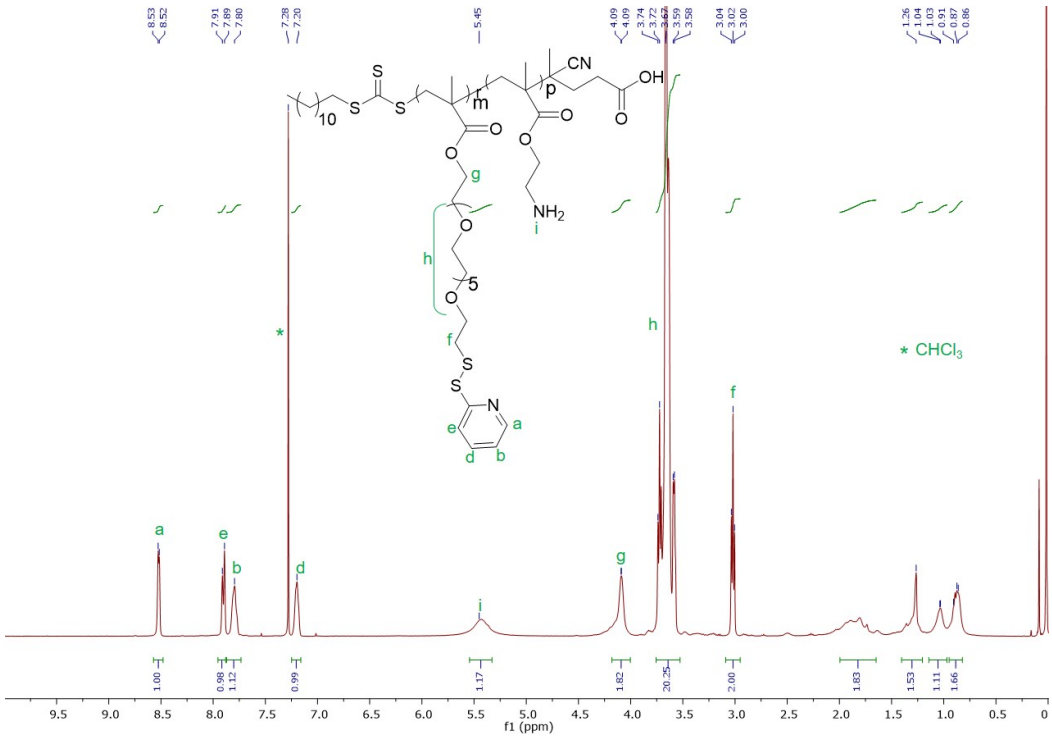



**Figure S51:** $^{1}H$ NMR (400 MHz) p(PDEGMMA-co-AEMA), $P_{PeriDS\text{-}NGs}$ polymer in $CDCl_3$.

## 11. References:


1. J.H. Ryu, R.T. Chako, S. Jiwpanich, S. Bickerton, R. P. Babu, S. Thayumanavan, *J. Am. Chem. Soc.* **2010**, *132*, 17227-17235.
2. J. Zhuang, S. Jiwpanich, V. D. Deepak, S. Thayumanavan, *ACS Macro Lett.* **2012**, *1*, 175-179.
3. L. Li, J. Ryu, S. Thayumanavan*, Langmuir* **2013,** *29,* 50-55.