I. Materials

Reagent grade maleic anhydride, furan, 4-dimethyl aminopyridine (DMAP), 1-(3-Dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDC), benzyl alcohol, 1,3-Di-Boc-2-(2-hydroxyethyl)guanidine, exo-5-Norbornenecarboxylic acid, 4-chloro-7-nitrobenzofurazan, N-Boc-ethylenediamine, ethyl vinyl ether, and trifluoroacetic acid (TFA) were purchased from Aldrich, Fluka, or Acros and used as received. 3rd generation Grubbs catalyst (Dichloro-di(3-bromopyridino)-N,N'-Dimesitylenoimidazolino-Ru=CHPh; G3) was synthesized as described previously by Grubbs et al (1). The ACS reagent grade solvents, ethyl acetate, tetrahydrofuran (THF), N,N-dimethylformamide (DMF), and pentane, were purchased from Fisher Scientific and used as received. Dichloromethane (DCM) (ACS reagent grade, Fisher Scientific) was distilled from CaH_2 under nitrogen. Deuterated solvents for NMR were purchased from Cambridge Isotope Laboratories. Spectra/Por® Biotech Cellulose Ester (CE) dialysis membranes with the molecular weight cut off (MWCO) of 100-500 were purchased from Spectrum Medical Industries. Media and supplements for cell culture were purchased from Lonza.

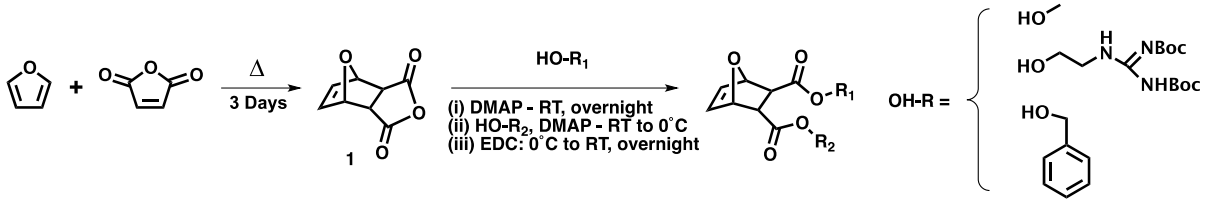
II. Instrumentation

^{1}H spectra were recorded at 300 MHz, using a Bruker DPX-300 NMR spectrometer. Chemical shifts (δ) are reported in ppm and coupling constants (*J*) in Hz. The abbreviations for splitting patterns are: s, singlet; d, doublet; dd, doublet of doublets; t, triplet; q, quartet; m, multiplet; br, broad. Gel permeation chromatography was measured on an Agilent 1260 series GPC setup with a PL Gel 5 μm guard column, two 5 μm analytical Mixed-C columns, and a 5 μm analytical Mixed-D column (Agilent), incubated at 40 °C, with RI detector. THF was used as the eluent at a flow rate of 1.0 mL/min. Polystyrene standards were used for the calibration and toluene was used as flow marker. Flow cytometry was performed on an LSRII flow cytometer and analyzed using the acquisition software FACSDiva (BD). Analysis of FACS data was performed using FlowJo (Tree Star) software. Images were taken using a FV1000 Olympus IX81 confocal laser scanning microscope (CLSM) at both 40 and 60X.

III. Synthesis

A. Monomers

All five monomers were synthesized following the procedure introduced by Lienkamp et al (2). In general, the Diels-Alder adduct **1** was obtained by the reaction of maleic anhydride with furan in toluene. As following Lienkamp et al., the monomers were synthesized from **1** under similar conditions:



dG: 1 was dissolved in the minimum amount of dry DCM together with 2 eq. of 1,2-Di-Boc-2-ethyl guanidine, and 10 mol% 4-dimethylaminopyridine (DMAP). The temperature was lowered to 0°C with an ice bath and 1 eq. of 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide

(EDC) was added, then stirred over night at room temperature. After completion of the reaction, the solution was concentrated and the product was purified via column chromatography with silica gel using DCM/ethyl acetate (8:2) as eluent. Vacuum evaporation of the solvent yielded the pure product with a yield of ~60% (3).

1H NMR (300 MHz, CD3CN): δ 11.54 (s, 2H), 8.35 (s, 2H), 6.44 (s, 2H), 5.17 (s, 2H), 4.33 – 3.97 (m, 4H), 3.72 – 3.41 (m, 4H), 2.82 (s, 2H), 1.48 (s, 9H), 1.42 (s, 9H); 13C NMR (75 MHz, CD3CN): δ 172.43, 164.51, 157.35, 153.72, 137.56, 84.05, 81.41, 79.50, 63.72, 47.49, 40.21, 28.38, 28.09; HR-MS (FAB) m/z [M+H]+: 755.3827 (calc.), 755.3824 (found).

MeG: 1 was dissolved in the minimum amount of dry DCM together with 1 eq. of 1,2-Di-Boc-2-ethyl guanidine, and 10 mol% DMAP The temperature was lowered to 0°C with an ice bath and 1 eq. of EDC was added, then stirred over night at room temperature. The half ester was precipitated from solution, isolated using vacuum filtration, and washed with cold DCM followed by drying on vacuum overnight. The half ester, one equivialent of MeOH. and 10% DMAP were dissolved in DCM and stirred at RT under nitrogen. After completion of the reaction, the solution was concentrated and the product was purified via column chromatography with silica gel using DCM/ethyl acetate (8:2) as eluent. Vacuum evaporation of the solvent yielded the pure product with a yield of ~80% (4).

1H-NMR (300 MHz, CDCl3): δ=11.50 (1H, s), 8.55 (1H, s), 6.46 (2H, s), 5.3 (2H, d, J=6.0 Hz), 4.25 (2H, m), 3.72 (5H, m), 2.84 (2H, s), 1.49 (18H, s).13C-NMR (75 MHz, CDCl3): δ= 171.7, 171.5, 163.4, 156.3, 153.1, 136.6, 83.2, 80.7, 80.6, 79.4, 63.5, 52.4, 47.1, 46.6, 39.4, 28.3, 28.1. HR-MS (FAB): calc. 483.22, found 484.23.

dPh: 1 was dissolved in the minimum amount of dry DCM together with 2 eq. of benzyl alcohol, and 10 mol% DMAP. The temperature was lowered to 0°C with an ice bath and 1 eq. of EDC was added, then stirred over night at room temperature. After completion of the reaction, the solution was concentrated and the product was purified via column chromatography with silica gel using DCM/ethyl acetate (8:2) as eluent. Vacuum evaporation of the solvent yielded the pure product with a yield of ~88% (3).

1H NMR (300 MHz, CD3CN): δ 7.34 (d, J = 3.0 Hz, 10H), 6.44 (s, 2H), 5.16 (s, 2H), 4.97 (dd, J = 45.5, 12.4 Hz, 4H), 2.87 (s, 2H); ^{13}C NMR (75 MHz, CD3CN): δ 172.37, 137.52, 137.03, 129.41, 129.14, 129.06, 81.30, 67.26, 47.58; HR- MS (FAB) m/z [M+H]+: 365.1389 (calc.), 365.1398 (found).

MePh: One equivalent of **1** and 1.25 equivalents of the corresponding substituted benzyl alcohol, and 10 mol% DMAP were dissolved in minimal amounts of distilled DCM and the reaction mizture was stired under nitrogen at room temperature over night. The half ester was precipitated from solution, isolated using vacuum filtration, and washed with cold DCM followed by drying on vacuum overnight. The half ester, one equivialent of MeOH. and 10% DMAP were dissolved in DCM and stirred at RT under nitrogen. The solution was then cooled down to 0˚C and one equivalent of EDC was added. The solution as stirred over night under nitrogen at room temperature. The reaction mixture was then collected by rotoevaporation and purified by column chromatography with DCM/ethyl acetate (8:2). the sample was dried under vacuum over night to obtain a pure white solid (yield 82%) (5).

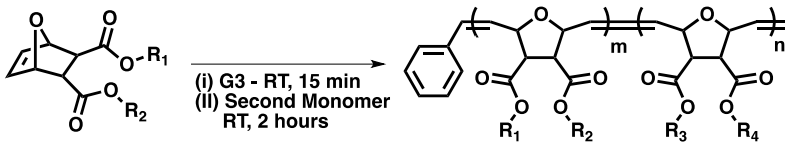
1H NMR (300 MHz, CD3CN): δ = 7.38 (comp, 5H), 6.44 (comp, 2H), 5.14 (d, 2H), 5.06 (comp, 2H), 3.50 (s, 3H), 2.84 (q, 2H).13C NMR (75 MHz, CD3CN): δ = 173.35, 172.84, 137.95, 137.91, 137.51, 129.84, 129.60, 129.50, 81.64, 81.63, 67.72, 52.75, 47.96, 47.89. HR-MS (FAB) m/z [M+H]+: 289.1076 (calc.), 289.1078 (found).

dMe: 1 was dissolved in the minimum amount of dry DCM together with 2 eq. of MeOH, and 10 mol% DMAP. The temperature was lowered to 0°C with an ice bath and 1 eq. of EDC was added, then stirred over night at room temperature. After completion of the reaction, the solution was concentrated and the product was purified via column chromatography with silica gel using DCM/ethyl acetate (8:2) as eluent. Vacuum evaporation of the solvent yielded the pure product with a yield of ~84% (6).

1H NMR (500 MHz, DMSO): δ = 6.46 (s, 2H), 5.11 (s, 2H), 3.55 (s, 6H), 2.82 (s, 2H). 13C NMR (125 MHz, DMSO): δ = 171.70, 136.61, 79.74, 51.63, 46.23. HR-MS (FAB) m/z [M+H]+: 213.0763 (calc.), 213.0749 (found).

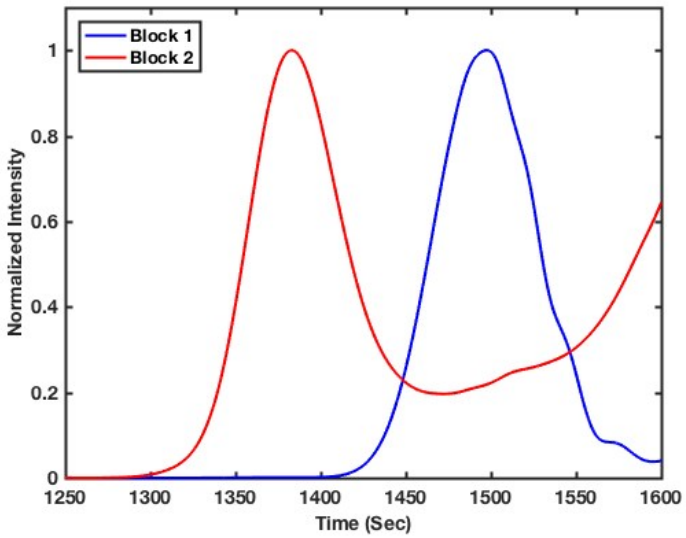
A. Polymers

Reaction conditions for block copolymer synthesis: Monomer **dG**, monomer **MePh**, and G3 catalyst were dissolved in dry DCM in respective schlenk flasks, purged with nitrogen, and subjected to three freeze-pump-thaw cycles. Monomer **dG** solution was added into the catalyst solution via syringe all at one time. The brown solution was stirred for 30 minutes at room temperature before monomer **MePh** was introduced. After stirring for an additional 2 hours, the reaction was terminated with 1 mL of ethyl vinyl ether and stirred for 30 minutes. DCM was evaporated and the product was redissolved in minimal DCM and loaded on a short silica gel column (7 cm length, 3 cm diameter). The unreacted end-group and any side products were washed from the column with DCM, while polymer remained on the column and was recovered with ethyl acetate. Ethyl acetate was evaporated to yield the pure product.

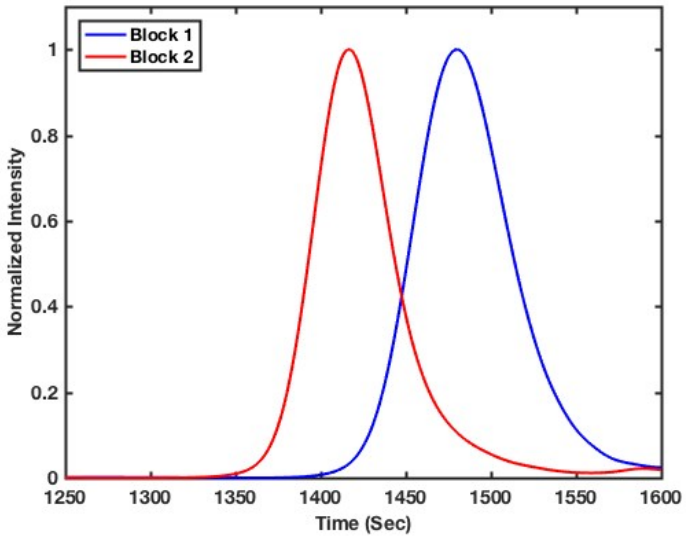


dPh$_5$-*b*-dG$_5$:

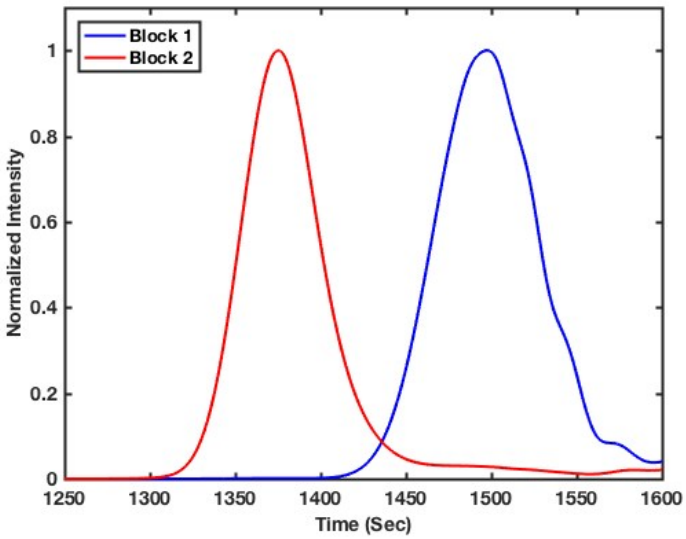
^{1}H-NMR (500 MHz, $(CD_3)_2CO$): δ 11.66 (2H, br), 8.47 (2H, br), 7.32 (12H, br), 5.98 (trans) and 5.68 (cis) (4H total, br), 5.21 (4H, br), 5.01 (cis) and 4.74 (trans) (4H total, br), 4.25 (4H, br), 3.69 (4H, br), 3.27 (4H, br), 1.46 (18H, s), 1.41 (18H, s).



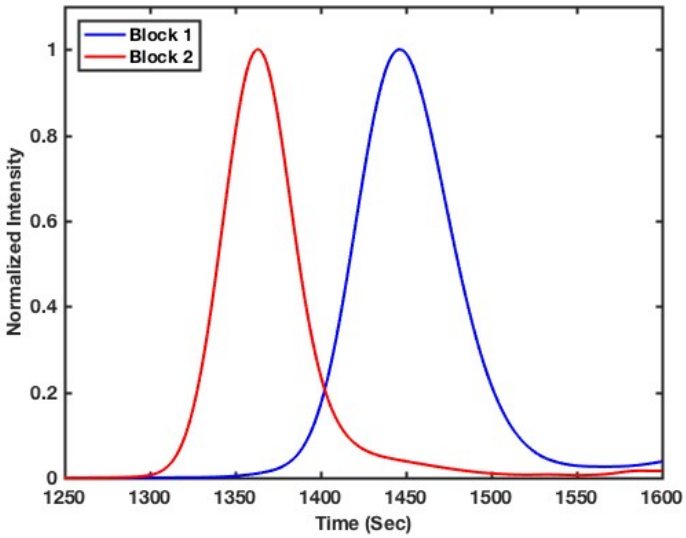
$MePh_{10}$-*b*-dG_5:
^{1}H-NMR (500 MHz, CDCl3): δ 11.57 (2H, br), 8.40 (2H, br), 7.37 (6H, br), 5.80 (trans) and 5.61 (cis) (4H total, br), 5.08 (2H, br), 5.00 (cis) and 4.77 (trans) (4H total, br), 4.66 (2H, br), 4.17 (4H, br), 3.64 (4H, br), 3.49 (3H, br), 3.17 (4H, br), 1.46 (18H, s), 1.41 (18H, s).



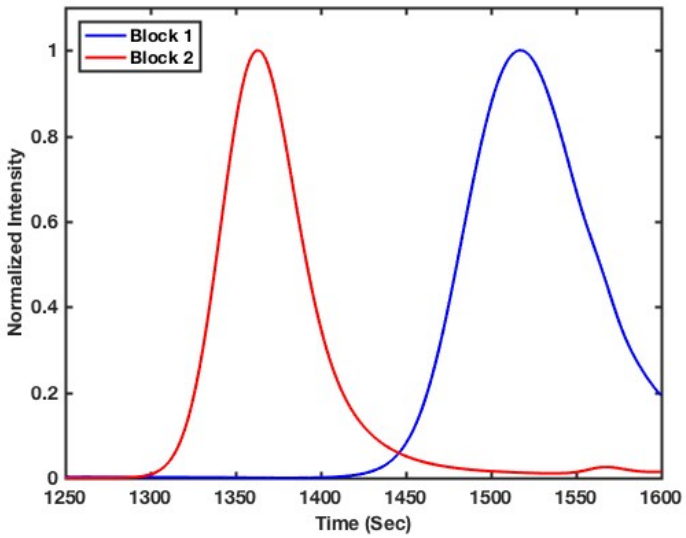
dPh_5-*b*-MeG_{10}:
^{1}H-NMR (500 MHz, CDCl3): δ 11.52 (1H, br), 8.31 (1H, br), 7.33 (12H, br), 5.84 (trans) and 5.60 (cis) (4H total, br), 4.99 (4H, br), 4.90 (cis) and 4.60 (trans) (4H total, br), 4.17 (2H, br), 3.62 (3H, br), 3.49 (2H, br), 3.13 (4H, br), 1.44 (9H, s), 1.41 (9H, s).



$MePh_{10}$-*b*-MeG_{10}:
^{1}H-NMR (500 MHz, CDCl3): δ 11.55 (1H, br), 8.42 (1H, br), 7.34 (6H, br), 5.82 (trans) and 5.61 (cis) (4H total, br), 5.03 (2H, br), 5.00 (cis) and 4.76 (trans) (4H total, br), 4.59 (2H, br), 4.14 (6H, br), 3.62 (2H, br), 3.51 (4H, br), 3.14 (9H, br), 1.44 (9H, s), 1.40 (18H, s).

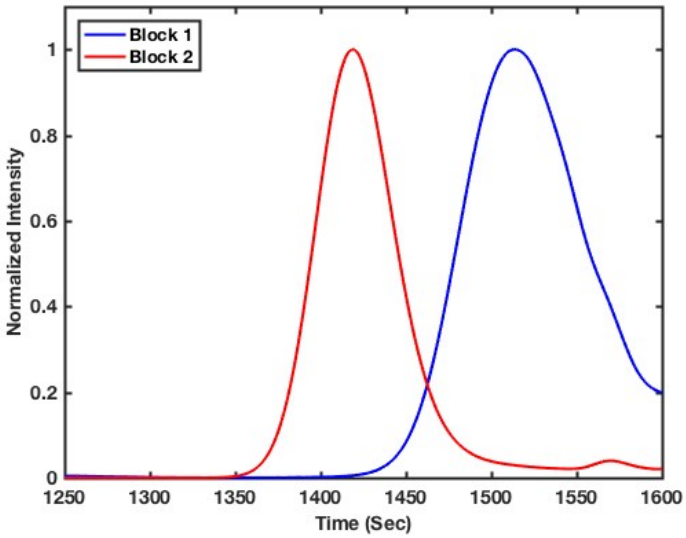


dPh_{10}-*b*-dG_5:
^{1}H-NMR (500 MHz, $(CD_3)_2CO$): δ 11.56 (2H, br), 8.39 (2H, br), 7.33 (12H, br), 5.88 (trans) and 5.62 (cis) (4H total, br), 5.10 (2H, br), 5.03 (cis) and 4.66 (trans) (4H total, br), 4.18 (2H, br), 3.60 (4H, br), 3.15 (4H, br), 1.46 (18H, s), 1.43 (18H, s).



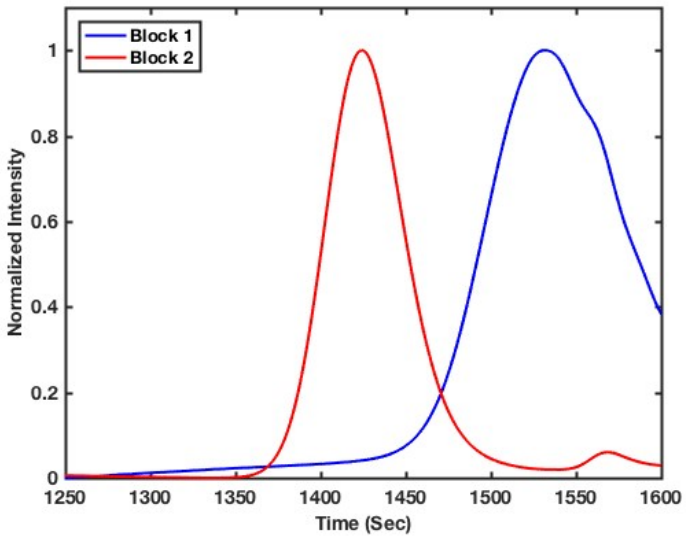
$MePh_5$-*b*-dG_5:

^{1}H-NMR (500 MHz, $(CD_3)_2CO$): δ 11.66 (2H, br), 8.47 (2H, br), 7.32 (6H, br), 5.98 (trans) and 5.68 (cis) (4H total, br), 5.21 (4H, br), 5.01 (cis) and 4.74 (trans) (4H total, br), 4.25 (4H, br), 3.69 (4H, br), 3.27 (4H, br), 1.46 (18H, s), 1.41 (18H, s).

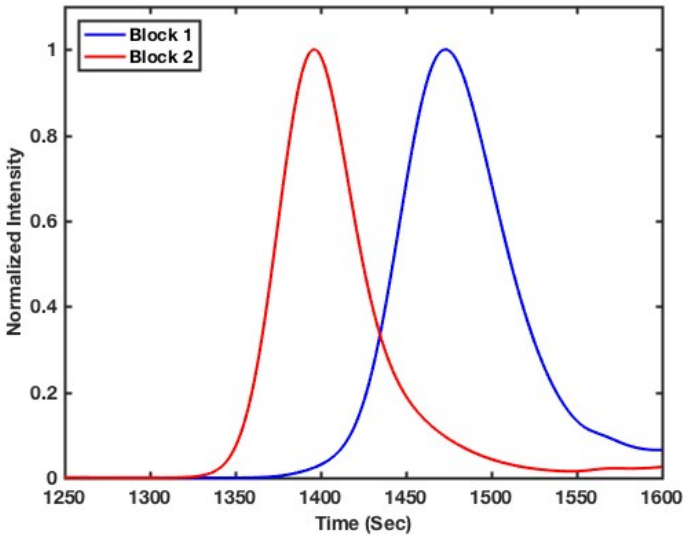


dMe_5-*b*-dG_5:

^{1}H-NMR (500 MHz, $(CD_3)_2CO$): δ 11.59 (2H, br), 8.39 (2H, br), 5.93 (trans) and 5.67 (cis) (4H total, br), 5.08 (4H, br), 4.90 (cis) and 4.70 (trans) (4H total, br), 4.23 (6H, br), 3.65 (4H, br), 3.18 (4H, br), 1.46 (18H, s), 1.41 (18H, s).



dMe_{10}-*b*-dG_5:
^{1}H-NMR (500 MHz, $(CD_3)_2CO$): δ 11.56 (2H, br), 8.38 (2H, br), 5.89 (trans) and 5.59 (cis) (4H total, br), 5.07 (4H, br), 5.96 (cis) and 4.69 (trans) (4H total, br), 4.21 (6H, br), 3.61 (4H, br), 3.22 (4H, br), 1.51 (18H, s), 1.47 (18H, s).



Polymer	M_n	M_w	M_p	PDI
dPh_5-b-dG_5	9488	10123	10103	1.067
$MePh_{10}$-b-dG_5	5612	6071	6166	1.082
dPh_5-b-MeG_{10}	9965	10609	10782	1.065
$MePh_{10}$-b-MeG_{10}	11063	12060	12277	1.090
dPh_{10}-b-dG_5	9496	10265	10565	1.081
$MePh_5$-b-dG_5	5412	5744	5743	1.061
dMe_5-b-dG_5	5078	5392	5438	1.062
dMe_{10}-b-dG_5	6366	7011	7380	1.10

B. Deprotection

The polymers were dissolved in 2 mL DCM and 2 mL trifluoroacetic acid (TFA) for deprotection. After stirring overnight, the excess acid was removed by azeotropic distillation with methanol. After complete evaporation of the acid, samples were dissolved in water/methanol mixture and dialyzed against RO water until the conductivity of water was ~0.1µS. The deprotected copolymer was recovered by lyophilization. Final deprotected polymer was stored at -20 ºC.

1. Love JA, Morgan JP, Trnka TM, & Grubbs RH (2002) A practical and highly active ruthenium-based catalyst that effects the cross metathesis of acrylonitrile. *Angew. Chem., Int. Ed.* 41(21):4035-4037.
2. Lienkamp K, *et al.* (2008) Antimicrobial polymers prepared by ROMP with unprecedented selectivity: A molecular construction kit approach. *J. Am. Chem. Soc.* 130(30):9836-9843.

3. Sgolastra F, Minter LM, Osborne BA, & Tew GN (2014) The Importance of Sequence Specific Hydrophobicity in Synthetic Protein Transduction Domain Mimics. *Biomacromolecules*.
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5. deRonde BM, Torres JA, Minter LM, & Tew GN (2015) Development of Guanidinium-Rich Protein Mimics for Efficient siRNA Delivery into Human T Cells. *Biomacromolecules 16*, 3172–9.
6. deRonde, BM, Posey, ND, Otter, R, Caffrey, LM, Minter, LM, & Tew, GN (2016). Optimal Hydrophobicity in Ring-Opening Metathesis Polymerization-Based Protein Mimics Required for siRNA Internalization. *Biomacromolecules 17*, 1969–77.

IV. Supplemental Figures

A. DLS

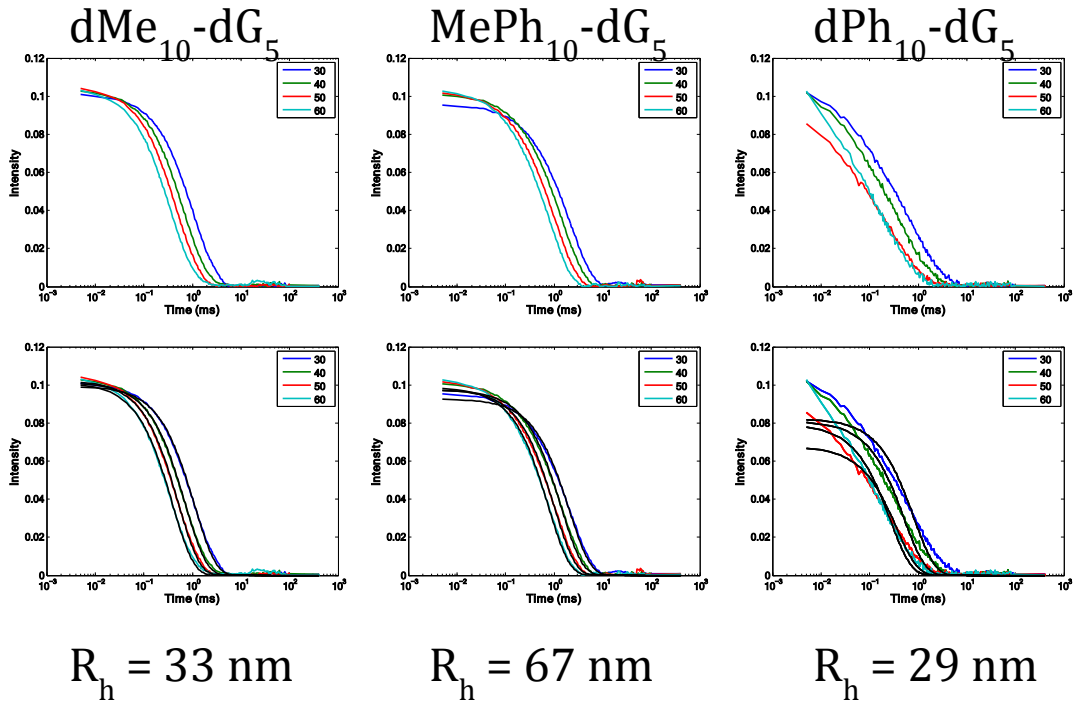

Figure 1. Dynamic light scattering curves (top) for dMe10-dG5, MePh10-dG5, and dPh10-dG5 overlayed with their CONTIN fits (bottom) used to predict the radius of hydration. Measurements were taken at 30˚, 40˚, 50˚, and 60˚ and used together for the prediction.

B. Viability

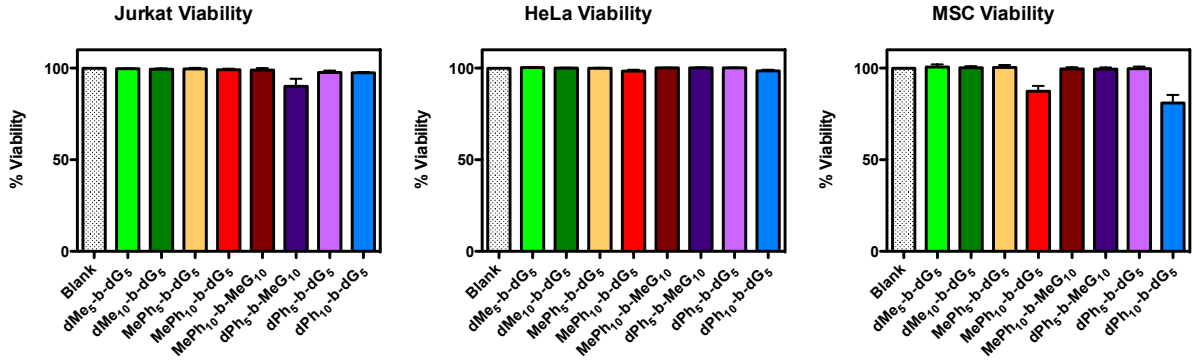

Figure 2. Cellular viability after cells were treated with 2 µg of protein at a 20:1 polymer to protein molar ratio for 4 hours, as determined by 7AAD staining compared with an untreated sample. Viability is above 90% in all cases, except with the $MePh_{10}$-dG_5 and dPh_{10}-dG_5 in the hTERT MSCs which are higher than 85%.