Supporting Information

Varying Hydrophobic Core Composition of Polymeric Nanoparticles Affects NLRP3 Inflammasome Activation

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Synthesis and Characterization of COOH-PEG$_{5000}$-b-HM$_{x,y}$ block copolymers

The typical synthetic protocol for the block copolymers has been outlined for the COOH-PEG$_{5000}$-b-HM$_{x,y}$, where x = 1 and y = 100, i.e, COOH-PEG$_{5000}$-b-BM$_{100}$. Similar procedure was followed for the other polymers with varying x = 1, 3, 5 and y = 100, 200, 400.

Synthesis of Macro chain transfer agent (CTA): In a round bottom flask, COOH-PEG$_{5000}$-NH$_2$ (500 mg, 0.1 mmol) was dissolved in 10 mL tetrahydrofuran followed by dropwise addition of triethylamine (101.19 mg, 1 mmol). The reaction mixture was stirred for 15 minutes under inert atmosphere. To this, a solution of 4-Cyano-4-(phenylcarbonothioylthio)pentanoic acid N-succinimidyl ester (376.45 mg, 1 mmol) in 5 mL dichloromethane was added dropwise. The reaction continued for 24 hours at room temperature and then concentrated in vacuo. The unreacted impurities were removed by dialyzing the resultant solution in a 3.5 kD membrane against dichloromethane/methanol (1:1) for 48 hours. Finally the macro **PEG-CTA** was yielded by concentration the solution in vacuo. Yield = 413 mg (83 %). ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm: 7.92 (t, 2.00 H), 7.56 (t, 1.00 H), 7.4 (m, 2.00 H), 6.4 (br s, 1.00 H), 4.16 (s, 2.00 H), 3.8 (m, 4.00 H), 3.76-3.56 (m, 505 H), 3.49 (m, 6.00 H), 2.57 (m, 3.00 H), 2.46 (m, 3.00 H), 1.95 (s, 3.00 H). ^{13}C NMR (101 MHz, $CDCl_3$) δ ppm: 170.4, 144.6, 133.0, 128.6, 126.7, 70.6, 70.3, 46.7, 34.8, 33.8, 25.8. GPC (DMF): Mn = 7800 g/mol, Mw = 8500 g/mol, Đ = 1.1

Synthesis of block copolymer COOH-PEG$_{5000}$-b-BM$_{100}$ (x = 1, y = 100): The PEG-CTA (50 mg, 0.0095 mmol), butyl methacrylate (135 mg, 0.95 mmol) and recrystallized azodiisobutyronitrile (AIBN) (0.31 mg, 0.0019 mmol) were dispersed in 0.4 mL anhydrous tetrahydrofuran in a Schlenk flask. This solution was subjected to five freeze-pump-thaw cycles followed by nitrogen purge to remove all the dissolved oxygen. The flask was then sealed and immersed in a preheated oil bath at 68 °C and continued stirring for 20 h. The flask was kept on ice to quench the polymerization. Further, the polymer was precipitated in ice-cold methanol, centrifuged at 4000 g for 20 min, decanted the supernatant and dried in vacuo to procure the block copolymer. Yield = 130 mg (70 %). ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm: 3.94 (br s, 207 H), 3.64 (s, 505 H), 1.90-1.81 (m, 196 H), 1.61 (br s, 250 H), 1.41 (s, 228 H), 1.03-0.87 (m, 605 H). ^{13}C NMR (101 MHz, $CDCl_3$) δ ppm: 177.9, 177.5, 176.8, 70.6, 64.7, 54.3, 45.1, 44.7, 30.9, 30.3, 30.2, 19.3, 18.3, 16.4, 13.8, 13.7.

COOH-PEG$_{5000}$-b-BM$_{200}$ (x = 1, y = 200): ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm: 3.95 (br s, 404 H), 3.64 (s, 505 H), 1.91-1.81 (m, 377 H), 1.61 (br s, 471 H), 1.41 (d, 434 H), 1.03-0.87 (m, 1248 H). ^{13}C NMR (101 MHz, $CDCl_3$) δ ppm: 176.8, 176.5, 175.8, 69.6, 63.7, 53.1, 51.3, 44.1, 43.7, 29.3, 29.2, 28.7, 18.3, 17.3, 15.4, 12.7, 12.7. GPC (THF): Mn = 30,200 g/mol, Mw = 36,200 g/mol, Đ = 1.2

COOH-PEG$_{5000}$-b-BM$_{400}$ (x = 1, y = 400): ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm: 3.95 (br s, 810 H), 3.64 (s, 505 H), 1.90-1.81 (m, 734 H), 1.61 (br s, 800 H), 1.41 (d, 877 H), 1.03-0.87 (m, 2439 H). ^{13}C NMR (101 MHz, $CDCl_3$) δ ppm: 176.8, 176.5, 175.8, 69.6, 63.7, 53.1, 44.1, 43.7, 29.9, 29.3, 29.2, 18.3, 17.3, 15.4, 12.7, 12.7. GPC (THF): Mn = 57,100 g/mol, Mw = 64,000 g/mol, Đ = 1.1

COOH-PEG$_{5000}$-b-OM$_{100}$ (x = 3, y = 100): ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm: 3.92 (br s, 207 H), 3.64 (s, 505 H), 1.89-1.80 (m, 185 H), 1.61 (br s, 230 H), 1.30 (br s, 1046 H), 1.02 (s, 103 H), 0.90 (m, 510 H). ^{13}C NMR (101 MHz, $CDCl_3$) δ ppm: 176.8, 176.4, 175.8, 69.6, 64.0, 44.1, 43.7, 30.8, 28.2, 28.2, 28.2, 27.2, 27.1, 25.0, 21.6, 15.4, 13.1.

COOH-PEG$_{5000}$-b-OM$_{200}$ (x = 3, y = 200): ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm: 3.92 (br s, 403 H), 3.64 (s, 505 H), 1.90-1.80 (m, 362 H), 1.61 (br s, 532 H), 1.30 (br s, 2155 H), 1.02 (s, 196 H), 0.90 (m, 992 H). ^{13}C NMR (101 MHz, $CDCl_3$) δ ppm: 176.8, 176.4, 175.7, 69.6, 64.0, 53.2, 44.1, 43.7, 30.8, 29.9, 28.2, 28.2, 28.2, 27.2, 27.1, 25.0, 21.6, 17.3, 15.5, 13.1. GPC (THF): Mn = 36,500 g/mol, Mw = 41,600 g/mol, Đ = 1.1

COOH-PEG$_{5000}$-b-OM$_{400}$ (x = 3, y = 400): ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm: 3.93 (br s, 807 H), 3.64 (s, 505 H), 1.89-1.80 (m, 744 H), 1.61 (br s, 969 H), 1.30 (br s, 4353 H), 1.02 (s, 405 H), 0.90 (m, 2001 H). ^{13}C NMR (101 MHz, $CDCl_3$) δ ppm: 176.7, 176.4, 175.7, 69.6, 64.8, 64.0, 44.1, 43.7, 30.8, 29.9, 28.2, 28.2, 28.2, 27.2, 27.1, 25.0, 21.6, 17.3, 15.5, 14.3, 13.1. GPC (THF): Mn = 51,100 g/mol, Mw = 64,000 g/mol, Đ = 1.3

COOH-PEG$_{5000}$-b-DM$_{100}$ (x = 5, y = 100): ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm: 3.92 (br s, 202 H), 3.64 (s, 505 H), 1.89-1.80 (m, 187 H), 1.61 (br s, 213 H), 1.27 (s, 2003 H), 1.02 (s, 101 H), 0.89 (m, 502 H). ^{13}C NMR (101 MHz, $CDCl_3$) δ ppm: 176.8, 176.5, 175.8, 69.6, 64.0, 63.8, 43.8, 30.9, 28.7, 28.6, 28.5, 28.4, 28.3, 28.2, 27.6, 27.2, 27.1, 25.1, 25.0, 21.7, 17.3, 13.1.

COOH-PEG$_{5000}$-b-DM$_{200}$ (x = 5, y = 200): ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm: 3.94 (br s, 409 H), 3.67 (s, 505 H), 1.91-1.82 (m, 380 H), 1.63 (br s, 460 H), 1.30 (s, 4200 H), 1.04-0.91 (m, 1303 H). ^{13}C NMR (101 MHz, $CDCl_3$) δ ppm: 176.8, 176.4, 175.8, 69.6, 64.0, 44.1, 43.8, 30.9, 28.7, 28.6, 28.4, 28.3, 27.6, 27.2, 27.1, 25.1, 21.7, 17.3, 15.5, 13.1. GPC (THF): Mn = 39,100 g/mol, Mw = 47,400 g/mol, Đ = 1.2

COOH-PEG$_{5000}$-b-DM$_{400}$ (x = 5, y = 400): ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm: 3.94 (br s, 807 H), 3.67 (s, 505 H), 1.91-1.81 (m, 767 H), 1.63 (br s, 811 H), 1.30 (s, 8121 H), 1.04-0.91 (m, 2561 H). ^{13}C NMR (101 MHz, $CDCl_3$) δ ppm: 176.8, 176.4, 175.8, 69.6, 64.0, 63.8, 44.1, 43.8, 30.9, 30.9, 28.7, 28.6, 28.5, 28.4, 28.3, 28.2, 27.6, 27.2, 27.1, 25.1, 25.0, 21.7, 17.3, 15.5, 13.1. GPC (THF): Mn = 82,300 g/mol, Mw = 94,100 g/mol, Đ = 1.1

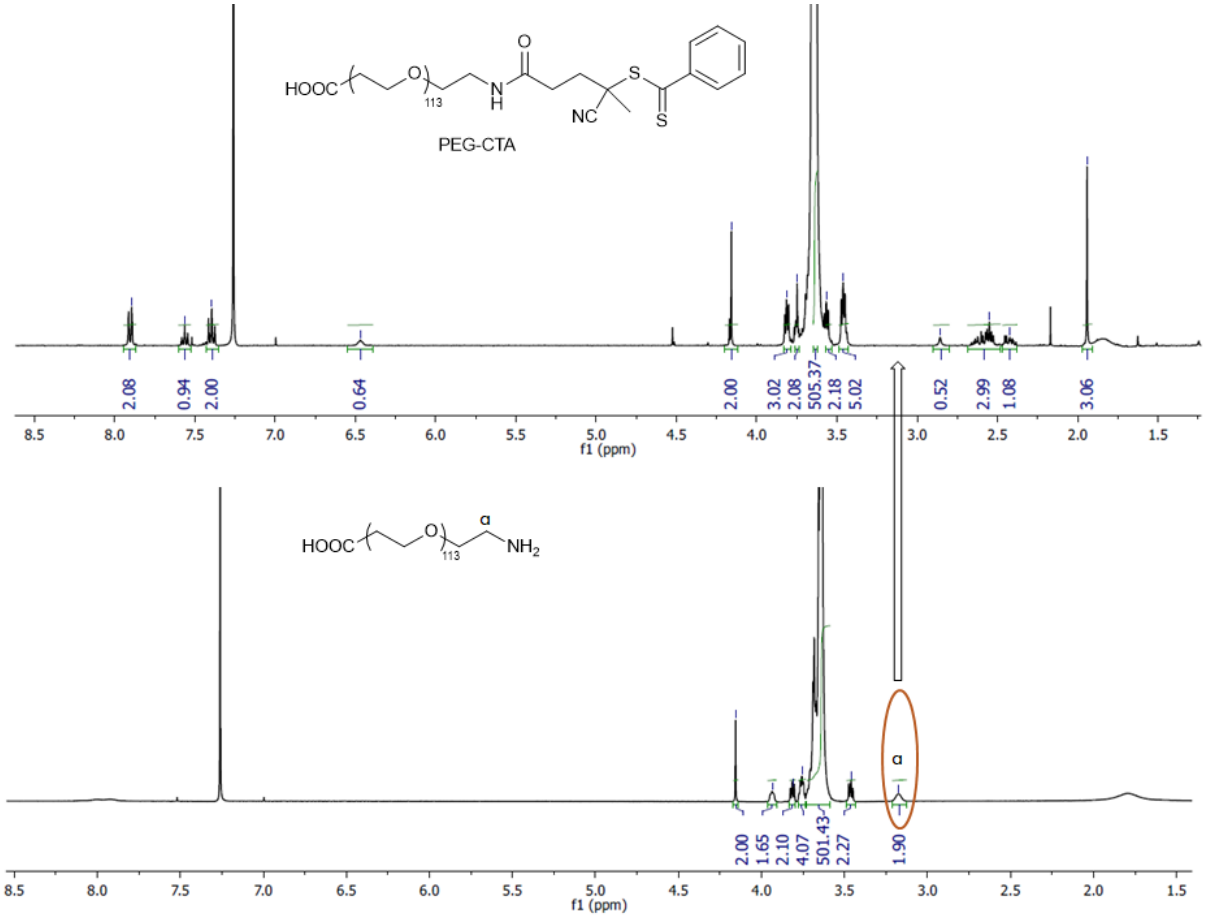

Figure S1. Structure and NMR characterization of PEG-CTA. (a) ^{1}H NMR stack plot of COOH-PEG$_{5000}$-NH$_2$ and COOH-PEG$_{5000}$-CTA in CDCl$_3$. The spectra are plotted up to 8.50 ppm for simplicity. The highlighted peak "a" corresponds to the NH$_2$ protons and vanishes upon the formation of the CTA confirming the reaction went 100 %

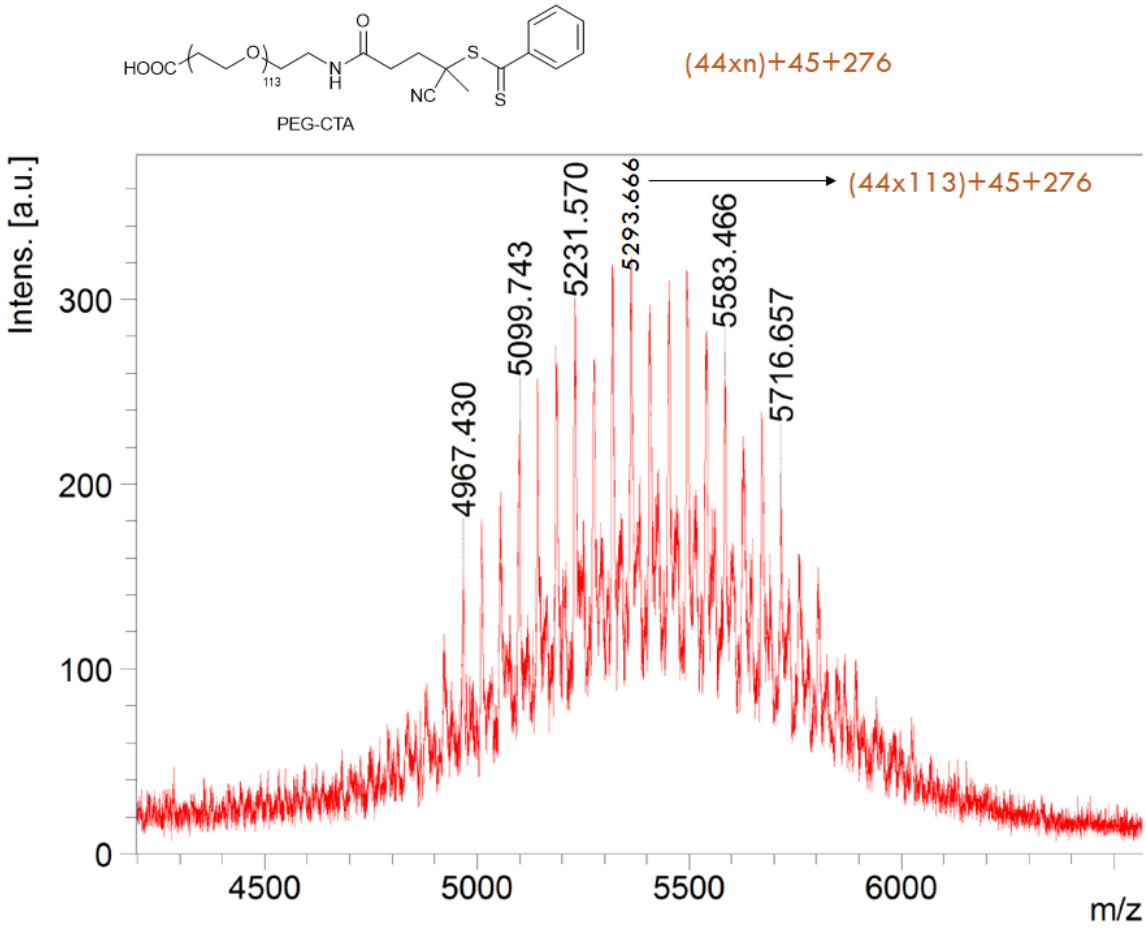

Figure S2. MALDI characterization of PEG-CTA. (a) MALDI-TOF mass spectra for the PEG-CTA explaining the mass breakdown of the peaks, corroborating the fromation of the desired product.

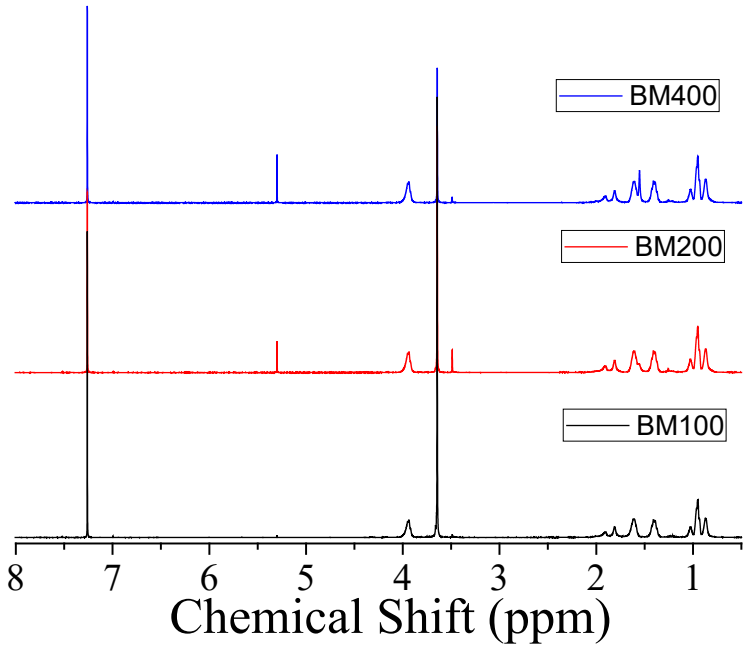

Figure S3. NMR characterization of COOH-PEG$_{5000}$-b-BM$_y$. ^{1}H NMR stack plot of COOH-PEG$_{5000}$-b-BM$_y$ (y = 100, 200, 400) in $CDCl_3$. The spectra are plotted up to 8.00 ppm for simplicity.

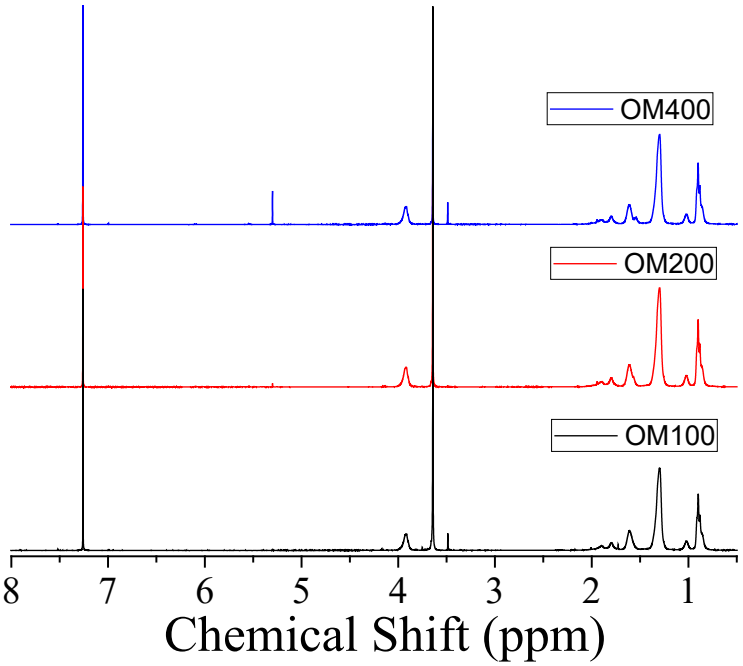

Figure S4. NMR characterization of COOH-PEG$_{5000}$-b-OM$_y$. ^{1}H NMR stack plot of COOH-PEG$_{5000}$-b-OM$_y$ (y = 100, 200, 400) in $CDCl_3$. The spectra are plotted up to 8.00 ppm for simplicity.

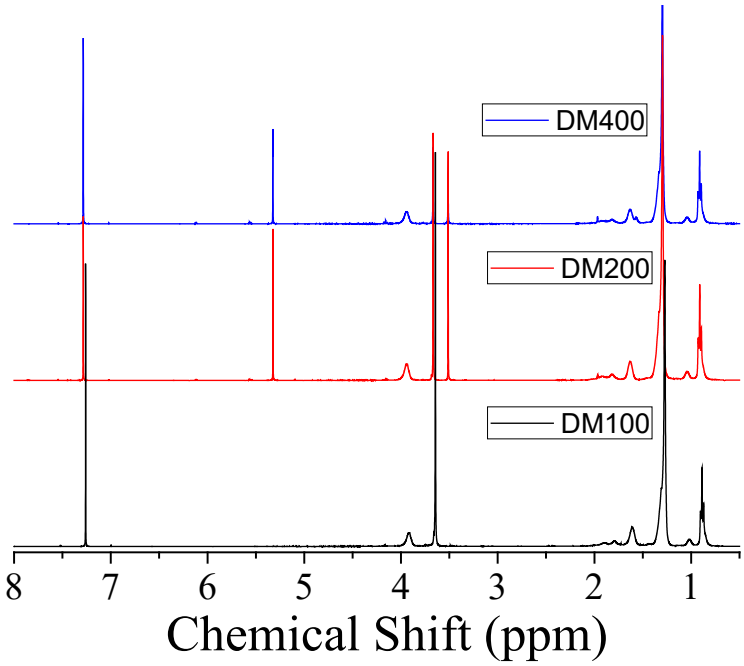

Figure S5. NMR characterization of COOH-PEG$_{5000}$-b-DM$_y$. ^{1}H NMR stack plot of COOH-PEG$_{5000}$-b-DM$_y$ (y = 100, 200, 400) in $CDCl_3$. The spectra are plotted up to 8.00 ppm for simplicity.

Note: In order to determine the degree of polymerization (X_n), the integration of the peak intensities from the PEG-CTA were compared to those of the hydrophobic polymer backbone in ^{1}H-NMR. The PEG protons peak at 3.64 ppm was given the reference integration of 505, and then detemined the integration of the peak at 3.90 ppm corresponding to the ester protons in the methacrylate polymer part.

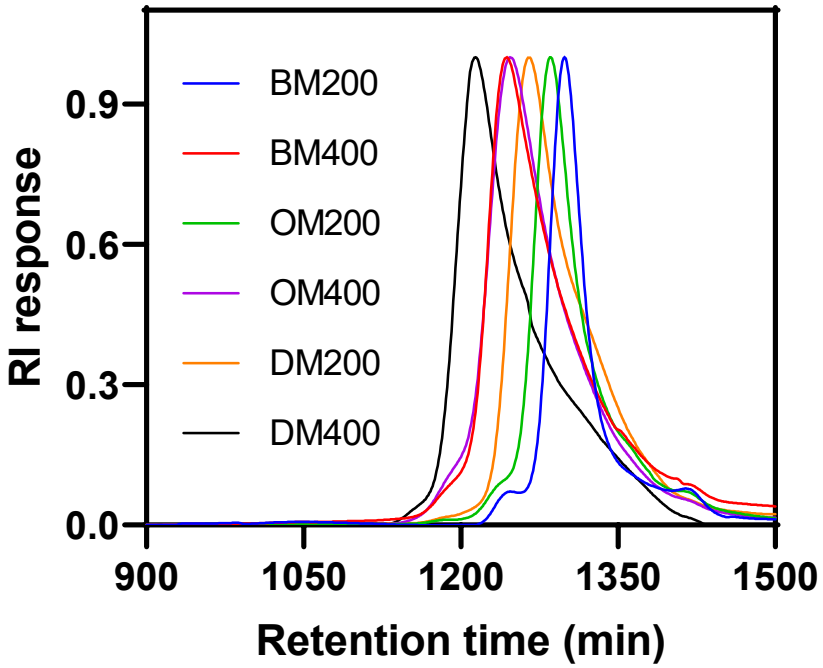

Figure S6. Gel Permeation chromatography (GPC) characterization of COOH-PEG$_{5000}$-b-HM$_{x,y}$. GPC chromatograms of COOH-PEG$_{5000}$-b-HM$_{x,y}$ (x = 1, 3, 5 and y = 200, 400) block copolymers in tetrahydrofuran (THF).

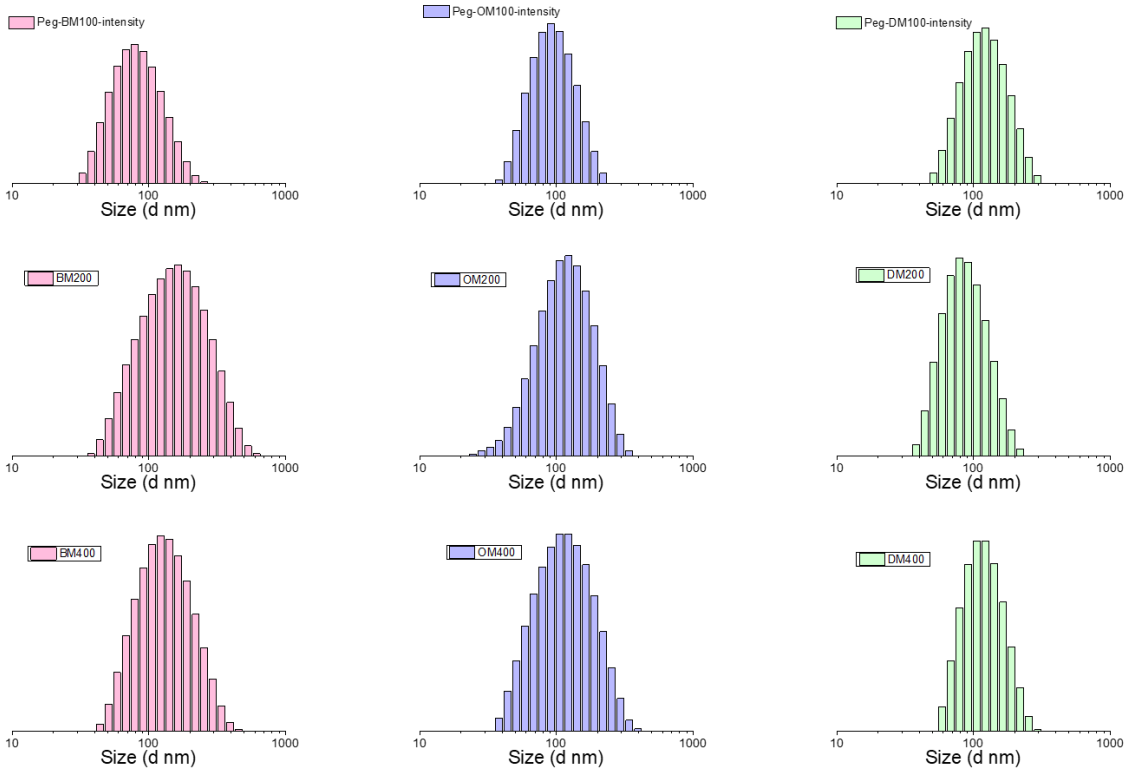

Figure S7. Size distribution analysis of COOH-PEG$_{5000}$-b-HM$_{x,y}$. Hydrodynamic diameter of the supramolecular polymer nanoparticle series COOH-PEG$_{5000}$-b-HM$_{x,y}$ (x = 1,3,5 and y = 100, 200, 400). SNP concentration in water is 0.1 mg/mL.

S.No.	Polymer Nanoparticles	Size (d nm) ± SD
1	BM100	78.12 ± 0.2
2	BM200	133.9 ± 2.50
3	BM400	118.9 ± 0.6
4	OM100	87.5 ± 0.4
5	OM200	101.9 ± 1.0
6	OM400	101.2 ± 0.8
7	DM100	111.5 ± 0.8
8	DM200	84.5 ± 1.5
9	DM400	109.7 ± 1.7

Table ST1. Size distribution of the SNP series

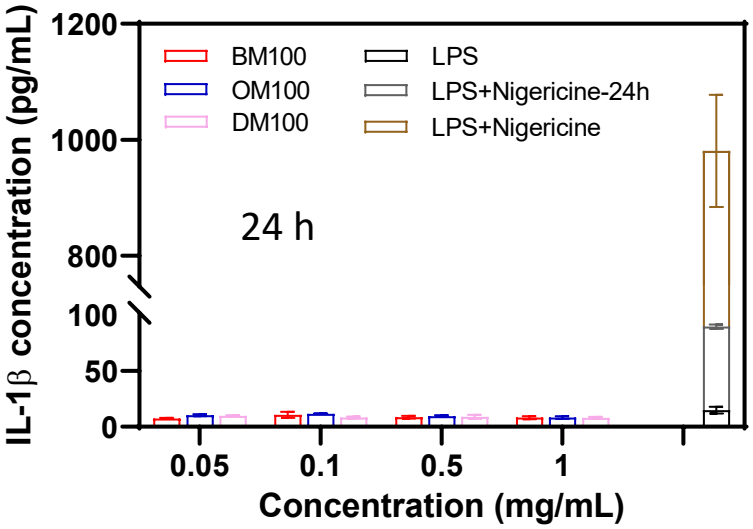

Figure S8. Determination of IL-1β release by COOH-PEG$_{5000}$-b-HM$_{x,100}$ SNPs. Concnetration dependent release of IL-1β by LPS-primed iBMDMs upon treatment with BM100, OM100 and DM100 polymer nanoparticles for 24 hours. Data is represented as mean ± SEM.

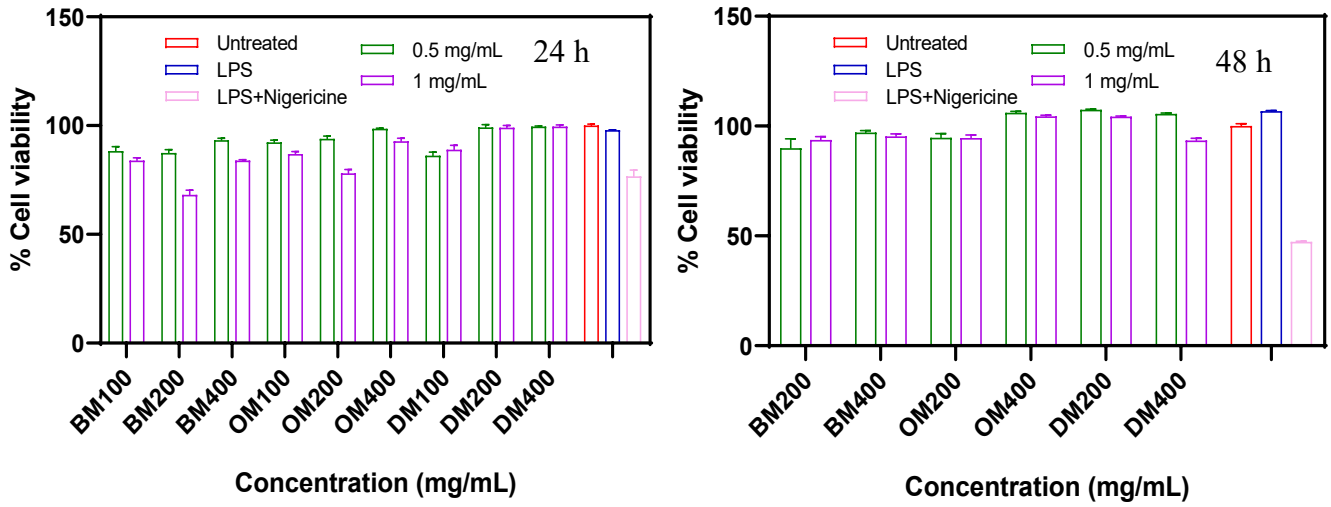

Figure S9. Cell viablity evaluation by MTT assay. MTT plot depicting the cytocompatibility of the SNP series at concnetrations of 0.5 and 1 mg/mL in iBMDMs incubated for 24 and 48 hours, respectively.

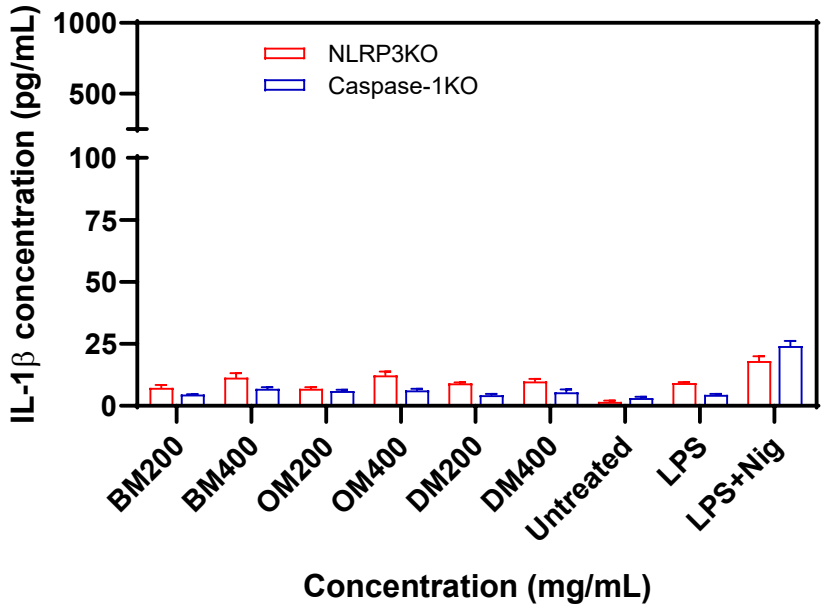

Figure S10. IL-1β release evaluation by COOH-PEG$_{5000}$-b-HM$_{x,y}$ SNPs in NLRP3 and caspase-1 knockout (KO) cells. Quantifucation of IL-1β release in NLRP3 KO and caspase-1 KO iBMDM cells upon stimulation with LPS followed by 24 hour incubation with SNPs at a concnetration of 0.5 mg/ml. Data is represented as mean ± SEM.

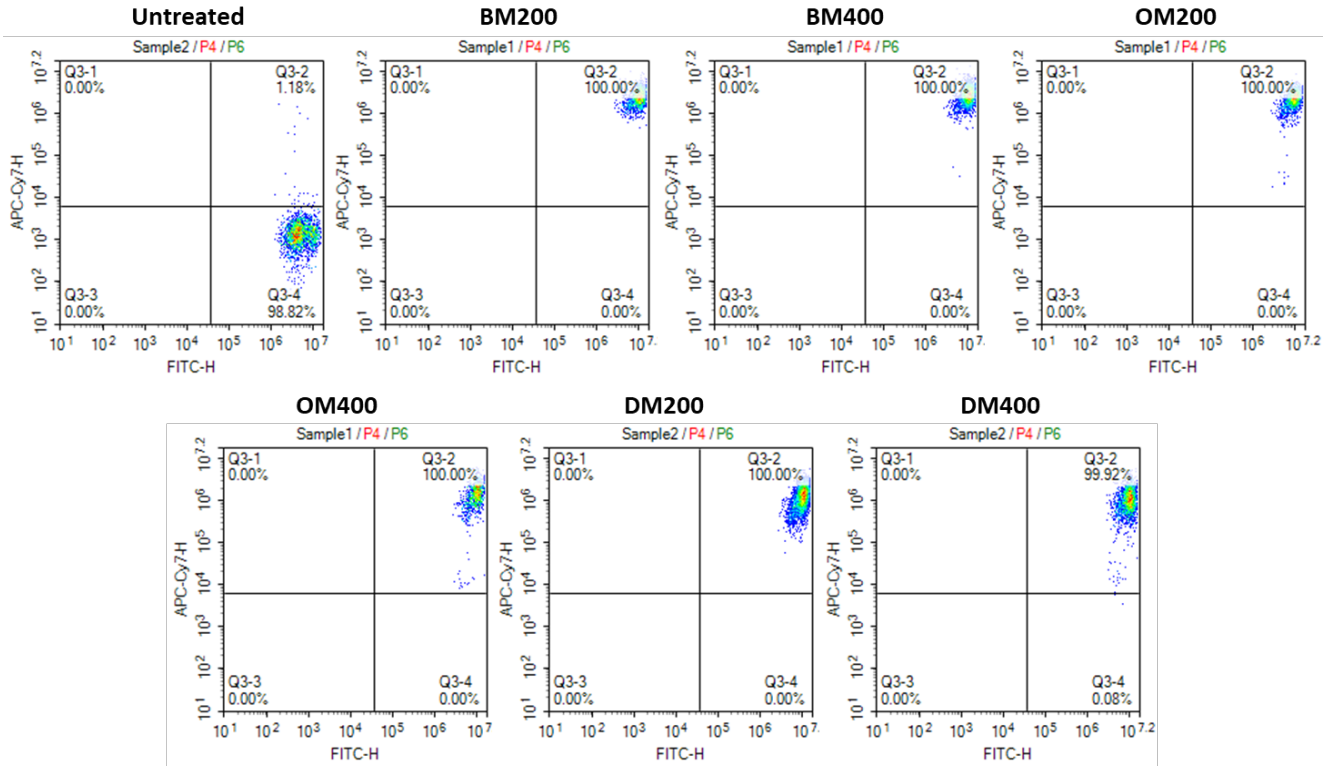

Figure S11. Cellular internalization of SNPs by WT-iBMDMs analyzed by flow cytometry. Representative dot plots for the cellular internalization by the CFSE-stained iBMDMs treated with DiR dye-loaded SNP series (0.5 mg/mL) for 4 hours. The cells in the lower right quadrnat represent only CFSE-positive cells while the cells in the upper right quadrant are depictive of dual CFSE and DiR positive cells, highlighting the cell population with internalized SNPs.

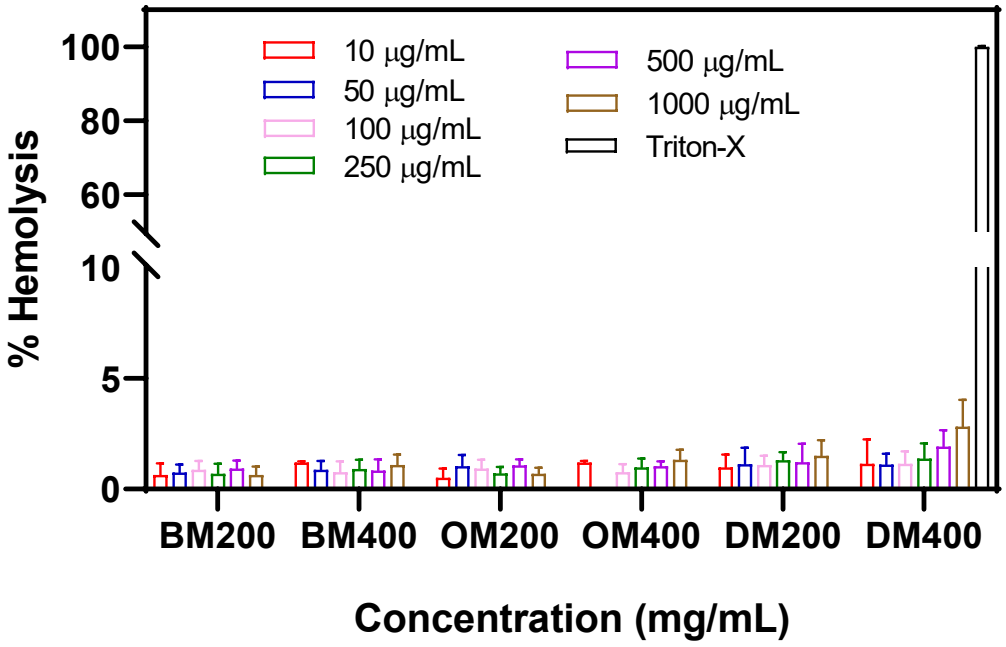

Figure S12. Hemolysis Assay. Plot of % hemolysis vs the polymer concentrations for COOH-PEG_{5000}-b-$HM_{x,y}$ SNP series (x = 1, 3, 5 and y = 200, 400) with concentration varying from 10 μg/mL to 1000 μg/mL. Triton-X was used as the positive control.

Sample name	Histopathological Interpretation
DM400 I- mouse liver	Focal mild parenchymal/lobular inflammation with lymphohistiocytes and eosinophils
DM400 I- mouse spleen	Negative for inflammation
DM400 II- mouse liver	Negative for inflammation
DM400 II- mouse spleen	Negative for inflammation
DM400 III- mouse liver	Focal perihepatic inflammation with lymphocytes and focal parenchymal/lobular inflammation with lymphocytes
DM400 III- mouse spleen	Negative for inflammation
DM400 - liver	Mild parenchymal/lobular inflammation with lymphocytes and neutrophils
DM400- spleen	Negative for inflammation
PBS I- mouse liver	Focal periportal and parenchymal/lobular inflammation with lymphocytes
PBS I- mouse spleen	Negative for inflammation
PBS II- mouse liver	Negative for inflammation
PBS II- mouse spleen	Negative for inflammation
PBS- liver	Negative for inflammation
PBS- spleen	Negative for inflammation
PBS IV- mouse liver	Mild parenchymal/lobular inflammation with lymphocytes and neutrophils
PBS IV- mouse spleen	Negative for inflammation

Table ST2. Histopathological Interpretation of Hematoxylin and eosin-stained tissue samples.