

Supporting Information

**Angiogenic Properties of Poly(NAG-co-NAPA)  
Nanoparticles: Assessment via Gray Level Co-occurrence  
Matrix based Image Processing**

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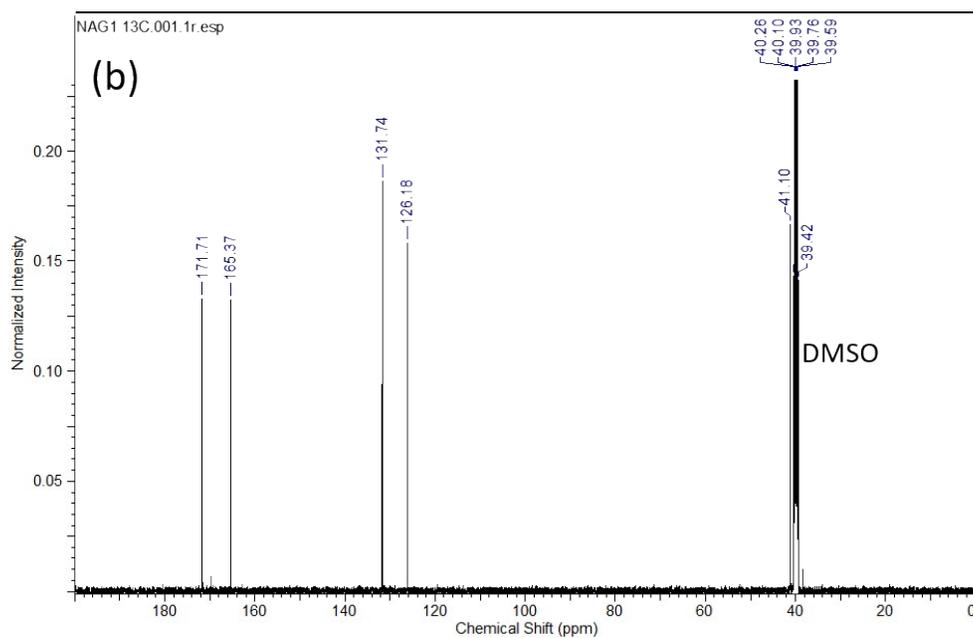
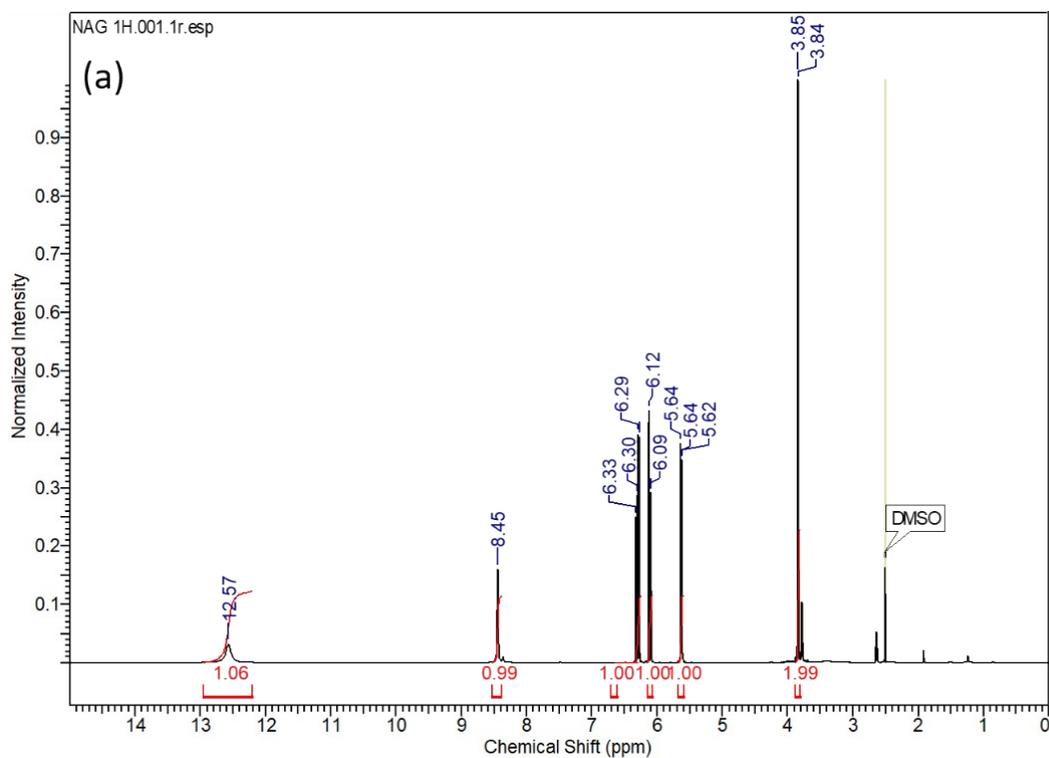
**Synthesis of NAG and NAPA monomers**

### Synthesis of NAG monomer

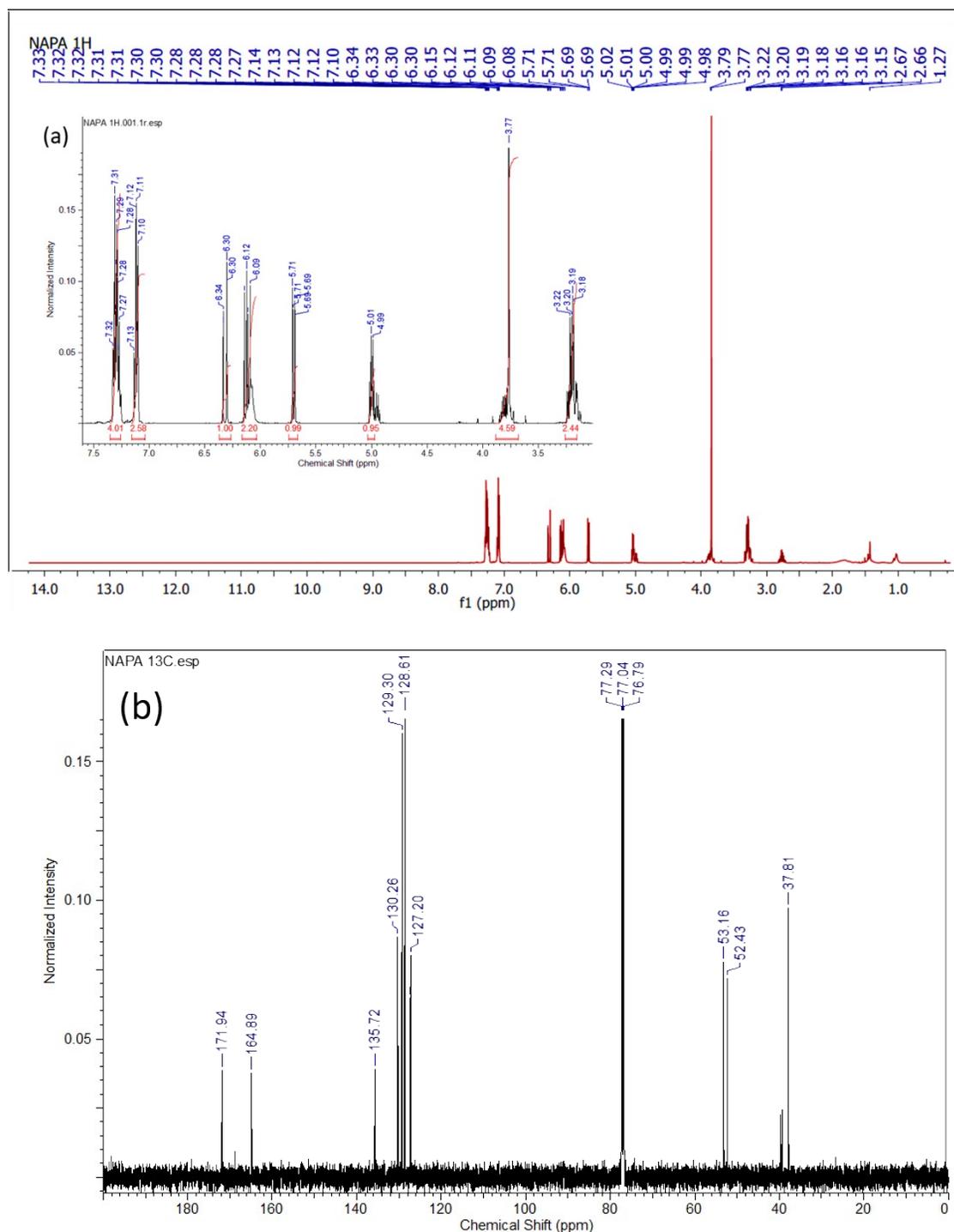
Solution A was prepared in a 100 mL round-bottom flask by combining 20 mmol of glycine with 20 mL of aqueous 2 M KOH, while maintaining the mixture in an ice bath and stirring continuously at 600 RPM. Meanwhile, Solution B was prepared in a 50 mL round-bottom flask, consisting of 20 mmol of acryloyl chloride and 5 mL of 1,4-dioxane, also in an ice bath with continuous stirring at 900 RPM. Over the course of one hour, Solution B was gradually added to Solution A, ensuring continuous stirring to facilitate the reaction. After the addition, the mixture was allowed to stir overnight at room temperature to ensure complete reaction. Following this period, the mixture was washed three times with 20 mL of diethyl ether to remove unreacted materials and by-products, collecting the bottom layer after each wash. The pH of the resulting solution was then adjusted to 2 through the dropwise addition of 5 M HCl. Subsequently, the solution was saturated with excess NaCl, and the aqueous phase was extracted five times with 20 mL of ethyl acetate to separate *N*-acryloyl glycine. The moisture was dried over magnesium sulfate (MgSO<sub>4</sub>), and the filtrate was then concentrated using rotary evaporation, yielding a white powder identified as *N*-acryloyl glycine (NAG).

### Synthesis of NAPA monomer

In a 100 mL round-bottom flask, Solution A was prepared by combining 1.6 g of L-phenylalanine methyl ester, 1.6 mL of triethylamine, and 60 mL of dry DCM, while maintaining the mixture in an ice bath with continuous stirring at 600 RPM. Solution B was prepared separately in a 50 mL round-bottom flask by dissolving 0.9 mL of acryloyl chloride in 5 mL of DCM. After one hour, Solution B was gradually added to Solution A, ensuring continuous stirring to facilitate the reaction and manage the exothermic nature of the process, which enhanced the formation of the desired product. After the complete addition, the mixture was stirred for an additional 24h. Following this period, DCM was removed using a rotary evaporator set at 30 °C, resulting in a white residue. To this residue, 100 mL of ethyl acetate was added, and the mixture was filtered through filter paper to eliminate insoluble impurities. The resulting solution underwent a series of washing steps: five times with 40 mL of sodium sulfate, followed by five washes with 40 mL of sodium bicarbonate, and finally three washes with 40 mL of brine solution. These washing steps were crucial for removing impurities. To ensure the removal of moisture, magnesium sulfate was added and then filtered through a 0.45-micron filter. The filtrate was subsequently concentrated using rotary evaporation, yielding a white powder identified as *N*-acryloyl-L-phenylalanine methyl ester (NAPA).

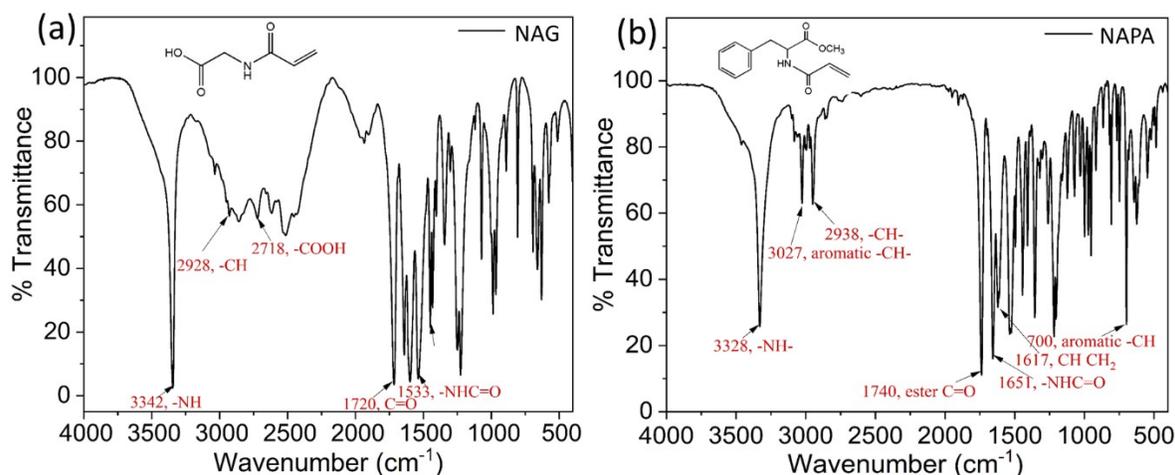


**Figure S1.** (a) <sup>1</sup>H-NMR and (b) <sup>13</sup>C-NMR of NAG monomer. NAG the bands are assigned as: <sup>1</sup>H NMR (500 MHz, DMSO-d<sub>6</sub>) δ ppm 3.84 (d, J=5.95 Hz, 2 H) 5.63 (dd, J=10.22, 2.14 Hz, 1 H) 6.11 (dd, J=17.17, 2.06 Hz, 1 H) 6.30 (dd, J=17.09, 10.22 Hz, 1 H) 8.45 (br. s., 1 H (NH)) 12.57 (br. s., 1 H (COOH)) and <sup>13</sup>C-NMR (500 MHz, DMSO-d<sub>6</sub>) δ ppm: 171.71 (-COOH), 165.37 (-C=O), 131.73 (-CH adjacent to carbonyl), and 126.18 (=CH<sub>2</sub>) Already attached in our previous research article. Only for reference, it has been attached here.

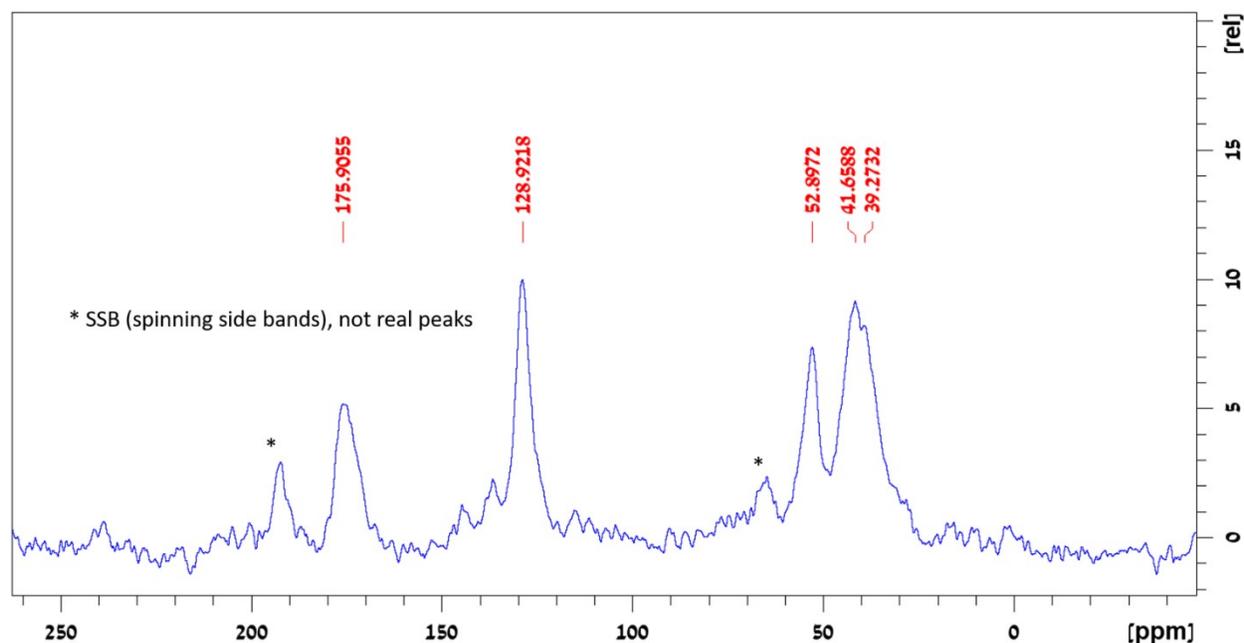


**Figure S2.** (a) <sup>1</sup>H-NMR and (b) <sup>13</sup>C-NMR of NAPA monomer. The characteristic bands are as follows: <sup>1</sup>H NMR (500 MHz, CHLOROFORM-d) δ ppm 3.20 (dd, J=11.14, 5.65 Hz, 3 H) 3.77 (m, 4 H) 5.00 (d, J=7.78 Hz, 1 H) 5.70 (dd, J=10.30, 1.30 Hz, 1 H) 6.04 - 6.18 (m, 2 H) 6.27 - 6.36 (m, 1 H) 7.05 - 7.16 (m, 2 H) 7.23 - 7.36 (m, 3 H) and <sup>13</sup>C-NMR (500 MHz, CHLOROFORM-d)

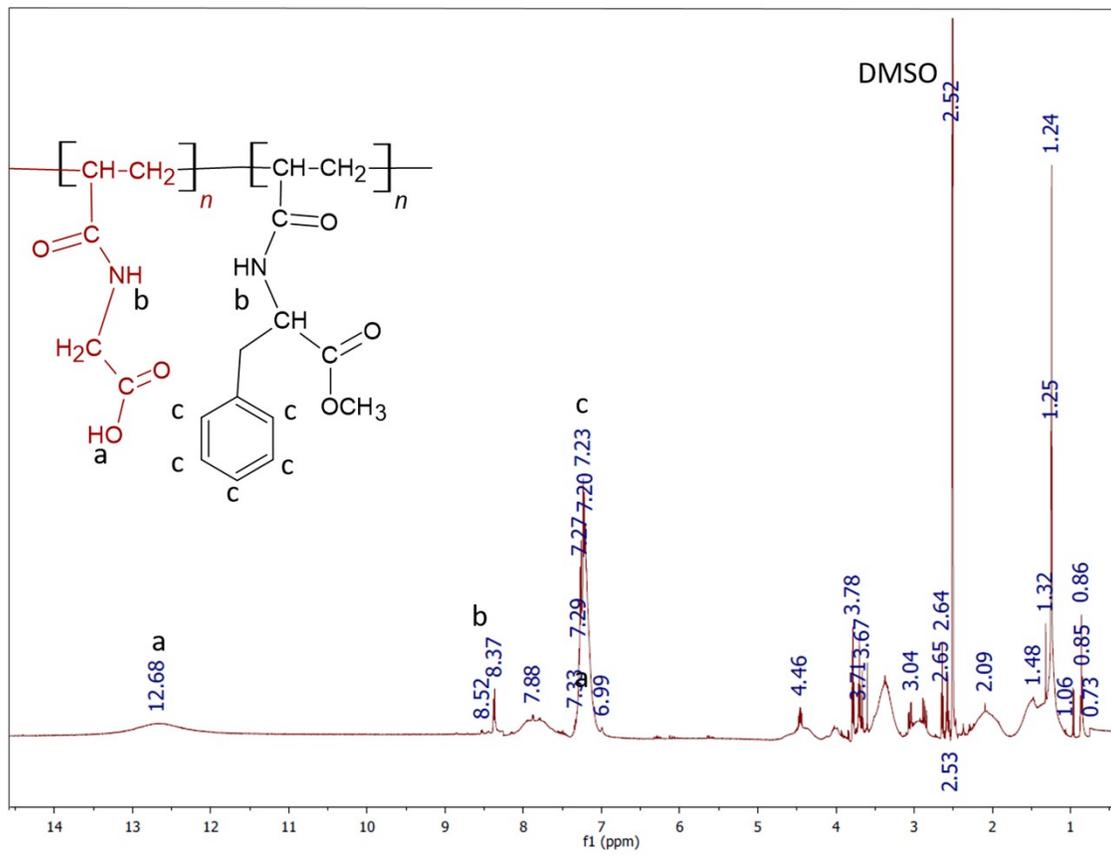
$\delta$  ppm: 171.94 (ester), 164.89 (-C=O), 135.72 (aromatic C attached to chain), 130.26 (C adjacent to =CH<sub>2</sub>), 129.30 and 128.61 (aromatic), 127.20 (=CH<sub>2</sub>), 53.16 (-CH adjacent to 2° amide), 52.43 (-OCH<sub>3</sub>), and 37.81 (-CH<sub>2</sub> adjacent to aromatic ring). Already attached in our previous research article. Only for reference, it has been attached here.



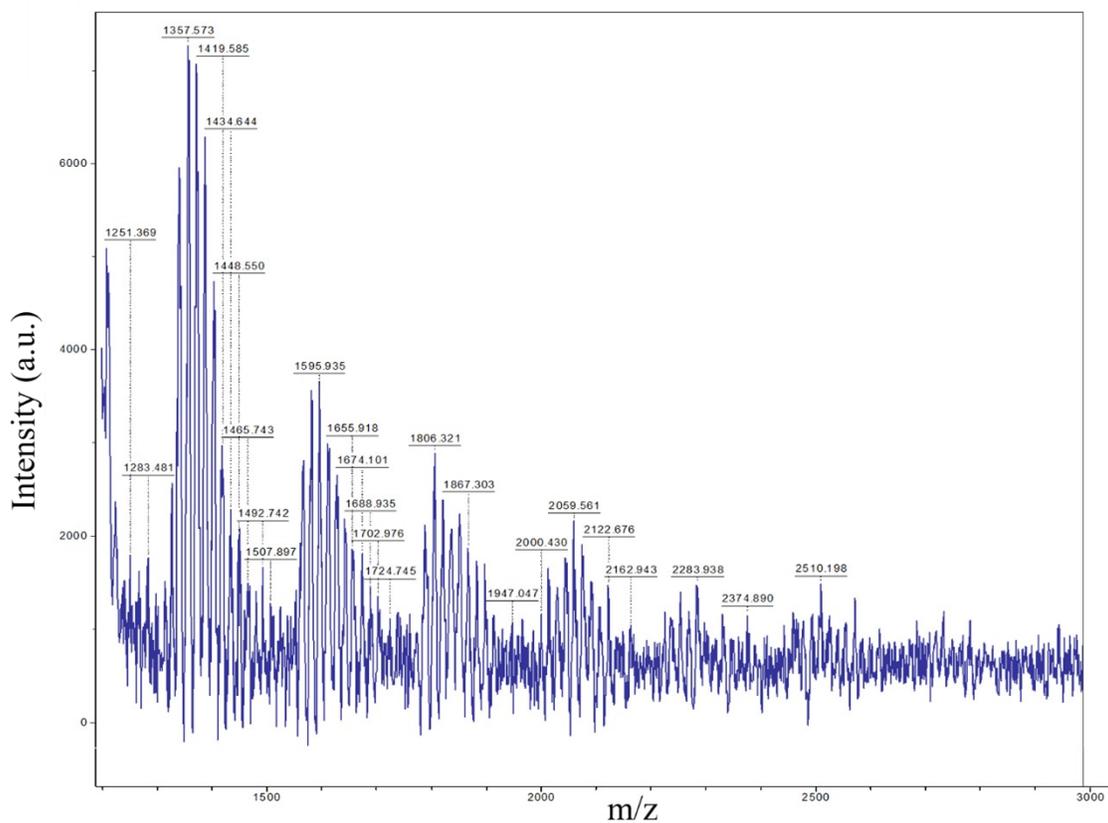
**Figure S3:** FTIR spectra of (a) NAG and (b) NAPA monomers.  $\nu = 3342$  (secondary -NH, s), 2928 (alkane, s), 2718 (-COOH), 1720 (ester C=O, s), and 1533 (-NH-C=O, s) for NAG and  $\nu = 3328$  (secondary -NH-, s), 3027 (aromatic -CH, s) and 2938 (alkane -CH, s), 1740 (ester C=O, s), 1651 (-NH-C=O, s), and 700 (aromatic -CH, b) for NAPA. Already attached in our previous research article. Only for reference, it has been attached here.



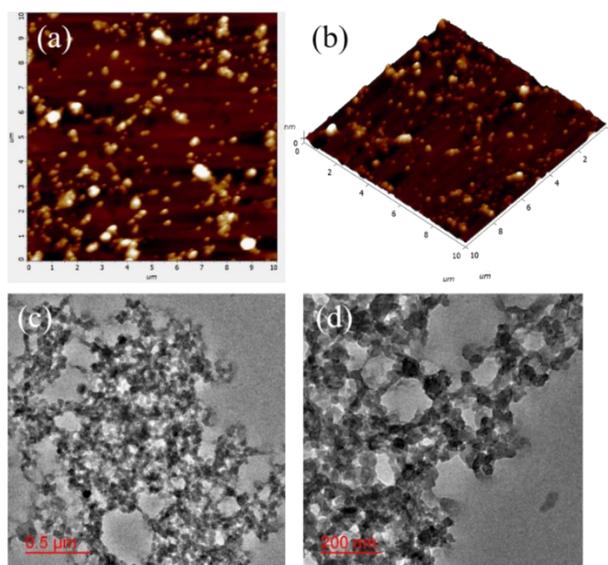
**Figure S4:** <sup>13</sup>C Solid-state NMR of p(NAG-co-NAPA) NPs.



**Figure S5:** <sup>1</sup>H NMR of p(NAG-co-NAPA)<sub>wc</sub> NPs.



**Figure S6.** Represents the MALDI-ToF spectrum of p(NAG-*co*-NAPA) NPs.

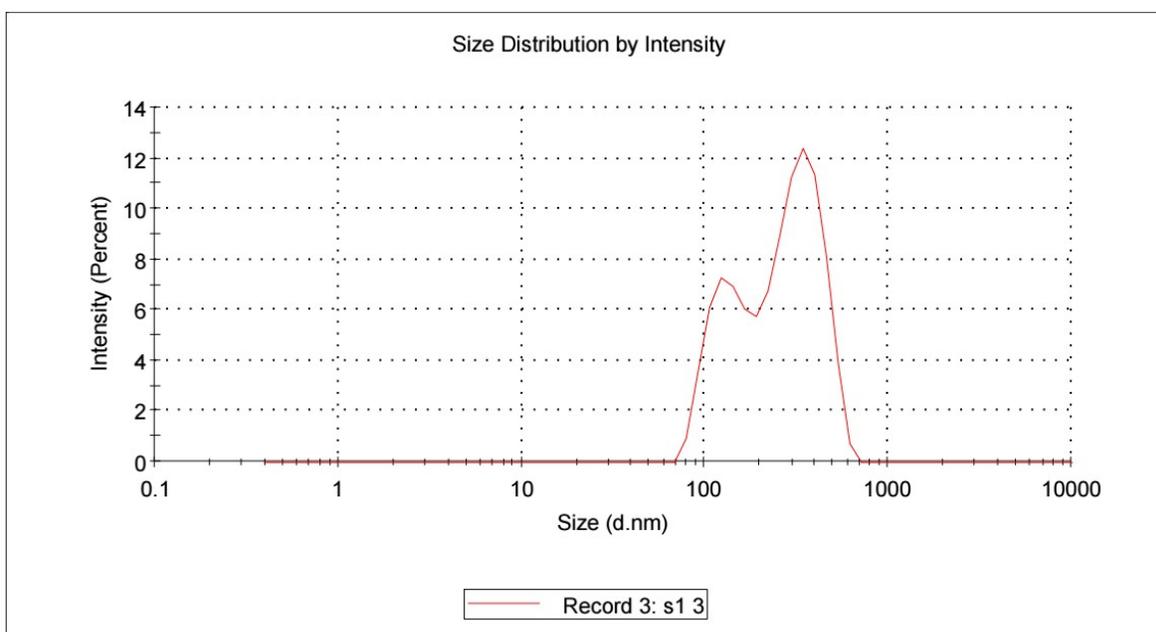


**Figure S7.** Represents the agglomeration behavior of p(NAG-*co*-NAPA) NPs. (a) 2D and (b) 3D AFM images of p(NAG-*co*-NAPA) NPs at 10µm scale, (c) and (d) HR-TEM images of p(NAG-*co*-NAPA) NPs at 500nm at 200nm scale.

## Results

	Size (d.n...	% Intensity:	St Dev (d.n...
<b>Z-Average (d.nm):</b> 339.6	<b>Peak 1:</b> 334.8	65.4	97.71
<b>Pdl:</b> 0.431	<b>Peak 2:</b> 136.7	34.6	32.63
<b>Intercept:</b> 0.979	<b>Peak 3:</b> 0.000	0.0	0.000

**Result quality** Refer to quality report



**Figure S8.** Represents DLS spectra of p(NAG-co-NAPA) NPs.

**Table S1:** Represents the Cell viability% calculated for p(NAG-co-NAPA) NPs with L929 and RAW 264.7 cells.

Concentration( $\mu\text{g/ml}$ )	L929	RAW 264.7
400	78.87 $\pm$ 2.96	96.77 $\pm$ 1.17
200	89.13 $\pm$ 1.49	99.91 $\pm$ 3.31
100	114.26 $\pm$ 1.33	112.40 $\pm$ 2.63
50	105.14 $\pm$ 2.07	114.76 $\pm$ 0.44
25	102.14 $\pm$ 0.97	101.08 $\pm$ 1.09
12.5	96.20 $\pm$ 3.43	101.83 $\pm$ 1.40
6.25	97.05 $\pm$ 3.63	99.96 $\pm$ 2.36
3.125	95.55 $\pm$ 1.41	101.88 $\pm$ 3.38

**Table S2:** Represents the Cell viability% calculated for p(NAG-co-NAPA) NPs with HUVEC cells.

Concentration( $\mu\text{g/ml}$ )	HUVECs
200	84.89 $\pm$ 0.74
100	86.09 $\pm$ 1.70
50	104.33 $\pm$ 0.92
25	105.10 $\pm$ 2.46
10	111.79 $\pm$ 0.58
5	114.36 $\pm$ 1.03

**Table S3:** Represents the Hemocompatibility% calculated for p(NAG-co-NAPA) NPs.

Concentration( $\mu\text{g/ml}$ )	2h	8h
500	0.97 $\pm$ 1.18	1.77 $\pm$ 1.18
250	1.06 $\pm$ 1.93	2.95 $\pm$ 1.93
125	0.47 $\pm$ 1.85	0.35 $\pm$ 1.85
62.5	0.53 $\pm$ 1.61	0.77 $\pm$ 1.61
31.25	0.71 $\pm$ 1.44	1.07 $\pm$ 1.44
15.625	2.01 $\pm$ 0.75	2.56 $\pm$ 0.75
7.8125	1.18 $\pm$ 1.61	1.16 $\pm$ 1.61

**Table S4:** Represents the error% calculated for the Cell Viability study.

Concentration( $\mu\text{g/ml}$ )	L929		RAW 264.7	
	p(NAPA)	p(NAG)	p(NAPA)	p(NAG)
400	**	*	***	***
200	***	NS	***	**
100	**	*	***	NS
50	NS	NS	**	NS
25	NS	*	**	NS
12.5	NS	**	***	NS
6.25	NS	NS	***	NS
3.125	NS	*	***	NS

**Table S5:** Represents the error% calculated for the Cell Viability study of HUVEC cells.

Concentration( $\mu\text{g/ml}$ )	HUVEC	
	p(NAPA)	p(NAG)
200	***	NS
100	***	NS
50	***	NS
25	***	NS
10	***	NS
5	***	***

**Table S6:** Represents the error% calculated for the number of pixels in blood vessels.

Number of pixels in blood vessels					
	Time	Control	1 $\mu\text{g}$	10 $\mu\text{g}$	100 $\mu\text{g}$
p(NAPA)	0	3.52675	5.93664	7.51567	4.6627
	2	6.62627	4.21846	8.43219	9.01171
	4	5.94178	7.60607	7.24897	8.83268
	8	5.87941	4.50518	6.12946	2.19371
	24	6.60758	6.49306	7.60039	4.22757
p(NAG)	0	3.52675	2.83613	5.20954	5.07186
	2	6.62627	6.21057	3.21637	8.93945
	4	5.94178	5.34573	7.41008	3.05839
	8	6.87941	3.5753	8.11069	1.41602
	24	6.60758	6.19848	2.19769	2.66008
p(NAG-co-NAPA)	0	3.52675	5.99279	5.93664	4.60627
	2	6.62627	8.5375	4.21846	6.69233
	4	5.94178	2.53397	7.60607	0.15441
	8	1.87941	6.56647	4.50518	6.80557
	24	6.60758	5.30111	5.47233	4.22757

**Table S7:** Represents the error% calculated for entropy.

Entropy					
	Time	Control	1µg	10µg	100µg
p(NAPA)	0	0.2687	3.00098	4.67479	5.62228
	2	0.23154	3.02027	1.54166	8.74816
	4	1.6339	0.80328	0.80328	0.23471
	8	6.11103	1.22809	1.22809	0
	24	2.23361	9.86319	1.98823	0.68837
p(NAG)	0	0.2687	1.77753	2.67502	8.75229
	2	0.23154	4.10935	5.91303	8.09166
	4	1.6339	3.69245	1.86901	7.3602
	8	6.11103	2.32792	8.16248	9.67659
	24	2.23361	2.24231	0.0456	3.0988
p(NAG-co-NAPA)	0	0.2687	1.84803	1.10491	1.30726
	2	0.23154	2.74257	1.75127	1.76149
	4	1.6339	2.5652	1.8854	1.05269
	8	1.11103	1.64681	1.20011	0.8244
	24	2.23361	1.87547	0.46794	1.00953

**Table S8:** Represents the error% calculated for energy.

Energy					
	Time	Control	1µg	10µg	100µg
p(NAPA)	0	0.32712	0.34674	0.48178	1.13853
	2	0.35567	0.10794	0.01964	1.75905
	4	0.48196	0.05104	0.18438	1.31191
	8	1.21795	0.71286	0.6693	0.90657
	24	0.70106	1.3345	0.73627	0.44313
p(NAG)	0	0.32712	0.48806	2.9025	5.90668
	2	0.35567	0.93403	3.276	3.57098
	4	0.48196	0.90663	2.30882	1.60044
	8	1.21795	0.62142	0.62142	2.43274
	24	0.70106	0.87051	0.437	1.15916
p(NAG-co-NAPA)	0	0.32712	0.9552	0.58454	0.9515
	2	0.35567	0.94671	0.62175	0.76885
	4	0.48196	0.4541	0.12302	0.23712
	8	0.21795	0.04989	0.55779	0.03195
	24	0.70106	0.67194	0.47245	0.09256

**Table S9:** Represents the error% calculated for mean.

Mean					
	Time	Control	1µg	10µg	100µg
p(NAPA)	0	0.37834	0.08858	0.08939	1.02228
	2	0.706	0.35098	0.29973	1.70297
	4	0.66174	0.79184	0.4511	1.18151
	8	1.32895	1.18121	0.80708	0.91522
	24	0.92243	0.761	1.00069	0.75472
p(NAG)	0	0.37834	0.51211	3.17444	5.57426
	2	0.706	0.93429	3.49606	3.37725
	4	0.66174	0.91782	2.32817	1.4705
	8	1.32895	0.64209	1.49766	2.45945
	24	0.92243	1.16451	0.31687	1.53266
p(NAG-co-NAPA)	0	0.37834	0.34478	0.89598	0.92002
	2	0.706	1.23804	0.59876	0.61953
	4	0.66174	2.44774	1.122	0
	8	1.32895	2.32455	0.74182	0.25276
	24	0.92243	0.70707	0.82282	0.59153

**Table S10:** Represents the error% calculated for contrast.

Contrast					
	Time	Control	1µg	10µg	100µg
p(NAPA)	0	8.27583	3.79401	19.78922	1.84325
	2	1.93783	2.56206	12.81691	1.72955
	4	4.73547	8.32263	9.05286	9.91213
	8	0.00879	1.72697	2.00665	3.8018
	24	3.97337	2.81178	3.48381	8.5931
p(NAG)	0	8.27583	2.17152	1.5521	3.79834
	2	1.93783	5.51736	2.86597	3.87353
	4	4.73547	5.31102	7.34164	6.1181
	8	0.00879	3.46937	2.85784	1.25676
	24	3.97337	3.12543	5.21121	3.36344
p(NAG-co-NAPA)	0	8.27583	8.34478	7.56905	0.62937
	2	1.93783	1.23804	3.30523	4.17939
	4	4.73547	2.44774	5.17778	2.10582
	8	0.00879	2.32455	3.80379	9.79665
	24	3.97337	0.70707	2.34642	9.63705

**Table S11:** Represents the error% calculated for dissimilarity.

<b>Dissimilarity</b>					
	<b>Time</b>	<b>Control</b>	<b>1µg</b>	<b>10µg</b>	<b>100µg</b>
<b>p(NAPA)</b>	0	1.83837	3.79399	9.78922	1.84325
	2	2.30176	2.56206	2.81689	1.72953
	4	8.00859	8.32263	9.05284	9.912142
	8	0.01535	4.72698	2.00666	3.801778
	24	6.75081	9.81176	2.84194	8.593099
<b>p(NAG)</b>	0	1.83837	2.17152	1.55213	9.92832
	2	9.30176	5.51734	2.86592	3.76089
	4	8.00859	5.311	7.34162	0.38691
	8	0.01535	3.46938	2.85787	2.48895
	24	6.75081	3.12541	3.25788	7.67158
<b>p(NAG-co-NAPA)</b>	0	1.83837	8.34478	7.56903	5.25809
	2	1.30176	1.23802	3.30524	7.90467
	4	8.00859	2.44773	5.1778	2.10582
	8	0.01535	2.32456	3.80384	9.79666
	24	6.75081	0.68832	2.34639	9.63705

**Table S12:** Represents the error% calculated for variance.

<b>Variance</b>					
	<b>Time</b>	<b>Control</b>	<b>1µg</b>	<b>10µg</b>	<b>100µg</b>
<b>p(NAPA)</b>	0	4.45313	0.69318	0.62504	5.52531
	2	8.37644	2.17898	2.02579	1.20718
	4	7.47005	4.99743	2.723	7.47934
	8	4.9442	7.17599	5.02566	5.94283
	24	8.0449	5.53646	4.52934	4.20075
<b>p(NAG)</b>	0	4.45313	0.69318	2.21287	4.68477
	2	8.37644	2.17898	7.00558	8.12007
	4	7.47005	4.99743	8.16824	2.67226
	8	4.9442	7.17599	9.62904	1.23786
	24	8.0449	5.03759	1.88097	3.24895
<b>p(NAG-co-NAPA)</b>	0	4.45313	4.00636	5.04335	4.3365
	2	8.37644	9.22113	3.62061	8.20283
	4	7.47005	1.8601	6.48963	7.89355
	8	4.9442	4.38192	3.76462	1.94106
	24	8.0449	1.42344	4.65162	3.63696

**Table S13:** Represents the error% calculated for from tube formation assay computed using Angiotool.

<b>p(NAG-co-NAPA) NPs</b>	<b>Control</b>	<b>1 µg</b>	<b>10 µg</b>	<b>100 µg</b>
Explant Area	0.628476	0.890086	2.813132	0.539732
Total number of junctions	3.957769	4.845815	3.450317	4.827586
Total vessel length	4.806807483	1.873105287	4.408096894	0.200604492
Mean-E-Lacunarity	3.018299546	1.176093	1.946974	1.209795

**Table S14:** Represents the error% calculated for from tube formation assay computed using Image processing tool.

<b>p(NAG-co-NAPA) NPs</b>	<b>Control</b>	<b>1 µg</b>	<b>10 µg</b>	<b>100 µg</b>
Number of pixels in blood vessels	2.052102	0.891457	3.939241	1.827812
Entropy	0.886685	0.482057	0.95784	0.466798
Energy	0.362912	0.184573	0.316621	0.363561
Mean	0.530265	0.298528	0.304386	0.692915