Supporting Information

Nucleus-targeted, echogenic polymersomes for delivering a cancer stemness inhibitor to pancreatic cancer cells

*Fataneh Karandish[†], Babak Mamnoon[†], Li Feng[†], Manas K. Haldar[†], Lang Xia[ǀ], Kara N. Gange[ǰ], Seungyong You[ǂ], Yongki Choi[ǂ], Kausik Sarkar[ǀ], and Sanku Mallik[†]***

†Department of Pharmaceutical Sciences, North Dakota State University, Fargo, ND 58108

ǀDepartment of Mechanical and Aerospace Engineering, The George Washington University, Washington DC, 20052

ǰDepartment of Health, Exercise, and Nutrition Sciences, North Dakota State University, Fargo, ND, 58108

‡Department of Physics, North Dakota State University, Fargo, ND 58108

Corresponding Author

*E-mail: Sanku.Mallik@ndsu.edu. Phone: 701-231-7888. Fax: 701-231-7831.

Present Address

Department of Pharmaceutical Sciences, North Dakota State, University, Fargo, North Dakota, 58108.

Table of Contents

Table of Contents

Determination of Copolymer Composition for PEG-S-S-PLA:

The repeating monomer number m of PLA was also estimated from ^{1}H NMR spectrum by comparing (-C**H**-C=O) (δ = 5.18 ppm) from the PLA block to $-(OCH_2CH_2)_n-$ (δ = 3.66 ppm) from the PEG block.

If m is the number of protons of -C**H**-C=O, 4n is the number of protons of $-(OCH_2CH_2)_n-$

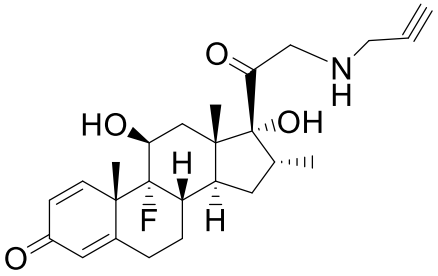
$m/4n = a_1/a_2$

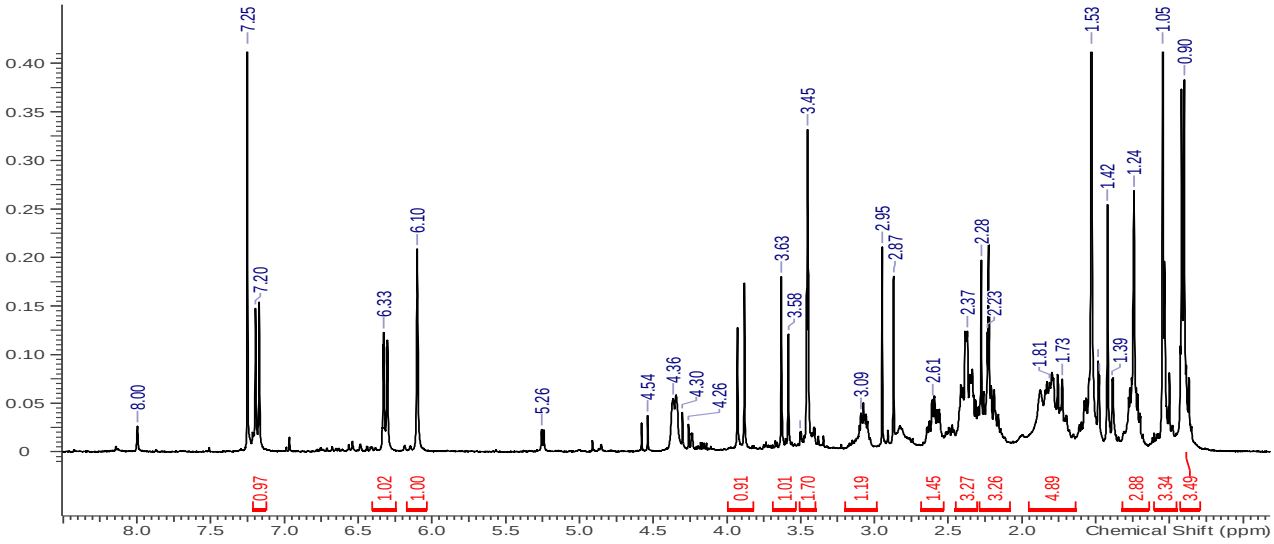
a_1 = area of peak at 5.18 ppm

a_2 = area of peak at 3.66 ppm

Molecular weight of PEG = 2000 (from the vendor) and n = 46. Therefore, 4n = 184, then m = 184 x a_1/a_2 = 184 x 90/184 = 90. Hence, the molecular weight of PLA = 72 x m = 6,480.

^{1}H NMR spectrum of alkyne-dexamethasone (400 MHz, $CDCl_3$):



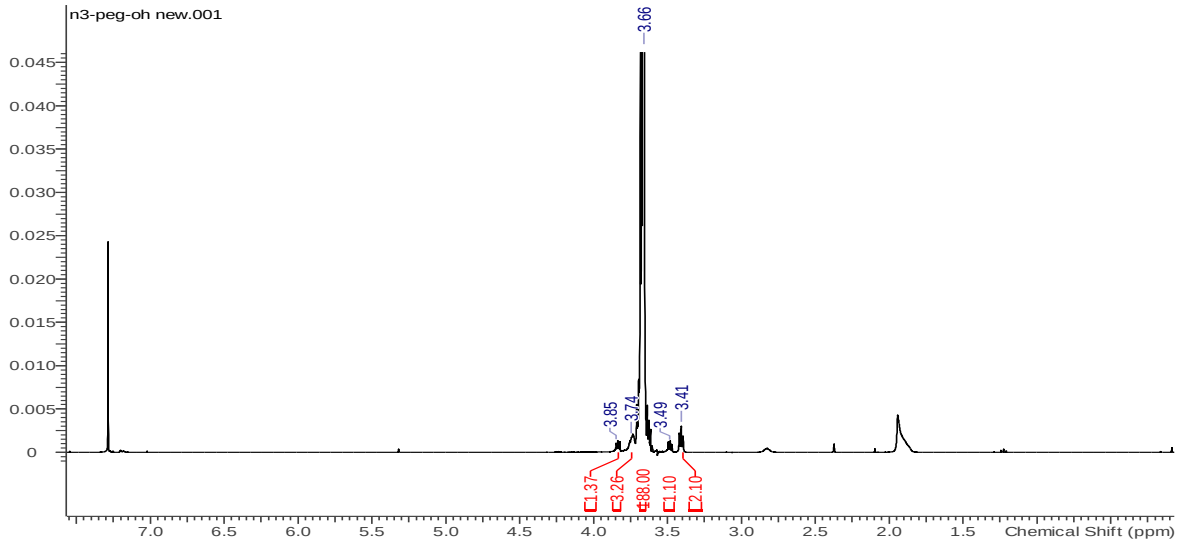


^{1}H NMR (400 MHz, chloroform-d) δ ppm: 7.20 ((C**H**=CH-C=O), d, 1H), 6.33 ((CH=C**H**-C=O), d, 1H), 6.10 ((C=C**H**-C=O), d, 1H), 3.93 ((NH-C**H$_2$**-C=O), d, 2H), 3.45 ((NH-C**H$_2$**- C≡CH), d, 2H), 3.09 ((C≡CH), s, 1H), 2.37 ((=C-C**H$_2$**-CH$_2$), t, 2H), 2.28 ((CH-C**H$_2$**-CH$_2$-C-OH), t, 2H), 1.81 ((C-C**H$_2$**-CH-OH), d, 2H), 1.42 ((C**H$_3$**-C-CH=CH), s, 3H), 1.05 ((C**H$_3$**-C-C-OH), s, 3H), 0.90 ((C**H$_3$**-CH-C-OH), s, 3H).

Synthesis and characterization of N_3-PEG_{1900}-PLA_{8000}:

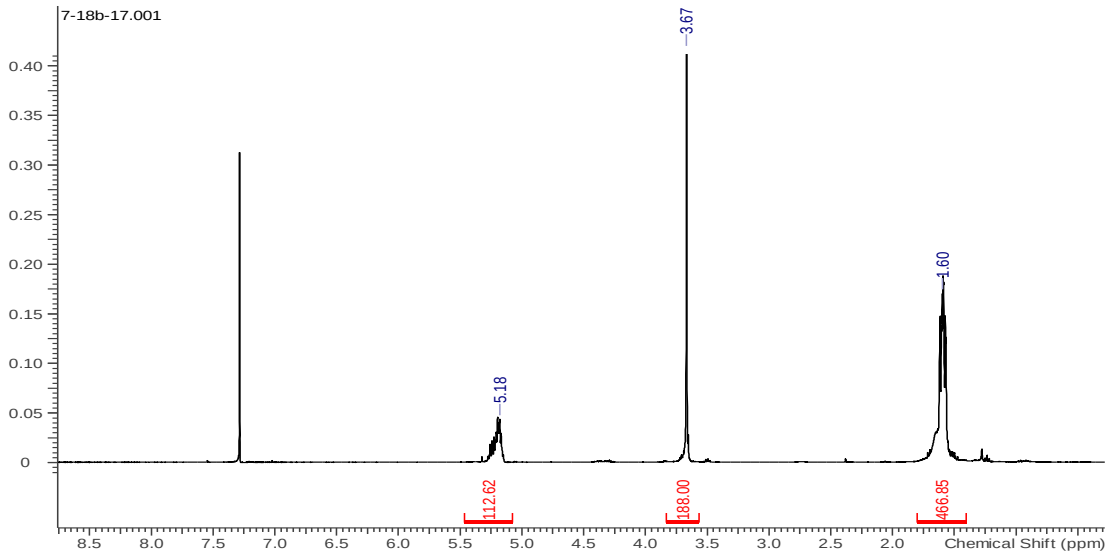
The hydroxy-PEG (2000)-azide (100 mg, 0.05mmol), D, L-lactide (480 mg, 3.36 mmol) and tin (II) ethoxyhexanoate (3 μL, 0.01 mmol) were dissolved in anhydrous toluene (5 mL), and heated at 120 ^{o}C for 24 h under nitrogen in a sealed pressure vessel. After cooling to room temperature, the reaction mixture was added dropwise to cold ether and centrifuged at 425 g for 2 minutes. The clear supernatant was decanted, and the precipitate was washed again with ether, dried under vacuum. The product was characterized by NMR spectroscopy and gel permeation chromatography for purity and molecular weight.

^{1}H NMR spectrum of N_3-PEG_{1900}-OH:



^{1}H NMR (400 MHz, chloroform-d) δ ppm: 3.74 ((O-C**H**$_2$-CH$_2$-N$_3$), t, 2H), 3.66 ((C**H**2-C**H**2-O), t, 4 H), 3.41 ((O-CH$_2$-C**H**$_2$-N$_3$), t, 2H).

^{1}H NMR spectrum of N_3-PEG_{1900}-PLA_{8000}:



^{1}H NMR (400 MHz, chloroform-d) δ ppm:5.18 ((-C**H**-C=O), q, 1 H), 3.67 ((C**H**2-C**H**2-O), t, 4 H), 1.60 ((C**H3**-CH-C=O), d, 3 H)

Determination of Copolymer Composition for N_3-PEG-PLA:

The repeating monomer number m of PLA was also estimated from ^{1}H NMR spectrum by comparing (-C**H**-C=O) (δ = 5.18 ppm) from the PLA block to $-(OCH_2CH_2)_n-$ (δ = 3.66 ppm) from the PEG block.

If m is the number of protons of -C**H**-C=O, 4n is the number of protons of $-(OCH_2CH_2)_n-$

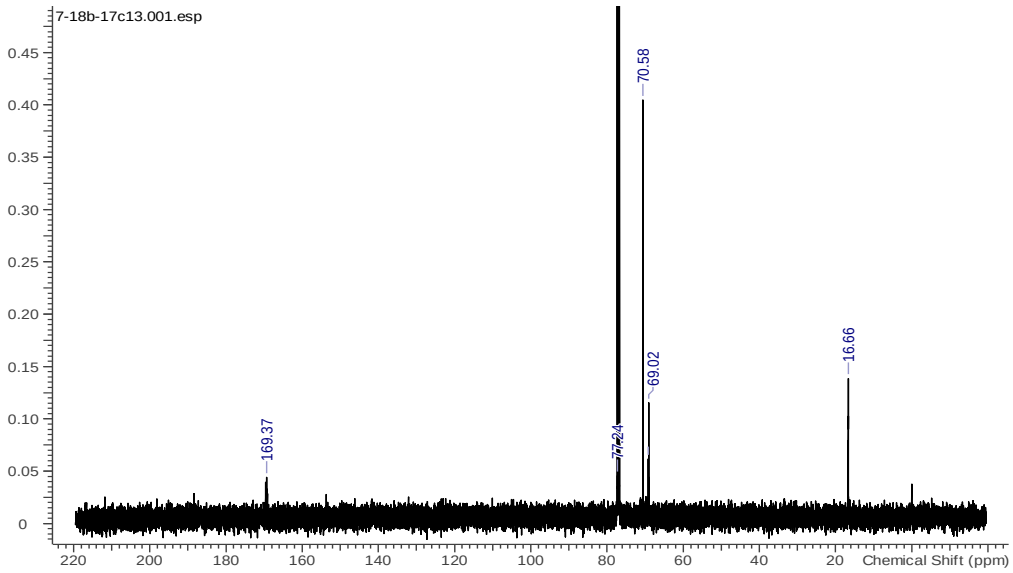
$m/4n = a_1/a_2$

a_1 = area of peak at 5.18 ppm

a_2 = area of peak at 3.67 ppm

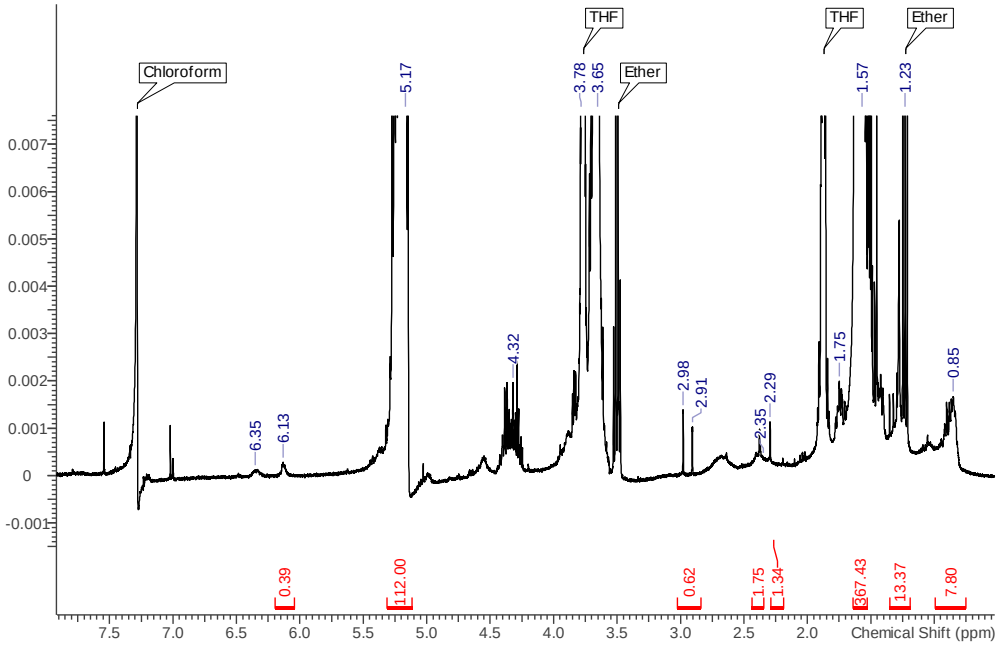
Molecular weight of PEG = 2000 (from the vendor) and n = 47. Therefore, 4n = 188, then m = 188 x a_1/a_2 = 188 x 112/188 = 112. Hence, the molecular weight of PLA = 72 x m = 8,064.

^{13}C NMR spectrum of N_3-PEG_{1900}-PLA_{8000}:



^{13}C NMR (400 MHz, chloroform-d) δ ppm: 169.37 ((-CH-C=O)), 69.02 ((-CH-C=O)), 70.58 ((CH_2-CH_2-O)), 16.66 ((CH_3-CH-C=O))

^{1}H NMR spectrum of dexamethasone–PEG$_{1900}$–PLA$_{8000}$:



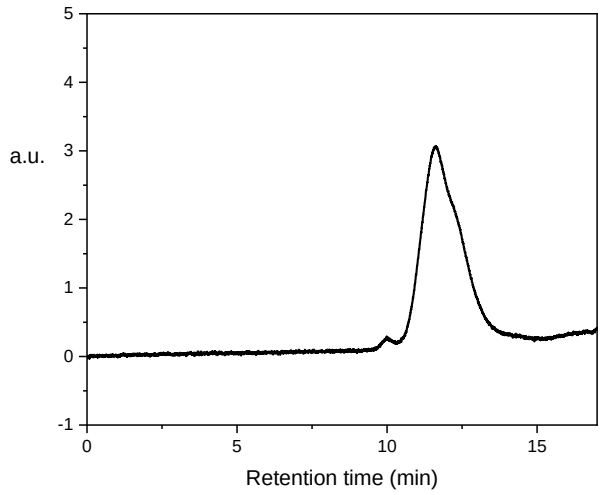
^{1}H NMR (400 MHz, chloroform-d) δ ppm: 6.35 ((CH=C**H**-C=O), d, 1H), 6.13 ((C=C**H**-C=O), d, 1H), 5.17 ((-C**H**-C=O), q, 1 H), 4.32 ((NH-C**H$_2$**-C=O), d, 2H),3.66 ((C**H$_2$**-C**H$_2$**-O), t, 4 H), 2.91 ((=C-C**H$_2$**-CH$_2$), t, 2H), 2.29 ((CH-C**H$_2$**-CH$_2$-C-OH), t, 2H),1.75 ((C**H$_3$**-CH-C=O), d, 3 H)， 1.05 ((C**H$_3$**-C-C-OH), s, 3H), 0.90 ((C**H$_3$**-CH-C-OH), s, 3H).

Conversion yield for the coupling reaction:

There is no peak at 3.09 ppm (C≡C-H); so no unreacted alkyne-dexamethasone left.

Using the integration of the peak at 6.13 ppm (from dexamethasone), we calculated the conversion of the Dex-PEG-PLA conjugates (using PLA peak at 5.17 ppm as integration reference). The conversion is 0.39/1=39%, meaning 39% of the PEG-PLA polymer has dexamethasone attached.

Gel Permeation Chromatography of DEX-PEG$_{1900}$-PLA$_{8000}$:

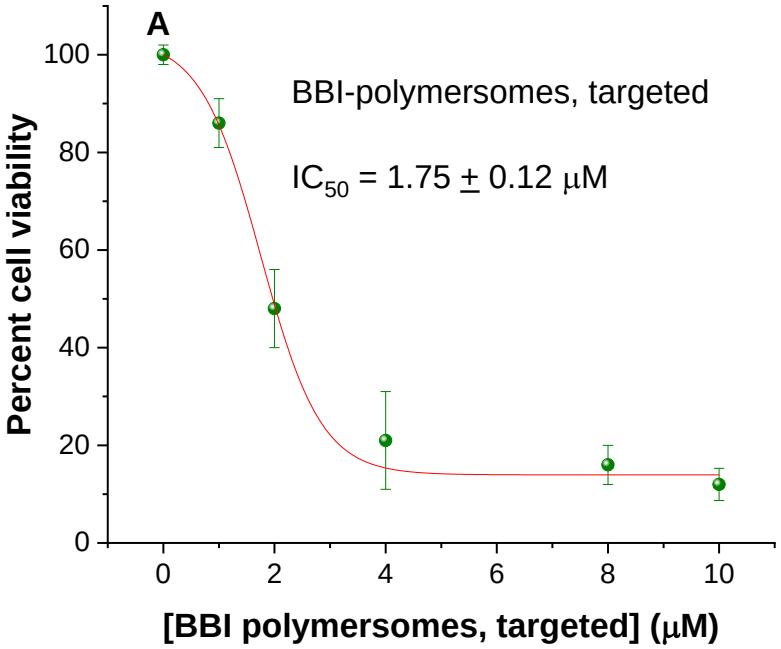


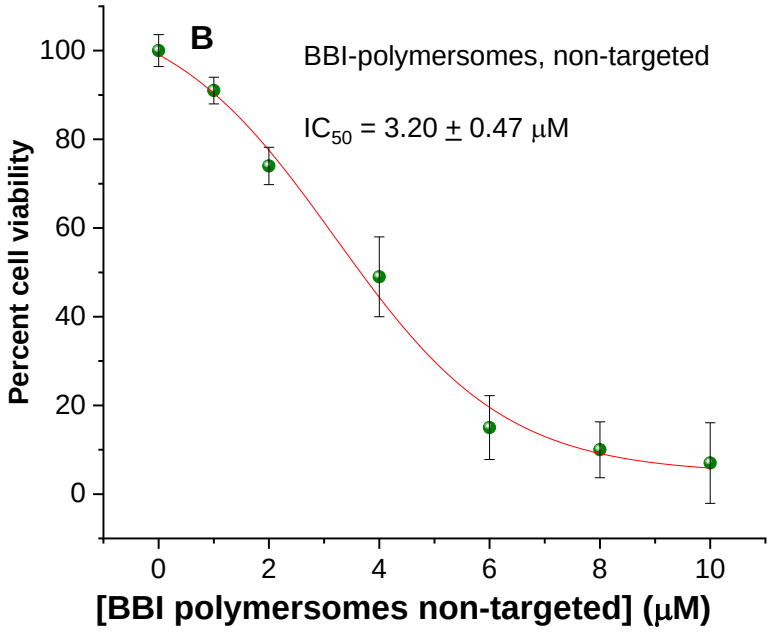
Mn	9,435
Mw	11,789
Mz	14,184
Mz+1	16,504
Mv	11,789
Mp	12,822
Mz/Mw	1.203
Mw/Mn	1.250
Mz+1/Mw	1.400

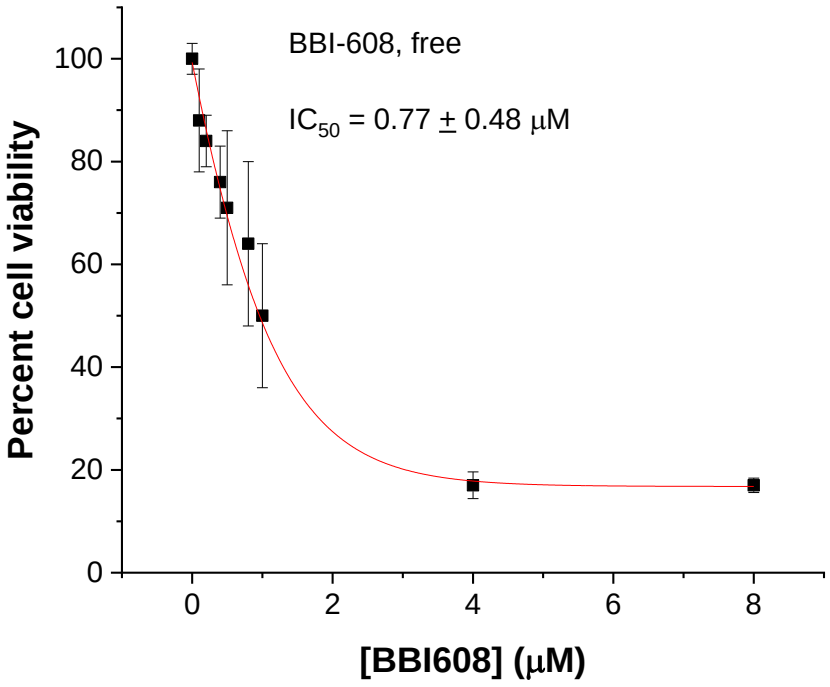
Analytical Comment

Column	TSKge; SupermultiporeHZ-M*2
Column no.	A, B
Flow rate	0.35ml/min
Detector	RI
Detector condition	pol.(+), Res.(0.5s)
Concentration	0.2wt%
Injection volume	10ul
Pressure	3.5MPa
Column temperature	40C
Pump temperature	40C
Solvent	THF

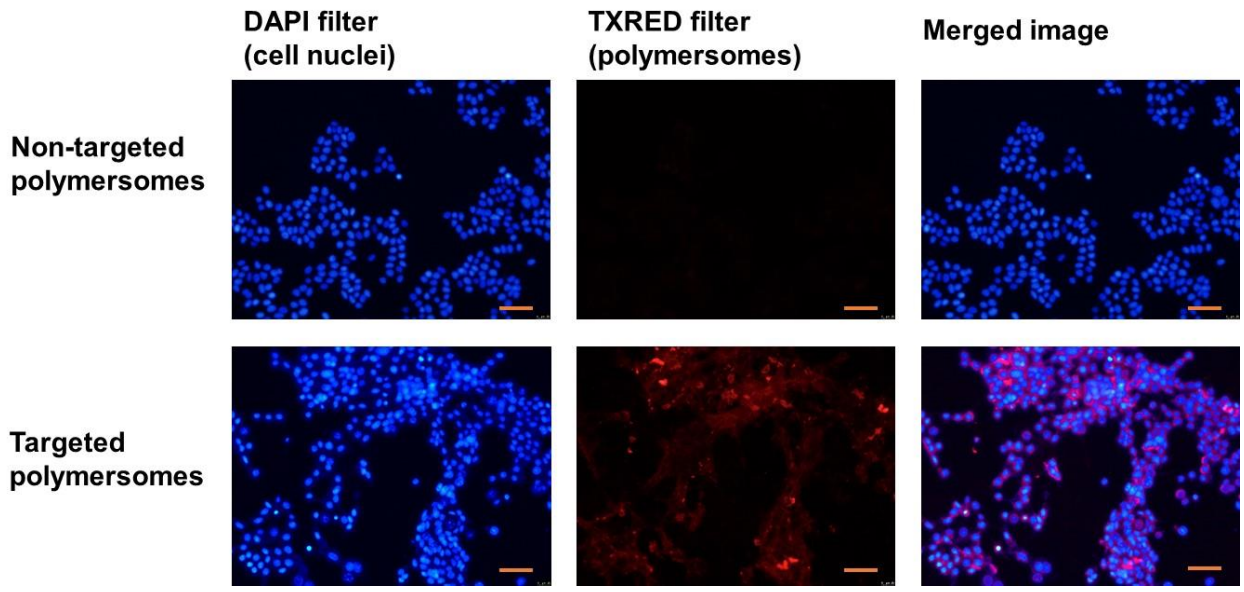
IC_{50} values of the formulations with BxPC3 pancreatic cancer cells:






BBI-608, free
IC50 = 0.77 ± 0.48 μM
Percent cell viability
[BBI608] (μM)
0
20
40
60
80
100
0
2
4
6
8

Cellular internalization studies of the polymersomes with breast cancer cells:



Cellular uptake studies with the MCF7 cells. The non-targeted polymersomes (top panel) did not enter the cell nucleus. The targeted polymersomes (bottom panel) were present in the cell nuclei after 3 hours of incubation (indicated by the overlapping blue and red colors in the Merged panel; scale bar: 50 μm).