SUPPORTING INFORMATION

Core-hydrophobicity of supramolecular nanoparticles induces NLRP3 inflammasome activation

Dipika Nandi[1,2], Manisha Shivrayan[3], Jingjing Gao[3], Jithu Krishna[3], Ritam Das[3], Bin Liu[3,6], S. Thanyumanavan[3,4,5]*, Ashish Kulkarni[1,2,3,4,5]*

[1]Department of Chemical Engineering, University of Massachusetts, Amherst, Massachusetts 01003, USA.

[2]Department of Veterinary and Animal Sciences, University of Massachusetts, Amherst, Massachusetts 01003, USA.

[3]Department of Chemistry, University of Massachusetts, Amherst, Massachusetts 01003, USA.

[4]Department of Biomedical Engineering, University of Massachusetts, Amherst, Massachusetts, 01003, USA

[5]Center for Bioactive Delivery, Institute for Applied Life Sciences, University of Massachusetts, Amherst, Massachusetts 01003, USA.

[6]Department of Chemistry, Massachusetts Institute of Technology, Cambridge, Massachusetts, 02139, USA

*Corresponding author: Email: akulkarni@engin.umass.edu; thai@umass.edu

Table of Contents

Scheme S1 – S3. Schematic illustration of synthetic approaches for different polymers.

Synthetic procedure for polymer constructs and their ^{1}H NMR and ^{13}C NMR characterization.

Figure S1. Chemical structure and morphology of hydrophobic series sNPs.

Figure S2. Size and ζ potential of R-PEG$_{5000}$-b-CM$_{10}$ polymer sNPs.

Figure S3. Size and ζ potential of NH$_2$-PEG$_{5000}$-b-CM$_{10}$-r-OTy polymer sNPs.

Figure S4. Stability studies for NH$_2$-PEG$_{5000}$-b-CM$_{10}$-r-OTy polymer sNPs.

Figure S5. IL-1β release quantification after 48 hours sNPs treatment.

Figure S6. Evaluation of cell death mediated by hydrophobic series sNPs, using MTT Assay.

Figure S7. IL-1β release by NLRP3 KO and caspase-1 KO cells treated with hydrophobic series sNPs.

Figure S8. Evaluation of dye leaching out from DiD encapsulated sNPs over time.

Figure S9. Evaluation of lysosomal rupture using acridine orange dye after treatment with core-hydrophobic series sNPs.

Figure S10. Schematics for estimation of intracellular calcium levels and mitochondrial ROS production in sNPs treated cells.

Figure S11. Quantification of intracellular Ca^{2+} levels and mitochondrial ROS production obtained via confocal microscopy.
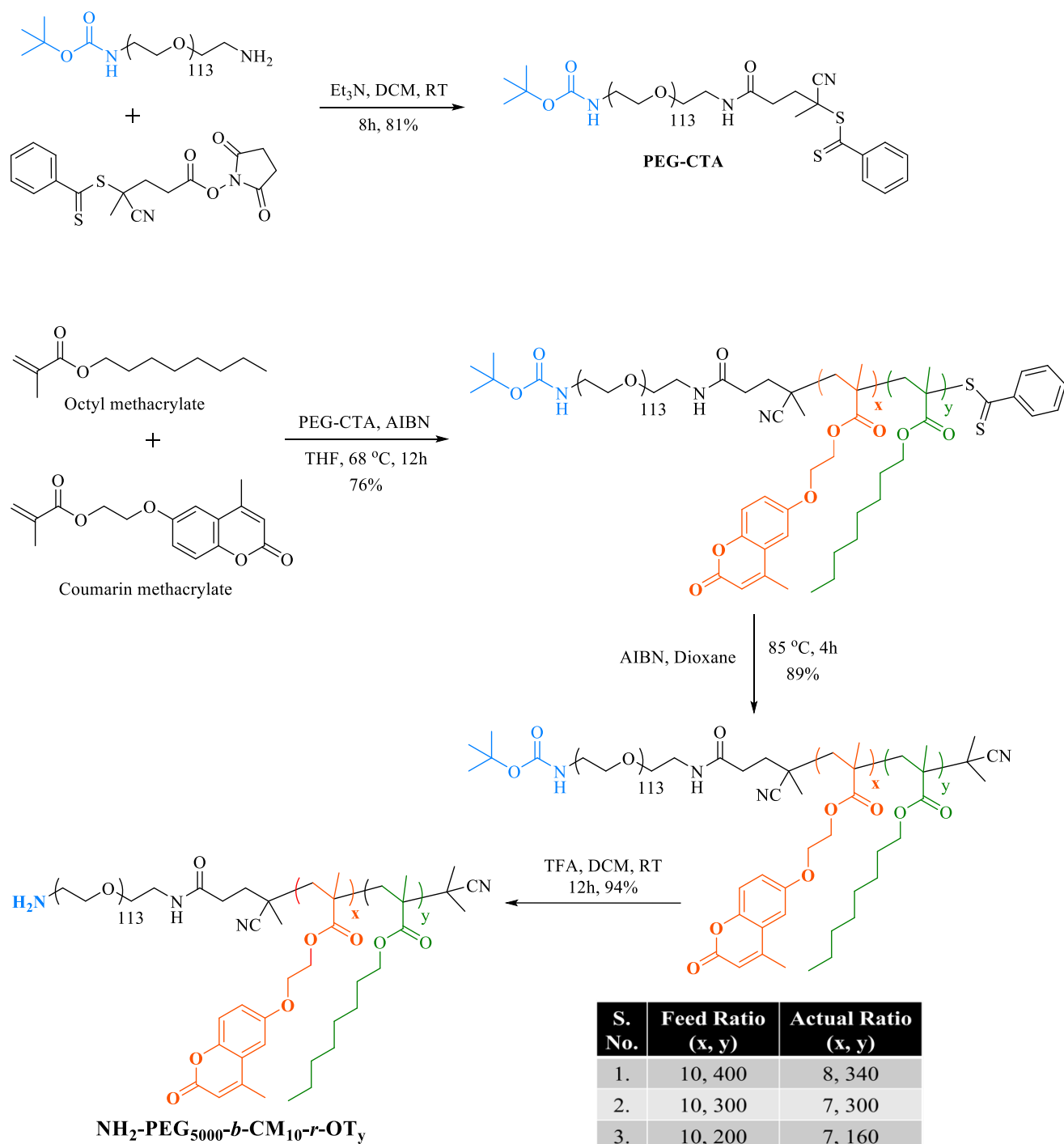
Supplemental Schemes
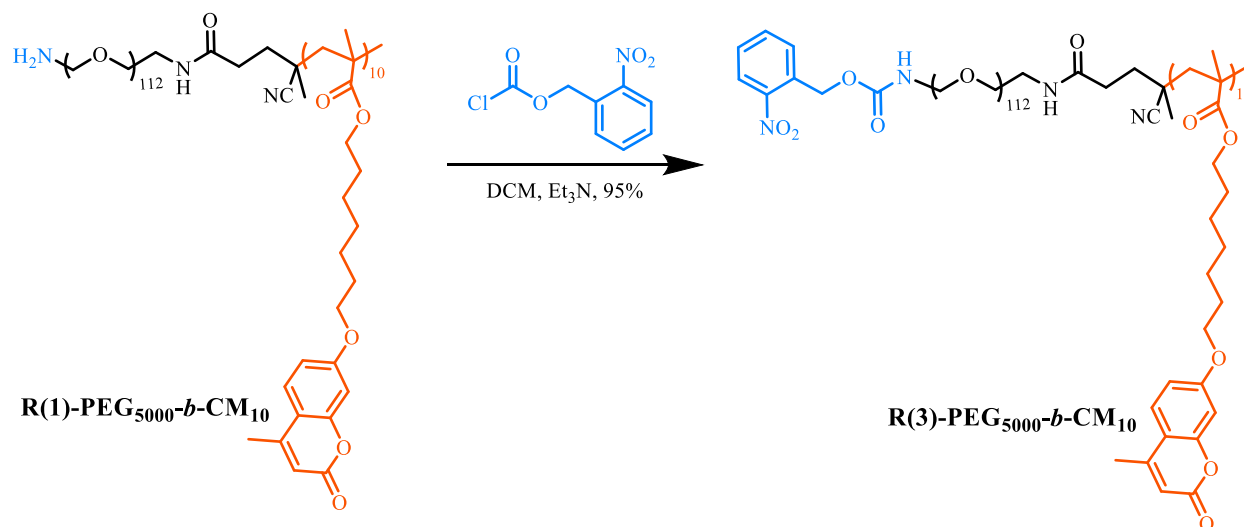
Scheme S1. Schematic illustration of synthetic approaches for R(3)-PEG$_{5000}$-*b*-CM$_{10}$ polymer construct (neutral charged).
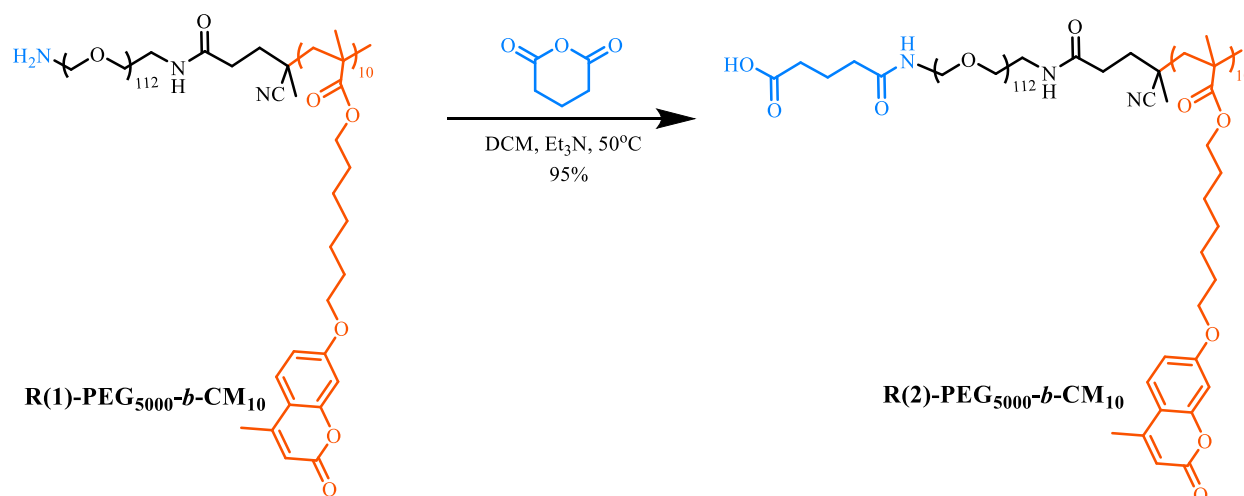
Scheme S2. Schematic representing synthetic approaches for R(2)-PEG$_{5000}$-*b*-CM$_{10}$ polymer construct (negatively charged).

Scheme S3. Synthetic approaches of precursors 1-4 for final NH_2-PEG_{5000}-b-CM_{10}-r-OT_y polymer construct.

The reaction scheme includes the following labels and conditions:

- Et_3N, DCM, RT, 8h, 81% → **PEG-CTA**
- Octyl methacrylate + Coumarin methacrylate, PEG-CTA, AIBN, THF, 68 °C, 12h, 76%
- AIBN, Dioxane, 85 °C, 4h, 89%
- TFA, DCM, RT, 12h, 94%
- NH_2-PEG_{5000}-b-CM_{10}-r-OT_y

S. No.	Feed Ratio (x, y)	Actual Ratio (x, y)
1.	10, 400	8, 340
2.	10, 300	7, 300
3.	10, 200	7, 160
4.	10, 100	9, 110
5.	10, 0	9, 0

4

Synthetic procedure for polymer constructs and their ^{1}H NMR and ^{13}C NMR characterization

Synthesis and characterization of R-PEG$_{5000}$-b-CM$_{10}$ polymer constructs.

R(4)-PEG$_{5000}$-b-CM$_{10}$ (hydrophobic patch) and R(1)-PEG$_{5000}$-b-CM$_{10}$ (positive charge) are synthesized according to previous procedure.(1)

Synthesis of R(2)-PEG$_{5000}$-b-CM$_{10}$ (negative charge), R(1)-PEG$_{5000}$-b-CM$_{10}$ (50 mg) was dissolved in 2 mL of dichloromethane with triethylamine (2 eq. per NH$_2$ group), then glutaric anhydride (2 eq. per NH$_2$ group) was added and the mixture was stirred at room temperature for overnight. The resulted solution was concentrated by Rotavap and precipitated three times in ether to afford R(2)-PEG$_{5000}$-b-CM$_{10}$ (95% yield).

GPC (THF): Mn= 8.2 K Da, Đ= 1.05. ^{1}H NMR (400 MHz, CDCl$_3$) δ 7.44, 6.80, 6.68, 6.06, 3.96, 3.83, 3.65, 3.55, 3.45, 3.11, 2.65, 2.59, 2.35, 1.81, 1.67, 1.46, 1.25, 1.05, 0.88. ^{13}C NMR (126 MHz, CDCl$_3$) δ 177.52, 177.24, 174.19, 172.96, 162.05, 161.20, 155.17, 152.68, 128.37, 126.56, 113.40, 112.54, 111.75, 101.16, 77.38, 77.06, 76.74, 70.54, 69.67, 68.36, 45.64, 45.08, 44.71, 39.47, 31.92, 31.03, 30.61, 29.70, 29.36, 28.97, 28.10, 25.96, 25.76, 18.65, 8.55.

Synthesis of polymer R(3)-PEG$_{5000}$-b-CM$_{10}$ (neutral charge): 2-nitrobenzyl alcohol (10 eq. per NH$_2$ of R(1)-PEG$_{5000}$-b-CM$_{10}$) was dissolved in dried THF and stir with argon protection at room temperature, triphosgene (15 wt% in toluene, 20 eq. per NH$_2$ of R(1)-PEG$_{5000}$-b-CM$_{10}$) was added to the mixture and stirred for 2 hours, then the solution was rotavaped to remove solvent and dried with vaccum pump for 3 hours to remove extra phosgene. The residue was redissolved in DCM and added to a solution of R(1)-PEG$_{5000}$-b-CM$_{10}$ (100 mg) and triethylamine (10 eq. per NH$_2$ of R(1)-PEG$_{5000}$-b-CM$_{10}$), the solution was stirred at room temperature for 8 hours and then dialyzed against DCM/MeOH to get purified R(4)-PEG$_{5000}$-b-CM$_{10}$ (95% yield).

GPC (THF): Mn= 8.6 K Da, Đ= 1.05. ^{1}H NMR (400 MHz, CDCl$_3$) δ 8.07, 7.62, 7.43, 7.26, 6.79, 6.68, 6.06, 5.51, 4.97, 3.96, 3.81, 3.63, 3.47, 3.45, 2.36, 1.97, 1.80, 1.66, 1.45, 1.24, 1.05, 0.87. ^{13}C NMR (101 MHz, CDCl$_3$) δ 166.30, 162.06, 161.18, 155.19, 152.64, 145.96, 137.71, 128.35, 127.82, 126.64, 125.55, 113.40, 112.53, 111.77, 101.17, 77.37, 77.05, 76.73, 70.56, 69.65, 68.36, 64.97, 45.61, 45.09, 44.68, 39.98, 29.70, 28.97, 28.11, 25.97, 25.76, 18.65.

General procedure for formulation of R-PEG$_{5000}$-b-CM$_{10}$ polymer sNPs:

To prepare nanoparticles, Polymers R(1)-PEG$_{5000}$-b-CM$_{10}$, R(2)-PEG$_{5000}$-b-CM$_{10}$, R(3)-PEG$_{5000}$-b-CM$_{10}$, and R(4)-PEG$_{5000}$-b-CM$_{10}$ (5 mg) were each dissolved in 100 µL acetone and 2 mL of H$_2$O was added dropwise. The solution was stirred overnight uncapped. The resulting micelle solution was then put into a UV-incubator with a 365 nm lamp for 3 minutes to generate the crosslinked nanoparticles.

Synthesis and characterization of NH₂-PEG₅₀₀₀-*b*-CM$_x$-*r*-OT$_y$ polymer constructs:

The general procedure for compound 1-4 has been published by our group for x = 10 and y = 400.(*2*) The same steps were followed for the synthesis here for variable x and y.

Synthesis of 1: 500 mg (0.001 mol) of t-BOC-NH-PEG-Amine, MW 5000 was taken in a round bottom flask and dissolved in 10 mL tetrahydrofuran under inert atmosphere. To this, triethylamine (101.19 mg, 0.01 mol) was added dropwise, and the solution was stirred for 15 minutes. Further, 4-Cyano-4-(phenylcarbonothioylthio)pentanoic acid N-succinimidyl ester (376.45 mg, 0.01 mol) was dissolved in 3 ml methylene chloride and added dropwise and the resultant solution was left to stir for 8h. The reaction mixture was concentrated in vacuo and dialyzed with 3500 MWCO dialysis tubing using methylene chloride and methanol for 24h. After dialysis, the solution was concentrated in vacuo to afford **1** in 81 % yield.

Synthesis of 2: To a Schlenk-flask, coumarin methacrylate (x equivalents), octyl methacrylate (y equivalents), recrystallized azodiisobutyronitrile (AIBN) (0.2 equivalent), and PEG-CTA **1** (1 equivalent) were mixed in anhydrous tetrahydrofuran. The solution mixture was subjected to three freeze-pump-thaw cycles. The sealed flask was immersed in a preheated oil bath at 78 °C and stirred for 12 h. The polymerization was quenched by cooling down the flask in ice water. The reaction mixture was concentrated and then dialyzed with 3500 MWCO dialysis tubing using methylene chloride and methanol for 48h. After dialysis, the polymer solution was concentrated in vacuo to afford **2**.

Synthesis of 3: Polymer **2** (1 equivalent) and AIBN (10 equivalent) were taken in a round bottom flask and dissolved in 1,4-dioxane under inert atmosphere. This reaction mixture was refluxed at 85 °C for 4h. Further, it was concentrated in vacuo and dialyzed with 3500 MWCO dialysis tubing using methylene chloride and methanol for 48h. After dialysis, the solution was concentrated in vacuo to afford **3**.

Synthesis of 4: Polymer **3** (1 equivalent) was taken in a round bottom flask and dissolved in methylene chloride under inert atmosphere. To the reaction mixture, trifluoroacetic acid (10 equivalent) was added dropwise and stirred overnight. Afterwards, it was concentrated in vacuo and dialyzed with 3500 MWCO dialysis tubing using methylene chloride and methanol for 24h. After dialysis, the solution was concentrated in vacuo to afford **4**.

1 (PEG-CTA) - ^{1}H NMR (CDCl₃, 400 MHz, TMS): δ (ppm) = 7.92-7.88 (m, 2H), 7.57-7.53 (m, 1H), 7.40-7.36 (m, 2H), 6.54 (s, 1H), 5.05 (s, 1H), 3.80-3.79 (m, 5H), 3.63-3.51 (m, 478 H), 3.46-3.44 (m, 7H), 3.30-3.29 (d, 3H), 2.84 (s, 1H), 2.63-2.52 (m, 3H), 2.45-2.42 (m, 1H), 1.93 (s, 3H), 1.43 (s, 9H). ^{13}C NMR (125 MHz, CDCl₃): δ (ppm) = 170.43, 156, 144.61, 132.97, 128.57, 126.68, 118.74, 107.40, 79.15, 70.58, 70.27, 69.68, 59.06, 46.13, 40.40, 39.48, 34.16, 31.63, 29.71, 28.44, 25.57, 24.15.

2 (x = 8, y = 340) - ^{1}H NMR (CDCl₃, 400 MHz, TMS): δ (ppm) = 7.86-7.84 (m, 2H), 7.54-7.52 (m, 11H), 6.96-6.93 (m, 8H), 6.82-6.80 (m, 7H), 6.15 (s, 8H), 4.32-4.22 (d, 50H), 3.91 (broad s,

691H), 3.64 (s, 478 H), 3.54-3.52 (m, 2H), 3.47-3.44 (m, 3H), 3.31-3.30 (d, 1H), 2.41 (s, 31H), 1.89-1.79 (m, 656H), 1.64-1.61 (m, 837H), 1.44-1.29 (m, 3579H), 1.02 (s, 402H), 0.89-0.88 (m, 1640H). ^{13}C NMR (125 MHz, CDCl$_3$): δ (ppm) = 178.15, 177.80, 177.46, 176.96, 176.95, 176.80, 176.74, 176.70, 161.37, 161.02, 155.27, 152.37, 125.72, 113.99, 112.84, 112.24, 101.13, 70.58, 65.01, 54.19, 45.14, 44.75, 31.86, 29.72, 29.26, 29.23, 28.44, 28.25, 28.16, 26.07, 22.67, 18.68, 18.38, 16.52, 14.13.

3 (x = 8, y = 340) - ^{1}H NMR (CDCl$_3$, 400 MHz, TMS): δ (ppm) = 7.54-7.52 (m, 10H), 7.00-6.91 (m, 8H), 6.82-6.80 (m, 7H), 6.15 (s, 8H), 4.32-4.23 (d, 63H), 3.91 (broad s, 701H), 3.64 (s, 478 H), 3.55-3.52 (m, 3H), 3.47-3.45 (m, 4H), 3.31-3.30 (d, 1H), 2.41 (s, 38H), 2.03-1.89 (m, 355H), 1.79 (s, 405H), 1.64-1.60 (m, 967H), 1.43-1.29 (m, 4135H), 1.01 (s, 487H), 0.89-0.88 (m, 1799H). ^{13}C NMR (125 MHz, CDCl$_3$): δ (ppm) = 177.80, 177.45, 176.78, 176.72, 161.37, 161.04, 155.27, 152.40, 125.71, 113.90, 112.25, 101.16, 70.58, 65.01, 54.24, 45.77, 45.14, 44.75, 31.86, 29.71, 29.26, 29.23, 29.21, 28.25, 28.16, 26.07, 22.67, 18.69, 18.35, 16.52, 14.13.

4 (x = 8, y = 340) NH$_2$-PEG$_{5000}$-*b*-CM$_8$-*r*-OT$_{340}$ - ^{1}H NMR (CDCl$_3$, 400 MHz, TMS): δ (ppm) = 7.83-7.78 (m, 1H), 7.61-7.52 (m, 10H), 7.00-6.91 (m, 7H), 6.82-6.75 (m, 6H), 6.15 (s, 5H), 4.32-4.22 (d, 63H), 3.91 (broad s, 705H), 3.64 (s, 478 H), 3.55-3.54 (m, 1H), 3.47-3.45 (m, 3H), 3.19 (s, 1H), 2.41 (s, 38H), 2.03-1.89 (m, 365H), 1.79 (s, 367H), 1.70 (s, 261H), 1.60 (s, 927H), 1.29-1.24 (m, 4172H), 1.01 (s, 506H), 0.89-0.88 (m, 1755H). ^{13}C NMR (125 MHz, CDCl$_3$): δ (ppm) = 177.80, 177.46, 176.76, 176.70, 161.34, 161.01, 155.27, 152.39, 113.89, 112.25, 101.01, 70.57, 65.01, 54.24, 45.14, 44.75, 31.86, 29.72, 29.26, 29.23, 29.22, 28.25, 28.16, 26.07, 22.67, 18.69, 18.36, 16.53, 14.14. GPC (THF): Mn = 50456 g mol^{-1}, PDI = 1.12.

2 (x = 7, y = 300) - ^{1}H NMR (CDCl$_3$, 400 MHz, TMS): δ (ppm) = 7.86-7.84 (m, 1H), 7.54-7.52 (m, 10H), 6.95-6.93 (m, 9H), 6.82-6.80 (m, 9H), 6.14 (s, 10H), 4.32-4.22 (d, 42H), 3.91 (broad s, 535H), 3.64 (s, 478 H), 3.54-3.52 (m, 1H), 3.47-3.44 (m, 2H), 3.31-3.29 (d, 1H), 2.41 (s, 30H), 1.93-1.88 (m, 216H), 1.79-1.74 (m, 306H), 1.60 (s, 551H), 1.43-1.29 (m, 2814H), 1.01 (s, 272H), 0.89-0.87 (m, 1329H). ^{13}C NMR (125 MHz, CDCl$_3$): δ (ppm) = 176.78, 176.43, 175.93, 175.92, 175.78, 175.76, 175.71, 175.69, 160.37, 160.02, 154.24, 151.36, 124.70, 112.88, 111.97, 111.22, 100.13, 69.55, 69.24, 64.69, 63.99, 61.72, 53.62, 53.18, 53.12, 44.75, 44.12, 43.73, 30.84, 28.24, 28.21, 28.18, 27.23, 27.13, 25.05, 21.65, 17.66, 17.32, 15.51, 13.11.

3 (x = 7, y = 300) - ^{1}H NMR (CDCl$_3$, 400 MHz, TMS): δ (ppm) = 7.56-7.54 (m, 7H), 6.98-6.95 (m, 8H), 6.84-6.82 (m, 8H), 6.17 (s, 6H), 4.34-4.24 (d, 47H), 3.93 (broad s, 561H), 3.66 (s, 478 H), 3.56-3.55 (m, 4H), 3.49-3.47 (m, 5H), 3.33-3.32 (d, 1H), 2.43 (s, 19H), 1.91 (s, 213H), 1.81 (s, 257), 1.74-1.72 (m, 68H), 1.62-1.56 (m, 569H), 1.31 (s, 2921H), 1.03 (s, 278H), 0.91-0.89 (m, 1452H). ^{13}C NMR (125 MHz, CDCl$_3$): δ (ppm) = 176.78, 176.44, 175.76, 175.67, 160.34, 160.02, 154.24, 151.35, 124.70, 121.08, 112.88, 111.18, 88.11, 81.37, 69.55, 69.24, 63.99, 53.61, 53.25, 53.19, 44.75, 44.12, 43.73, 30.84, 30.67, 28.24, 28.21, 28.18, 27.22, 27.13, 25.05, 21.65, 17.66, 17.31, 15.51, 13.11.

4 (x = 7, y = 300) NH$_2$-PEG$_{5000}$-*b*-CM$_7$-*r*-OT$_{300}$ - ^{1}H NMR (CDCl$_3$, 400 MHz, TMS): δ (ppm) = 7.83 (s, 1H), 7.55-7.52 (m, 8H), 6.96-6.93 (m, 9H), 6.80-6.75 (m, 8H), 6.15 (s, 6H), 4.32-4.22 (d, 43H), 3.91 (broad s, 600H), 3.65-3.64 (m, 478 H), 3.55-3.54 (m, 2H), 3.47-3.45 (m, 5H), 3.18 (s, 1H), 2.41 (s, 21H), 1.89 (s, 236H), 1.79 (s, 362H), 1.60 (s, 600H), 1.29 (s, 3099H), 1.01 (s, 306H),

0.89-0.87 (m, 1482H). ^{13}C NMR (125 MHz, CDCl$_3$): δ (ppm) = 177.81, 177.46, 176.75, 176.69, 161.46, 161.02, 155.26, 154.57, 135.04, 112.22, 101.14, 72.54, 70.56, 70.18, 65.01, 61.60, 54.65, 54.41, 54.22, 45.77, 45.14, 44.75, 31.86, 29.71, 29.26, 29.23, 29.20, 28.25, 28.16, 26.07, 22.67, 18.68, 18.43, 18.35, 16.50, 14.13. GPC (THF): Mn = 45501 g mol^{-1}, PDI = 1.3.

2 (x = 7, y = 160) - ^{1}H NMR (CDCl$_3$, 400 MHz, TMS): δ (ppm) = 7.85-7.83 (m, 1H), 7.53-7.51 (m, 9H), 6.95-6.92 (m, 9H), 6.81-6.74 (m, 9H), 6.13 (s, 9H), 4.31-4.21 (d, 38H), 3.90 (broad s, 355H), 3.63 (s, 478 H), 3.53-3.51 (m, 2H), 3.46-3.43 (m, 3H), 3.30-3.29 (d, 1H), 2.40 (s, 25H), 1.89 (s, 174H), 1.78 (s, 157H), 1.59 (s, 345H), 1.42-1.24 (m, 1879H), 1.00 (s, 185H), 0.88-0.86 (m, 962H). ^{13}C NMR (125 MHz, CDCl$_3$): δ (ppm) = 178.15, 177.81, 177.46, 176.96, 176.80, 176.75, 175.69, 176.61, 161.39, 161.03, 155.26, 152.38, 125.72, 113.90, 112.99, 112.24, 101.15, 70.58, 70.26, 65.71, 65.01, 62.78, 54.63, 54.23, 45.77, 45.14, 44.75, 40.38, 31.86, 29.70, 29.26, 29.23, 29.20, 28.25, 28.16, 26.07, 22.67, 18.68, 16.52, 14.13.

3 (x = 7, y = 160) - ^{1}H NMR (CDCl$_3$, 400 MHz, TMS): δ (ppm) = 7.54-7.52 (m, 9H), 6.95-6.93 (m, 8H), 6.82-6.80 (m, 9H), 6.15 (s, 7H), 4.32-4.22 (d, 39H), 3.91 (broad s, 378H), 3.64 (s, 478 H), 3.54-3.53 (m, 4H), 3.47-3.44 (m, 3H), 3.31-3.30 (d, 1H), 2.41 (s, 26H), 1.89 (s, 153H), 1.79-1.71 (m, 254H), 1.61-1.53 (m, 394H), 1.29 (s, 1984H), 1.01 (s, 198H), 0.89-0.87 (m, 933H). ^{13}C NMR (125 MHz, CDCl$_3$): δ (ppm) = 177.80, 177.46, 176.80, 176.75, 161.39, 161.04, 155.26, 152.39, 125.70, 113.91, 113.08, 112.25, 101.13, 84.05, 70.58, 70.26, 65.71, 65.01, 54.63, 52.22, 45.77, 45.14, 44.75, 31.86, 29.26, 29.23, 29.20, 28.25, 28.16, 26.07, 22.67, 18.68, 18.48, 18.34, 16.53, 14.13.

4 (x = 7, y = 160) NH$_2$-PEG$_{5000}$-*b*-CM$_7$-*r*-OT$_{160}$ - ^{1}H NMR (CDCl$_3$, 400 MHz, TMS): δ (ppm) = 7.92 (s, 1H), 7.56-7.54 (m, 7H), 6.98-6.96 (m, 7H), 6.82-6.77 (m, 7H), 6.17 (s, 6H), 4.34-4.25 (d, 35H), 3.93 (broad s, 319H), 3.67-3.66 (m, 478 H), 3.57-3.56 (m, 2H), 3.50-3.47 (m, 3H), 3.21 (s, 1H), 2.43 (s, 21H), 1.91 (s, 176H), 1.81 (s, 154H), 1.62 (s, 321H), 1.31 (s, 1621H), 1.04 (s, 160H), 0.91-0.90 (m, 765H). ^{13}C NMR (125 MHz, CDCl$_3$): δ (ppm) = 177.81, 177.46, 176.95, 176.75, 161.39, 161.03, 155.26, 152.38, 125.72, 113.90, 112.24, 101.16, 72.58, 70.57, 70.23, 65.70, 65.01, 61.65, 54.66, 54.28, 54.19, 45.77, 45.14, 44.75, 31.86, 31.69, 29.71, 29.26, 29.23, 29.21, 28.25, 28.16, 26.07, 22.67, 18.68, 18.36, 16.53, 14.13. GPC (THF): Mn = 38399 g mol^{-1}, PDI = 1.32.

2 (x = 9, y = 110) - ^{1}H NMR (CDCl$_3$, 400 MHz, TMS): δ (ppm) = 7.85-7.84 (m, 1H), 7.51 (s, 11H), 6.95-6.93 (m, 9H), 6.79-6.74 (m, 9H), 6.13 (s, 10H), 4.31-4.22 (d, 40H), 3.90 (broad s, 208H), 3.63 (s, 478 H), 3.54-3.51 (m, 3H), 3.46-3.44 (m, 4H), 3.30-3.29 (d, 2H), 2.40 (s, 30H), 1.87 (s, 125H), 1.78 (s, 93H), 1.60 (s, 208H), 1.43-1.28 (m, 1087H), 1.01 (s, 115H), 0.88-0.87 (m, 525H). ^{13}C NMR (125 MHz, CDCl$_3$): δ (ppm) = 178.15, 177.81, 177.46, 176.96, 176.80, 176.75, 175.69, 176.61, 161.39, 161.03, 155.26, 152.38, 125.72, 113.90, 112.99, 112.24, 101.15, 70.58, 70.26, 65.71, 65.01, 62.78, 54.63, 54.23, 45.77, 45.14, 44.75, 40.38, 31.86, 29.70, 29.26, 29.23, 29.20, 28.25, 28.16, 26.07, 22.67, 18.68, 16.52, 14.13.

3 (x = 9, y = 110) - ^{1}H NMR (CDCl$_3$, 400 MHz, TMS): δ (ppm) = 7.52 (m, 9H), 6.95-6.93 (m, 8H), 6.82-6.80 (m, 9H), 6.14 (s, 7H), 4.31-4.22 (d, 44H), 3.91 (broad s, 224H), 3.64 (s, 478 H), 3.54-3.53 (m, 1H), 3.47-3.44 (m, 3H), 3.31-3.29 (d, 1H), 2.40 (s, 30H), 1.89 (s, 103H), 1.78 (s, 151H), 1.60-1.53 (m, 231H), 1.43-1.28 (m, 1138H), 1.01 (s, 117H), 0.89 (s, 535H). ^{13}C NMR (125 MHz, CDCl$_3$): δ (ppm) = 177.80, 177.46, 176.81, 176.68, 161.39, 161.01, 155.26, 152.39, 125.80,

125.73, 113.90, 113.07, 112.24, 72.61, 70.58, 70.26, 65.69, 65.01, 62.79, 54.63, 54.16, 45.14, 44.75, 39.35, 31.86, 29.26, 29.23, 29.20, 28.25, 28.15, 28.05, 26.07, 22.67, 18.68, 16.50, 14.13.

4 (x = 9, y = 110) NH$_2$-PEG$_{5000}$-b-CM$_9$-r-OT$_{110}$ - ^{1}H NMR (CDCl$_3$, 400 MHz, TMS): δ (ppm) = 7.95 (s, 2H), 7.53 (s, 9H), 6.94-6.91 (m, 9H), 6.81-6.76 (m, 9H), 6.15 (s, 8H), 4.32-4.23 (d, 44H), 3.92 (broad s, 227H), 3.64 (s, 478 H), 3.55-3.54 (m, 2H), 3.46-3.43 (m, 4H), 3.35-3.32 (d, 1H), 2.41 (s, 25H), 1.89 (s, 93H), 1.80 (s, 161H), 1.60 (s, 222H), 1.29 (s, 1159H), 1.02 (s, 122H), 0.89 (s, 586H). ^{13}C NMR (125 MHz, CDCl$_3$): δ (ppm) = 177.80, 177.46, 176.91, 176.74, 161.39, 161.03, 155.25, 152.38, 125.71, 113.90, 112.96, 112.22, 101.29, 72.57, 70.56, 70.18, 70.00, 69.86, 65.72, 65.02, 62.77, 61.60, 54.56, 54.22, 50.87, 45.78, 45.14, 44.75, 40.16, 31.86, 29.71, 29.26, 29.23, 29.20, 28.25, 28.15, 26.07, 22.67, 18.68, 16.66, 16.52, 14.13. GPC (THF): Mn = 24221 g mol^{-1}, PDI = 1.14.

2 (x = 9, y = 0) - ^{1}H NMR (CDCl$_3$, 400 MHz, TMS): δ (ppm) = 7.96-7.90 (m, 1H), 7.37 (s, 10H), 6.79-6.75 (m, 10H), 6.66-6.61 (m, 9H), 6.02 (s, 9H), 4.29-4.19 (d, 35H), 3.81-3.79 (m, 3H), 3.63 (s, 478 H), 3.53-3.51 (m, 4H), 3.46-3.44 (m, 4H), 3.30-3.29 (d, 2H), 2.30 (s, 29H), 2.06 (s, 21H), 1.89 (s, 11H), 1.43 (s, 11H), 1.31-1.24 (m, 9H), 1.13 (s, 9H), 1.00 (s, 13H). ^{13}C NMR (125 MHz, CDCl$_3$): δ (ppm) = 177.60, 177.35, 177.30, 161.30, 161.17, 160.86, 156.03, 154.92, 152.52, 125.74, 113.77, 112.06, 107.38, 101.60, 70.56, 70.25, 69.74, 65.88, 65.72, 63.12, 62.91, 59.05, 45.07, 44.72, 40.37, 39.45, 39.36, 29.70, 28.44, 18.61, 16.65, 16.48.

3 (x = 9, y = 0) - ^{1}H NMR (CDCl$_3$, 400 MHz, TMS): δ (ppm) = 7.43 (s, 9H), 6.80 (s, 9H), 6.66-6.62 (m, 7H), 6.03 (s, 6H), 4.31-4.20 (d, 41H), 3.82-3.80 (m, 5H), 3.63 (s, 478 H), 3.54-3.53 (m, 4H), 3.47-3.44 (m, 6H), 3.31-3.29 (d, 2H), 2.31 (s, 25H), 1.92 (s, 44H), 1.43 (s, 9H), 1.32-1.24 (m, 10H), 1.13 (s, 10H), 0.98 (s, 16H). ^{13}C NMR (125 MHz, CDCl$_3$): δ (ppm) = 160.30, 160.16, 159.98, 154.05, 153.91, 151.60, 151.51, 124.76, 112.80, 111.22, 111.03, 100.63, 71.60, 70.22, 69.55, 69.28, 69.24, 67.85, 67.05, 65.26, 64.68, 62.03, 60.70, 47.48, 44.06, 43.69, 38.34, 28.69, 27.43, 27.29, 27.02, 17.61.

4 (x = 9, y = 0) NH$_2$-PEG$_{5000}$-b-CM$_9$-r-OT$_0$ - ^{1}H NMR (CDCl$_3$, 400 MHz, TMS): δ (ppm) = 7.95 (s, 1H), 7.40 (s, 9H), 6.80 (s, 9H), 6.62 (s, 8H), 6.03 (s, 6H), 4.29-4.20 (d, 39H), 3.81 (s, 13H), 3.73-3.60 (m, 478 H), 3.54 (s, 3H), 3.48-3.46 (m, 5H), 3.35-3.32 (m, 1H), 2.31 (s, 26H), 1.93-1.87 (m, 94H), 1.24 (s, 11H), 1.12 (s, 10H), 0.99-0.87 (m, 17H). ^{13}C NMR (125 MHz, CDCl$_3$): δ (ppm) = 161.23, 161.18, 154.96, 154.90, 152.60, 125.76, 113.85, 113.79, 112.08, 112.04, 101.57, 72.58, 70.56, 70.22, 69.86, 67.08, 61.64, 50.88, 44.71, 29.71, 29.51, 18.64. GPC (THF): Mn = 3974 g mol^{-1}, PDI = 1.55.

General procedure for formulation of NH$_2$-PEG$_{5000}$-b-CM$_x$-r-OT$_y$ polymer sNPs:

2 mg of polymer was dissolved in 150 μL acetone. 1 ml water was added to this solution dropwise and stirred overnight to obtain the polymer nanoparticles dispersed in water. These nanoparticles were concentrated via 3k MWCO centrifugal filters and were redispersed in PBS buffer and were utilized for further experiments.

Supplemental Figures

a.


b.

y=100   y=200   y=300   y=400
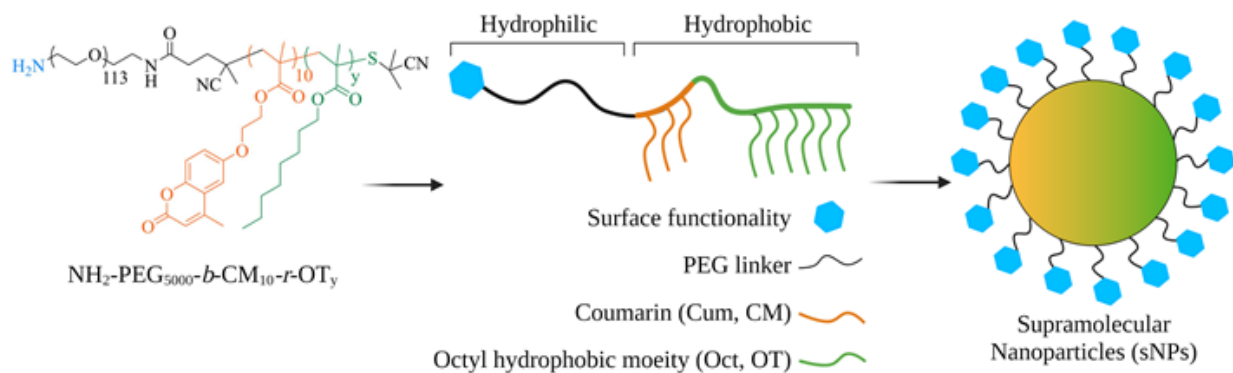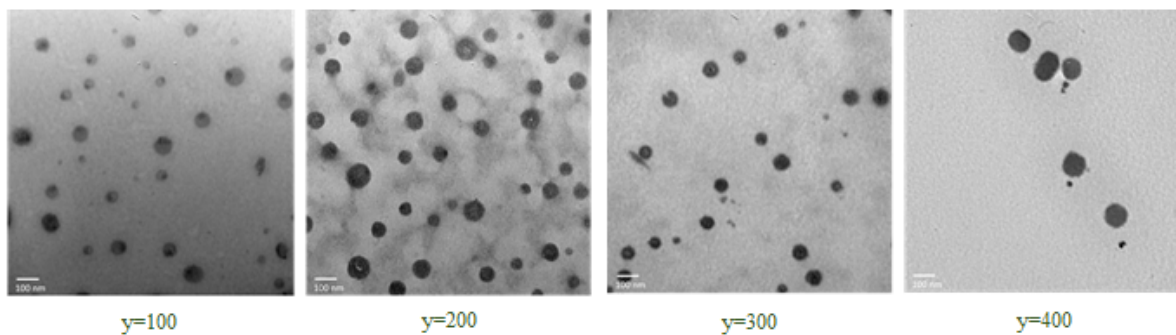
Figure S1. Chemical structure and morphology of hydrophobic series sNPs. (a) Schematic illustration of supramolecular nanoparticles (sNPs) assembling from NH_2-PEG_{5000}-b-CM_{10}-r-OT_y polymer. The figure displays the distribution of different polymer components (surface functional group, PEG linker, CM and OT) in the nanoparticle assembly. (b) TEM images of y=100, y=200, y=300 and y=400 hydrophobic series sNPs. Scale bar: 100 nm.

a.

b.
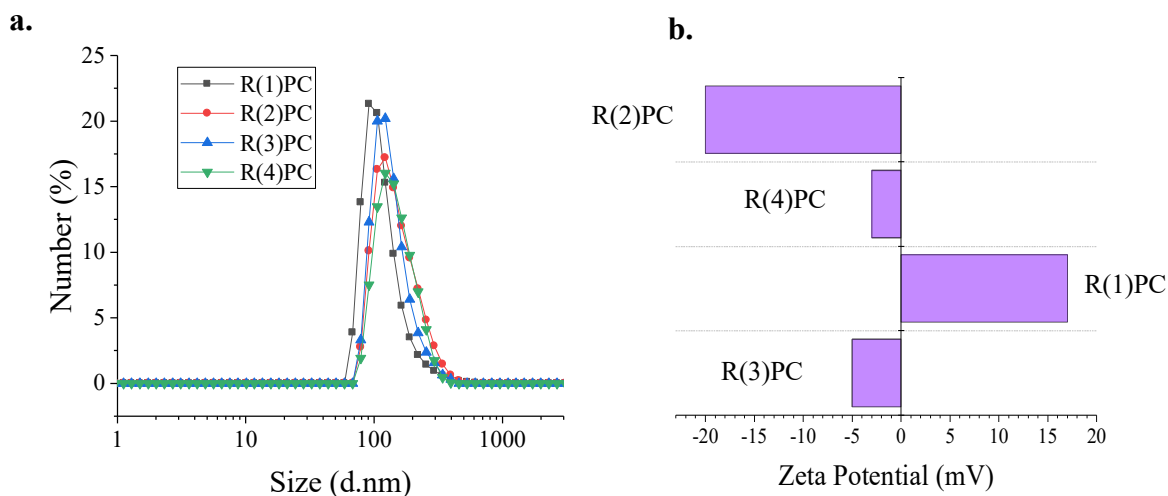
c.

| S.No.	Short forms	Polymer used to synthesize sNPs	NAMPs	Size (nm)	ζ Potential (mV)
1.	R(1)PC	R(1)-PEG$_{5000}$-*b*-CM$_{10}$	Positive surface	130	+17
2.	R(2)PC	R(2)-PEG$_{5000}$-*b*-CM$_{10}$	Negative surface	130	-20
3.	R(3)PC	R(3)-PEG$_{5000}$-*b*-CM$_{10}$	Neutral surface	130	-5
1.	R(1)PC	R(1)-PEG$_{5000}$-*b*-CM$_{10}$XL	Positive surface, rigid core	130	+17
2.	R(2)PC	R(2)-PEG$_{5000}$-*b*-CM$_{10}$XL	Negative surface, rigid core	130	-20
3.	R(3)PC	R(3)-PEG$_{5000}$-*b*-CM$_{10}$XL	Neutral surface, rigid core	130	-5
4.	R(4)PC	R(4)-PEG$_{5000}$-*b*-CM$_{10}$	Hydrophobic patch	130	-3

Figure S2. Characterization of R-PEG$_{5000}$-*b*-CM$_{10}$ polymer nanoparticles. (a) Graph showing hydrodynamic diameters of all R-PEG$_{5000}$-*b*-CM$_{10}$ polymer sNPs. (b) Graph depicting zeta potential of all R-PEG$_{5000}$-*b*-CM$_{10}$ polymer sNPs. (c) Table listing all the polymer constructs used to synthesize sNPs, varying NAMPs, size and zeta potential. Data shown here are mean (n =3).

a.

b.
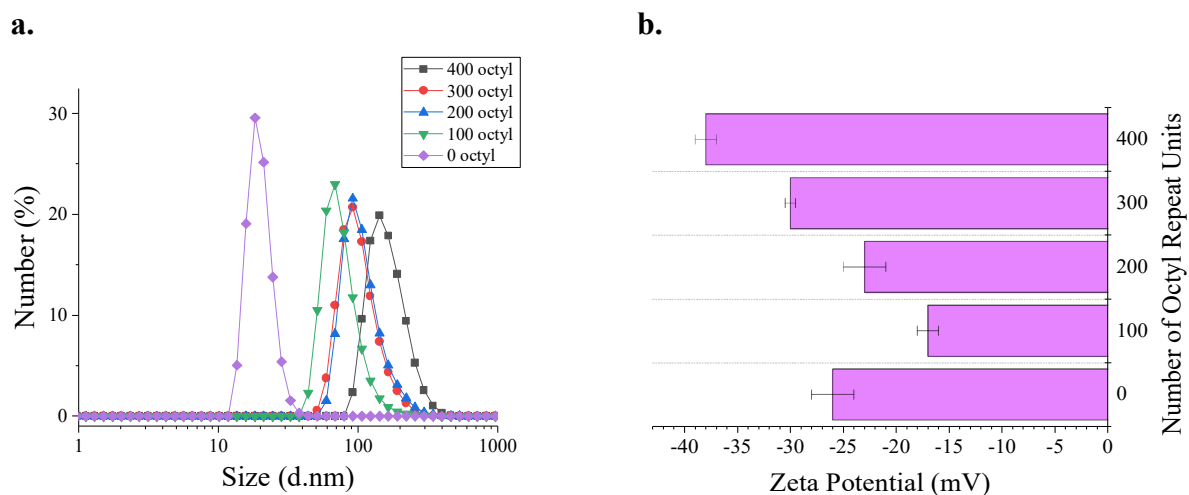
c.

S. No.	Number of Octyl Repeat Units, "y"	Polymer used to synthesize sNPs	Size (nm)	ζ Potential (mV)
1.	400	$NH_2\text{-}PEG_{5000}\text{-}b\text{-}CM_{10}\text{-}r\text{-}OT_{400}$	165	-38
2.	300	$NH_2\text{-}PEG_{5000}\text{-}b\text{-}CM_{10}\text{-}r\text{-}OT_{300}$	104	-30
3.	200	$NH_2\text{-}PEG_{5000}\text{-}b\text{-}CM_{10}\text{-}r\text{-}OT_{200}$	110	-23
4.	100	$NH_2\text{-}PEG_{5000}\text{-}b\text{-}CM_{10}\text{-}r\text{-}OT_{100}$	76	-17
5.	0	$NH_2\text{-}PEG_{5000}\text{-}b\text{-}CM_{10}\text{-}r\text{-}OT_{0}$	20	-26

Figure S3. Characterization of $NH_2\text{-}PEG_{5000}\text{-}b\text{-}CM_{10}\text{-}r\text{-}OTy$ polymer nanoparticles. (a) Size of sNPs varying in their core hydrophobicity from y=0 to y=400, revealed by DLS measurements. (b) Zeta potentials of $NH_2\text{-}PEG_{5000}$-b-CM_{10}-r-OTy polymer sNPs. (c) Table listing all the polymer constructs used to synthesize core hydrophobic series sNPs, varying in their octyl repeat units "y", size and zeta potential. Data shown here are mean ± S.D. (n =3).

a. NH₂-PEG₅₀₀₀-b-CM₁₀-r-OT₄₀₀

b. NH₂-PEG₅₀₀₀-b-CM₁₀-r-OT₃₀₀

c. NH₂-PEG₅₀₀₀-b-CM₁₀-r-OT₂₀₀

d. NH₂-PEG₅₀₀₀-b-CM₁₀-r-OT₁₀₀

e. NH₂-PEG₅₀₀₀-b-CM₁₀-r-OT₀
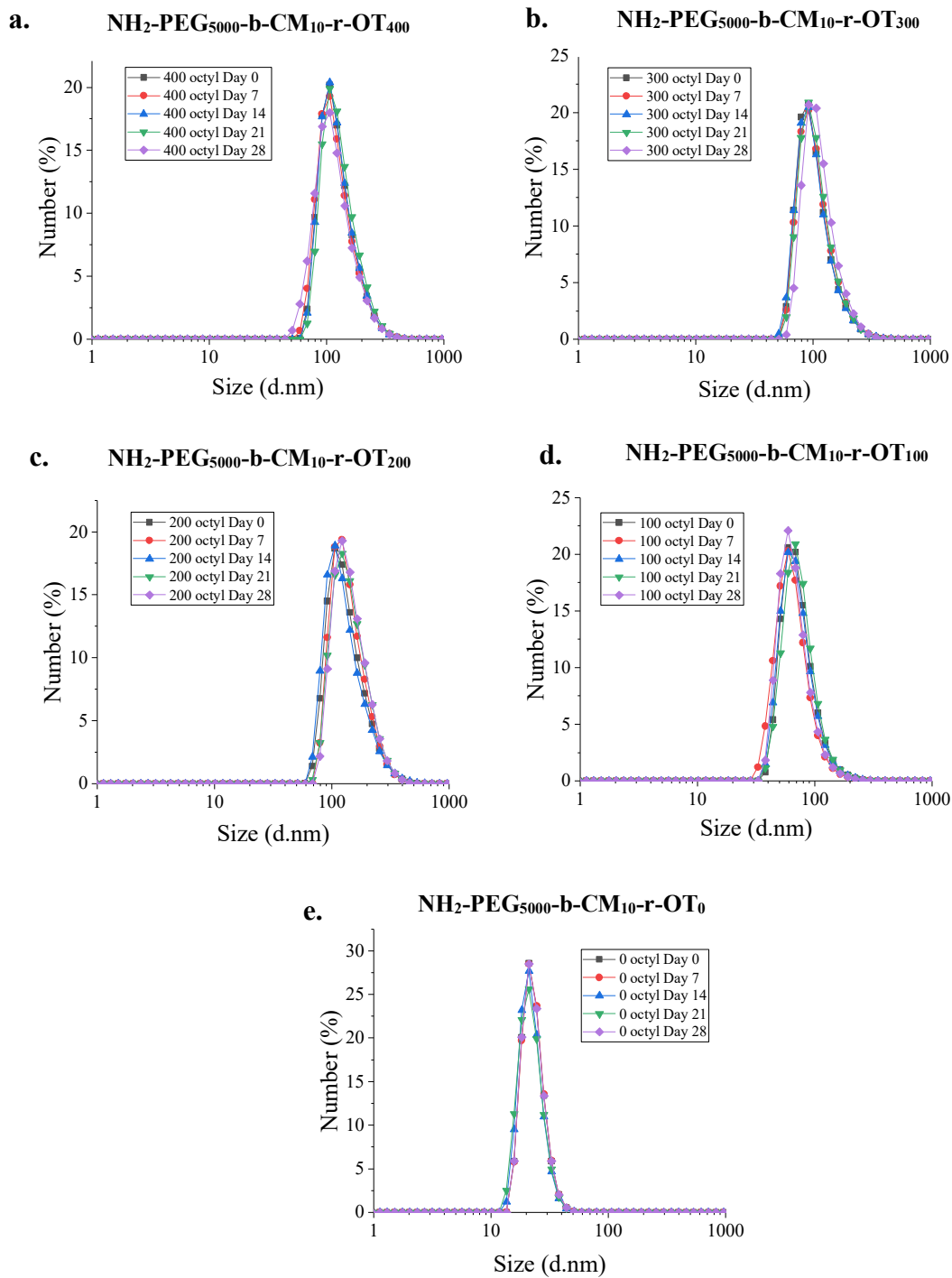
Figure S4. Stability studies for NH₂-PEG₅₀₀₀-b-CMx-r-OTy polymer nanoparticles. Size of all sNPs with varying core hydrophobicity y=0 – 400, measured at indicated days, for a period of about 1month. sNPs stored in PBS at 4°C. a, b, c, d, e represents DLS size plots for NH₂-PEG₅₀₀₀-*b*-CM₁₀-*r*-OT₄₀₀, NH₂-PEG₅₀₀₀-*b*-CM₁₀-*r*-OT₃₀₀, NH₂-PEG₅₀₀₀-*b*-CM₁₀-*r*-OT₂₀₀, NH₂-PEG₅₀₀₀-*b*-CM₁₀-*r*-OT₁₀₀ and NH₂-PEG₅₀₀₀-*b*-CM₁₀-*r*-OT₀, respectively. Readings were taken at Days 0, 7, 14, 21 and 28. Data shown here are mean (n =3).
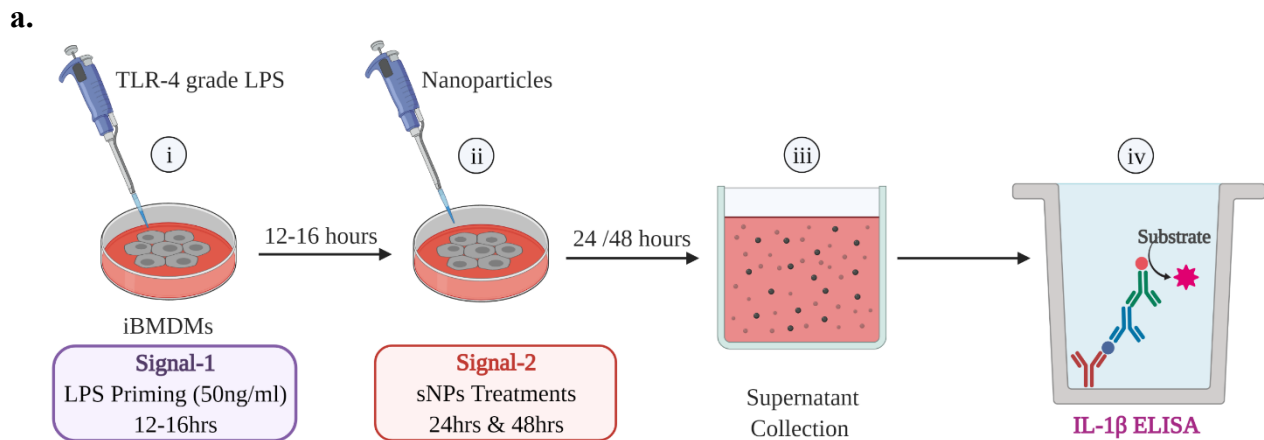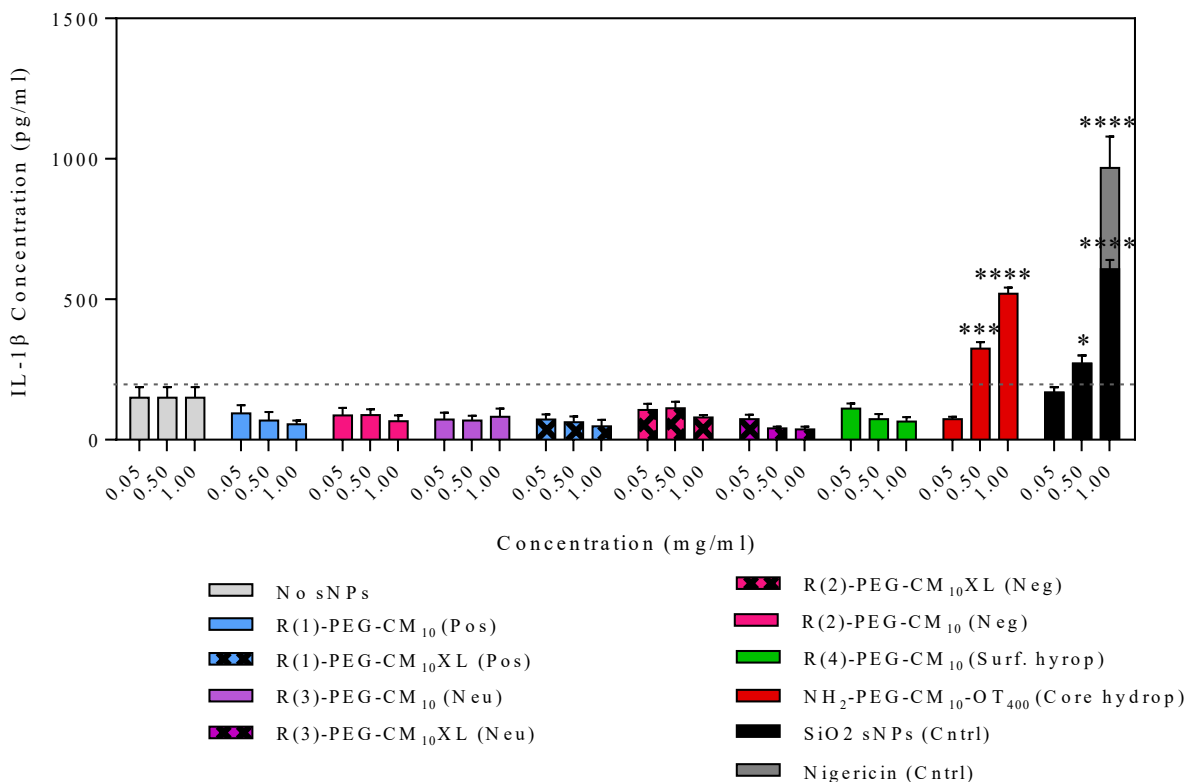
Figure S5. IL-1β release stimulated by 48hours sNPs treatment. (a) Schematic representation of IL-1β release quantification for screening four series of sNPs. Immortalized bone-marrow-derived macrophages (iBMDMs) were primed with TLR-grade LPS for 12-16 hours, following which nanoparticles were added in fresh medium. Supernatant were collected at 24 or 48 hours and then subjected to IL-1β ELISA for detection. **(b)** Quantification of IL-1β release by primed iBMDMs after 48hrs treatment with four series of sNPs in a concentration-dependent manner. Different concentrations tested were 0.05 mg/ml, 0.5 mg/ml, and 1mg/ml. Data shown are mean ± S.E.M. (n=3). Statistical significance was determined using Ordinary two-way ANOVA and Dunnett's multiple comparisons test. $*p < 0.05$, $**p<0.01$, $***p<0.001$, $****p<0.0001$.
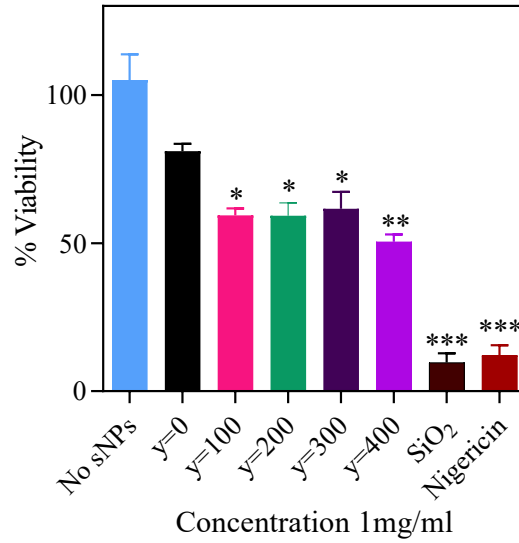
Figure S6. Evaluation of cell death mediated by hydrophobic series sNPs. Graph denotes percent viability of 1mg/ml sNPs treated iBMDMs using MTT assay to identify their pyroptosis level. Data shown are mean ± S.E.M. (n=6). Statistical significance was determined using Ordinary one-way ANOVA and Dunnett's multiple comparisons test. *p < 0.05, **p<0.01, ***p<0.001, ****p<0.0001.
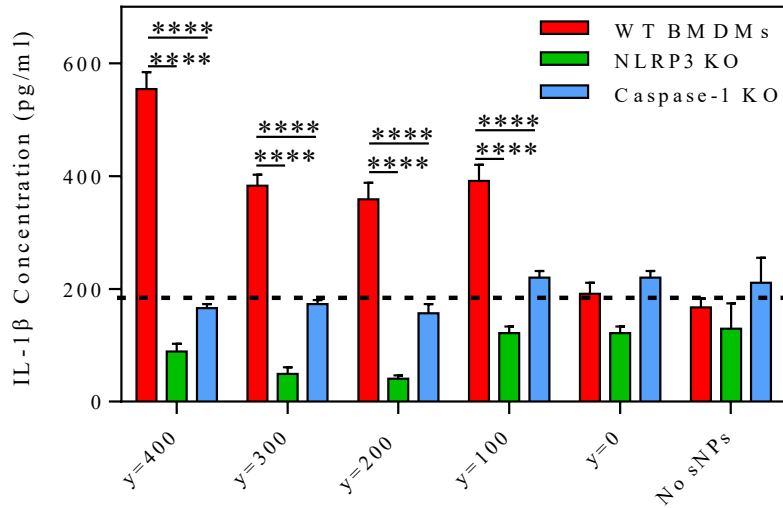
Figure S7. IL-1β release by NLRP3 KO and caspase-1 KO cells treated with hydrophobic series sNPs. Graph represents concentration of IL-1β released by LPS primed WT, NLRP3 KO and Caspase-1 KO iBMDMs incubated with 1 mg/mL hydrophobic series sNPs (y=0 to y=400) for 24 hours. Data shown are mean ± S.E.M. (n=6). Statistical significance was determined using Ordinary two-way ANOVA and Dunnett's multiple comparisons test. *p < 0.05, **p<0.01, ***p<0.001, ****p<0.0001.
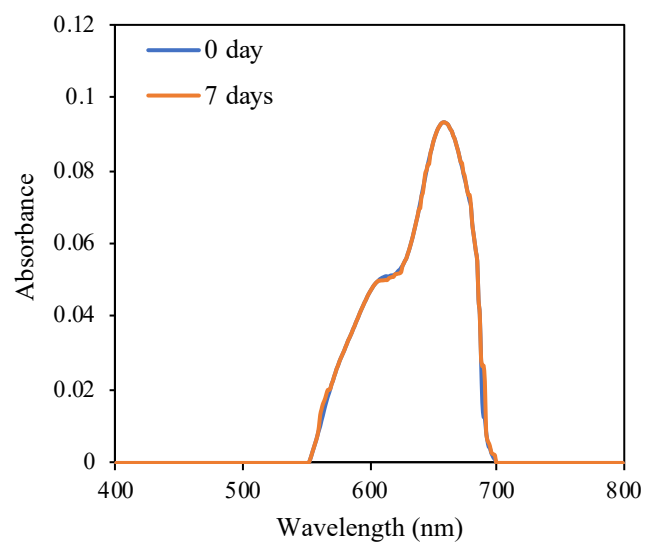
Figure S8. Evaluation of dye leaching out from DiD encapsulated sNPs over time. The graph represents absorption spectra of DiD loaded nanoparticles (y= 400) stored for 7 days.
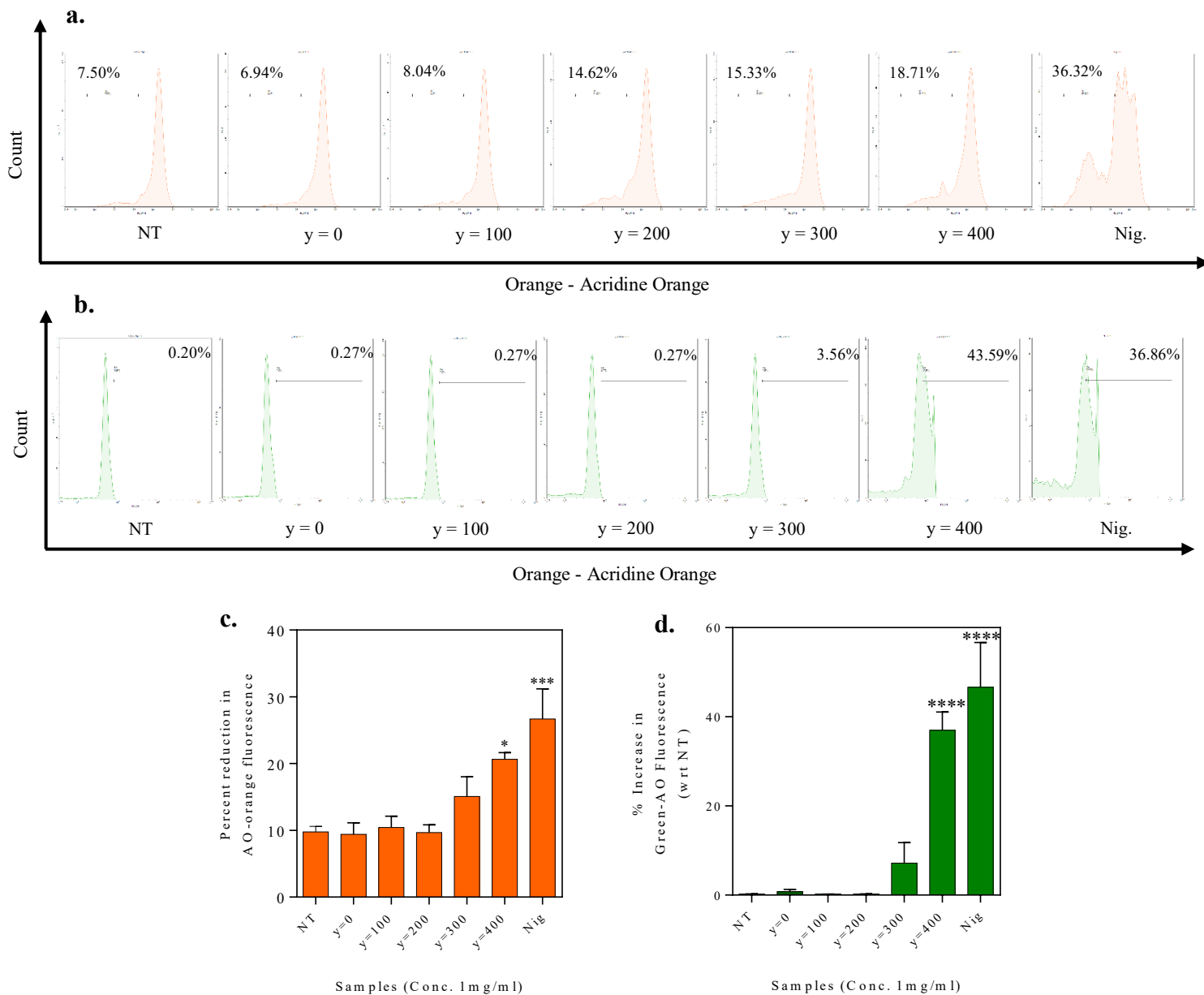
Figure S9. Evaluation of lysosomal rupture using acridine orange (AO) dye. (a) Representative flow cytometry histograms representing percent shift in AO-orange signal towards left with increasing core hydrophobic sNPs, y=0 to 400. NT: Non-treated, LPS only; Nig.: Nigericin treated positive control. (Left shift: decrease in fluorescence intensity) (b) Plots showing percent shift in AO-green signal towards the right with increasing core hydrophobic sNPs, y=0 to 400. NT: Non-treated, LPS only; Nig.: Nigericin treated positive control. (Right shift: increase in fluorescence intensity) (c) Quantitation of lysosomal rupture via percent reduced orange signal (orange and green) measured by flow cytometry in sNPs (y=0 to 400, hydrophobic series) treated iBMDMs. (d) Quantification of lysosomal rupture via percent increase in green signal of AO, measured by flow cytometry in sNPs (y=0 to 400, hydrophobic series) treated iBMDMs. Data shown in (c) and (d) are mean ± S.E.M. (n=3). Statistical significance was calculated by Ordinary one-way ANOVA and Dunnett's multiple comparisons test. 'ns', not significant; *p < 0.05, **p<0.01, ***p<0.001, ****p<0.0001.
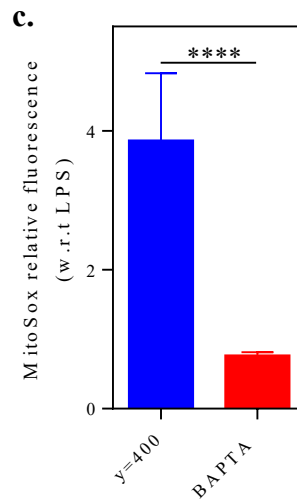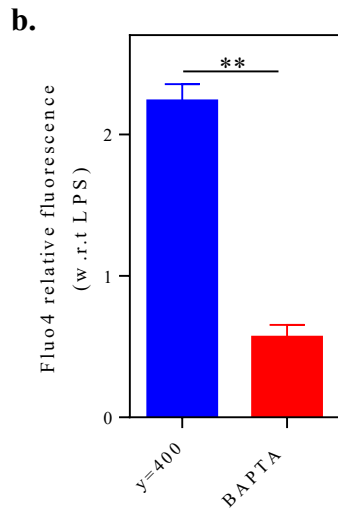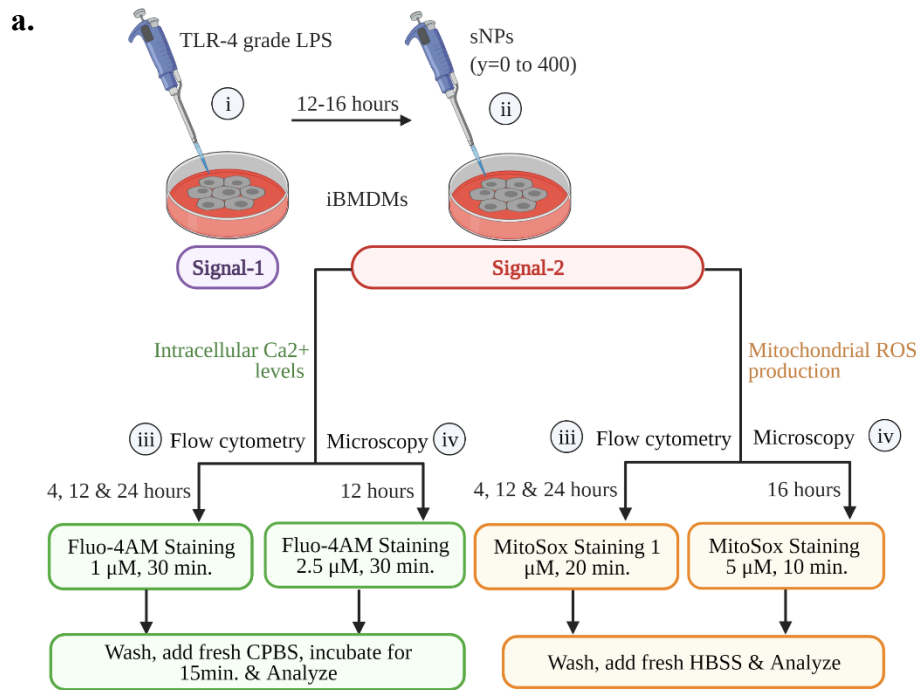
Figure S10. Schematics for estimation of intracellular calcium levels and mitochondrial ROS production in sNPs treated cells. (a) Schematics representation of treatments and fluo-4AM or mitosox staining of treated iBMDMs for flow and microscopy analysis at indicated time points. **(b)** Graph displays the effect of BAPTA inhibitor on Fluo-4 relative fluorescence intensity (due to calcium influx) generated in y=400 sNPs treated cells , measured by flow cytometry. **(c)** Graph represents inhibition response of BAPTA treatment on MitoSox relative fluorescence generated in y=400 sNPs treated cells due to mitochondrial ROS production. Data shown in (b) and (c) are mean ± S.E.M. (n=3). Statistical significance was calculated by Mann Whitney test; $*p < 0.05$, $**p<0.01$, $***p<0.001$, $****p<0.0001$.
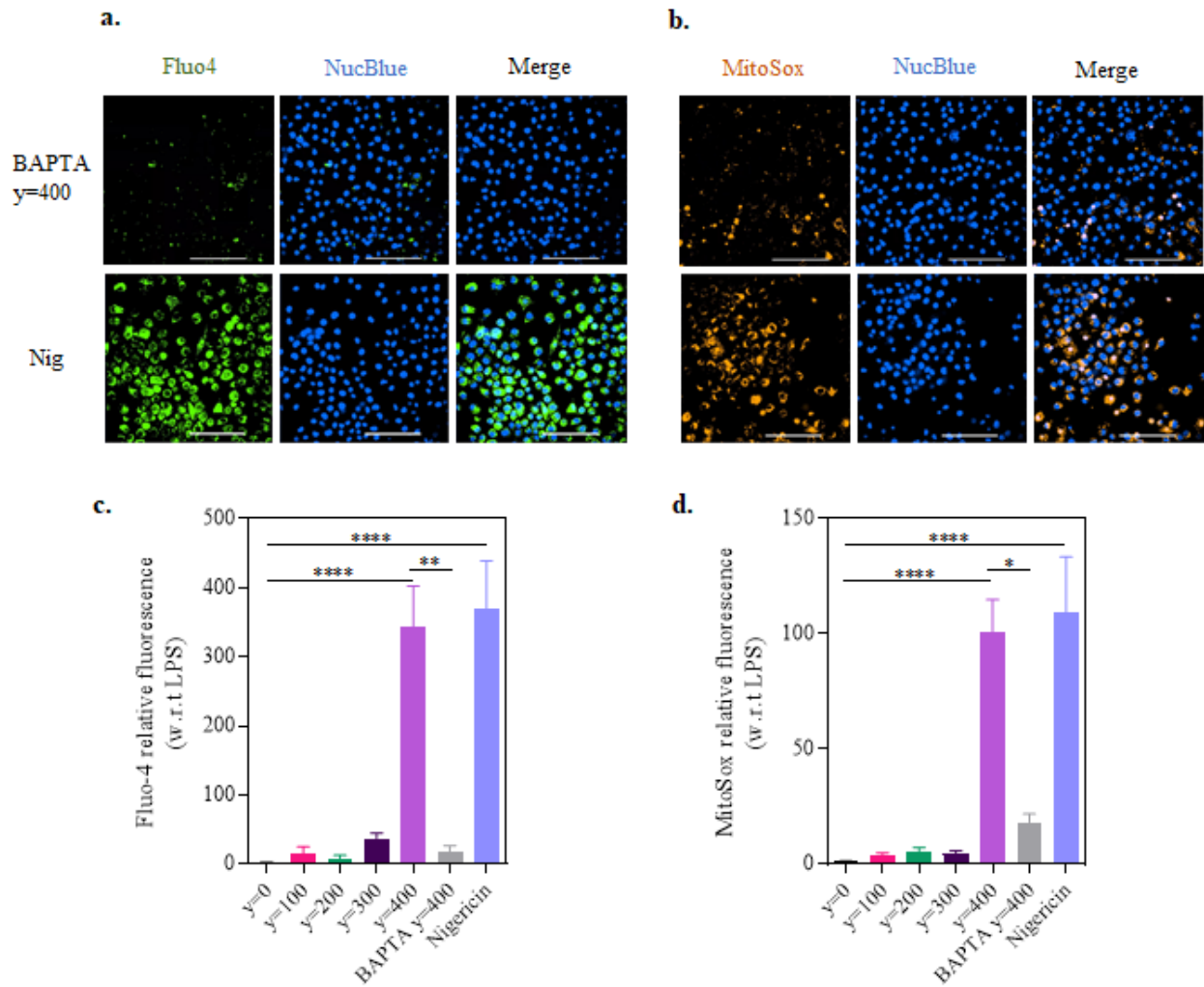
Figure S11. Quantification of intracellular Ca²⁺ levels and mitochondrial ROS production obtained via confocal microscopy. (a) Top part represents microscopic image of fluo-4AM stained iBMDMs treated with y=400 sNPs and incubated with BAPTA-AM inhibitor. Bottom part displays the image of fluo-4AM stained iBMDMs treated with nigericin (positive control). Nuclei were stained with NucBlue Live ReadyProbes Reagent. Green shows intracellular calcium and blue denotes live cell nucleus. Images are of 60x magnification (Scale bar: 100 μm). **(b)** Upper part represents confocal image of iBMDMs treated y=400 sNPs and incubated with BAPTA-AM inhibitor, followed by MitoSox and NucBlue staining. Lower part displays the image of MitoSox stained nigericin treated iBMDMs (positive control). MitoSox (Orange) represents mitochondrial ROS and NucBlue (Blue) shows live cell nucleus. Images are of 60x magnification (Scale bar: 100 μm).**(c)** Graph shows the quantification of intracellular Ca²⁺ in sNPs treated iBMDMs compared to untreated ones, as measured by relative fluo-4 fluorescence intensity at 12 hours, by fluorescence microscopy. **(d)** Graph representing relative mean fluorescence intensity in sNPs treated cells as compared to untreated (LPS-primed) at 16 hours time-point obtained via confocal microscopy. Data shown in (c) and (d) are mean ± S.E.M. (n=6). Statistical significance was calculated by Ordinary one-way ANOVA and Dunnett's multiple comparisons test. 'ns', not significant; *p < 0.05, **p<0.01, ***p<0.001, ****p<0.0001.

References

1. J. Gao, P. Wu, A. Fernandez, J. Zhuang, S. Thayumanavan, Cellular AND Gates: Synergistic Recognition to Boost Selective Uptake of Polymeric Nanoassemblies. *Angewandte Chemie International Edition* **59**, 10456-10460 (2020).

2. A. Fernandez, C. A. Zentner, M. Shivrayan, E. Samson, S. Savagatrup, J. Zhuang, T. M. Swager, S. Thayumanavan, Programmable Emulsions via Nucleophile-Induced Covalent Surfactant Modifications. *Chemistry of Materials* **32**, 4663-4671 (2020).