Electronic Supplementary Material (ESI) for ChemComm.

Supporting Information for:

An organometallic swap strategy for bottlebrush polymer–protein conjugate synthesis

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1. Supplementary Figures

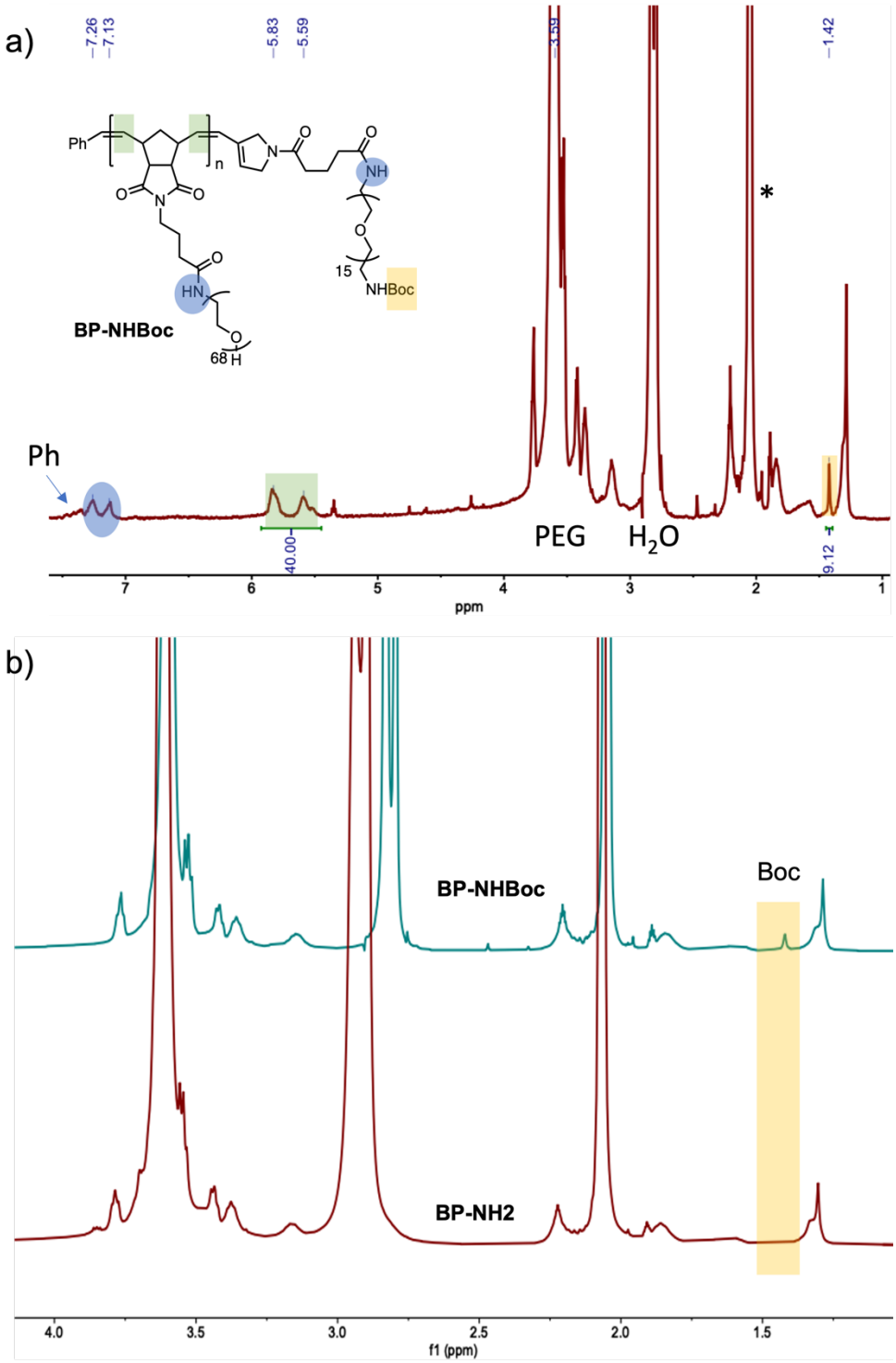

Figure S1. a) Detailed analysis of ^{1}H NMR spectrum of BP-NHBoc. * Indicates the solvent residue from acetone-d_6. b) ^{1}H NMR spectra comparison of BP-NHBoc and BP-NH_2. The results showed the clear disappearance of the Boc group after deprotection.

Sample						105 Pd [He-mode]			
Rjct	Data File	Acq. Date-Time	Type	Level	Sample Name	CPS	CPS RSD	Conc. [ppb]	Conc. RSD
####	001SMPL.d	6/3/21 16:29	Sample		blank	17816.82667	2.5418291		
####	002SMPL.d	6/3/21 16:32	Sample		blank	6508.966667	1.7968745		
####	003SMPL.d	6/3/21 16:34	Sample		blank	5216.196667	1.3343067		
####	004CALB.d	6/3/21 16:36	CalBlk	1	0ppb	4597.09	6.7075659	0	N/A
####	005CALS.d	6/3/21 16:39	CalStd	2	10ppb	245906.07	0.5848353	8.897748308	0.595976742
####	006CALS.d	6/3/21 16:41	CalStd	3	50ppb	1227961.723	0.5539796	45.10893294	0.556061346
####	007CALS.d	6/3/21 16:43	CalStd	4	100ppb	2665845.36	1.196253	98.12779156	1.198319398
####	008CALS.d	6/3/21 16:45	CalStd	5	500ppb	13342902.43	0.5394997	491.8212483	0.539685646
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####	010CALS.d	6/3/21 16:50	CalStd	7	5000ppb	135674645	1.2987365	5002.540473	1.298780529
####	011SMPL.d	6/3/21 16:52	Sample		blank	60580.91667	0.4849731	2.064282892	0.524796472
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####	024SMPL.d	6/3/21 17:22	Sample		blank	26583.39333	1.2794469	0.810697527	1.546964907

Figure S2. ICP-MS analysis of the Pd content on each chain of BP-[Pd]. We used a BP-[Pd] sample with theoretical concentration of 1000 ppb for measurements. After calibration with standard solutions, our sample showed a concentration of 1086 ppb. These results suggested the high fidelity of the Pd OACs content on each bottle brush polymer chain end.

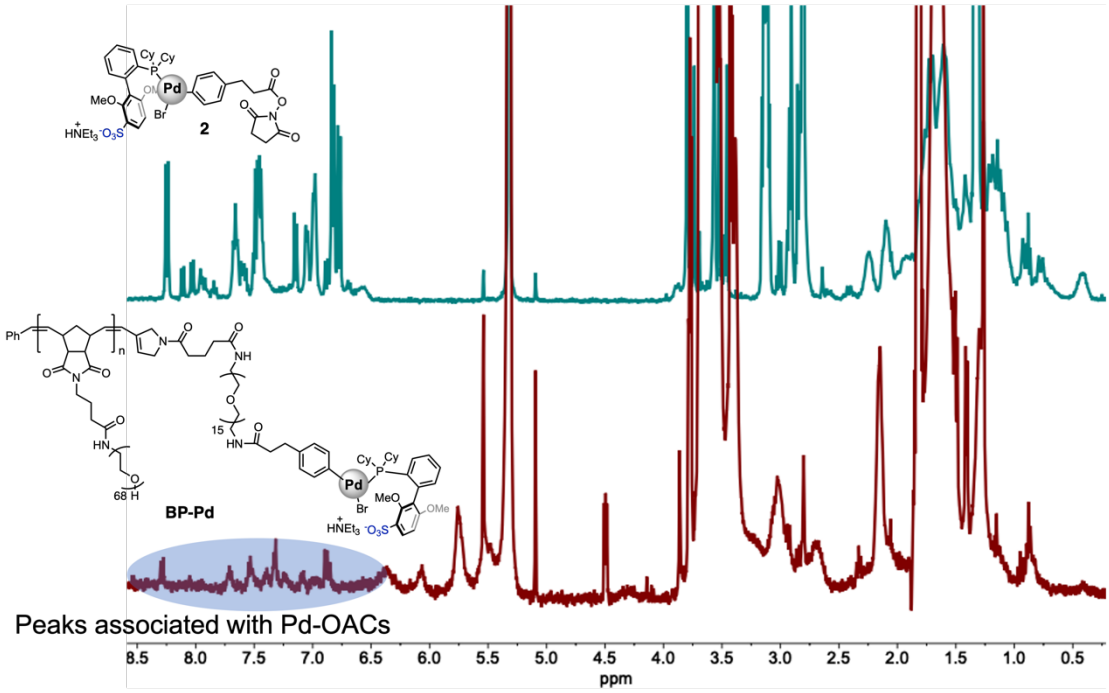


Figure S3. ^{1}H NMR spectra of BP-Pd and Pd-OACs compound **2**. The results showed the clear presence of the Pd-OACs signals in the BP-Pd sample.

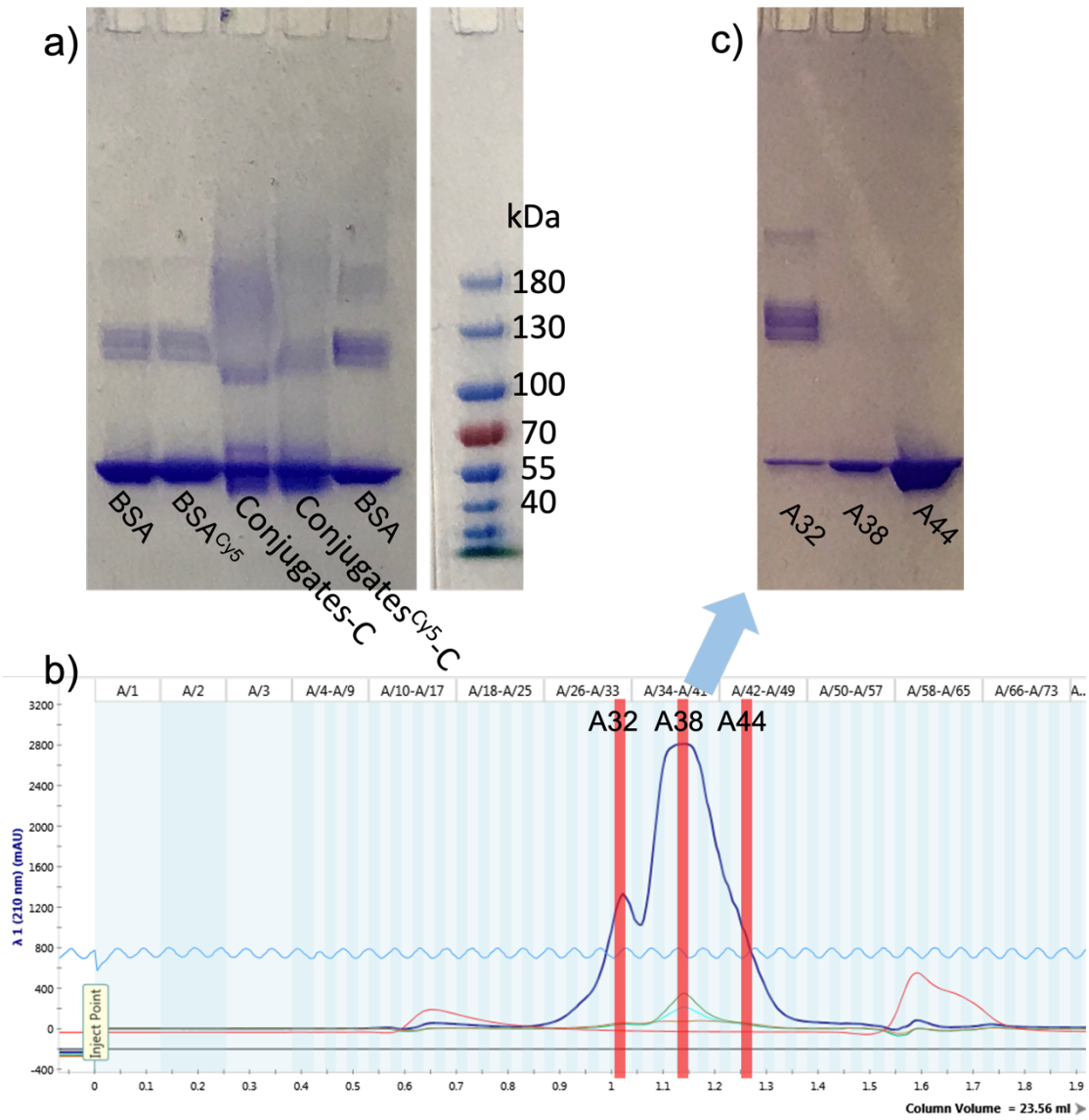


Figure S4. a) SDS PAGE gel for monitoring the conjugation process. Conjugates-C presents crude product from the conjugation reaction between BSA and BP, and Conjugates^{Cy5}-C represents crude product from the conjugation reaction between BSA^{Cy5} and BP. b) FPLC trace for BSA under an SEC column. c) SDS-PAGE gel of BSA at different fractions after SEC. BSA protein dimer is presented at ~120 kDa in the SDS-PAGE gel.

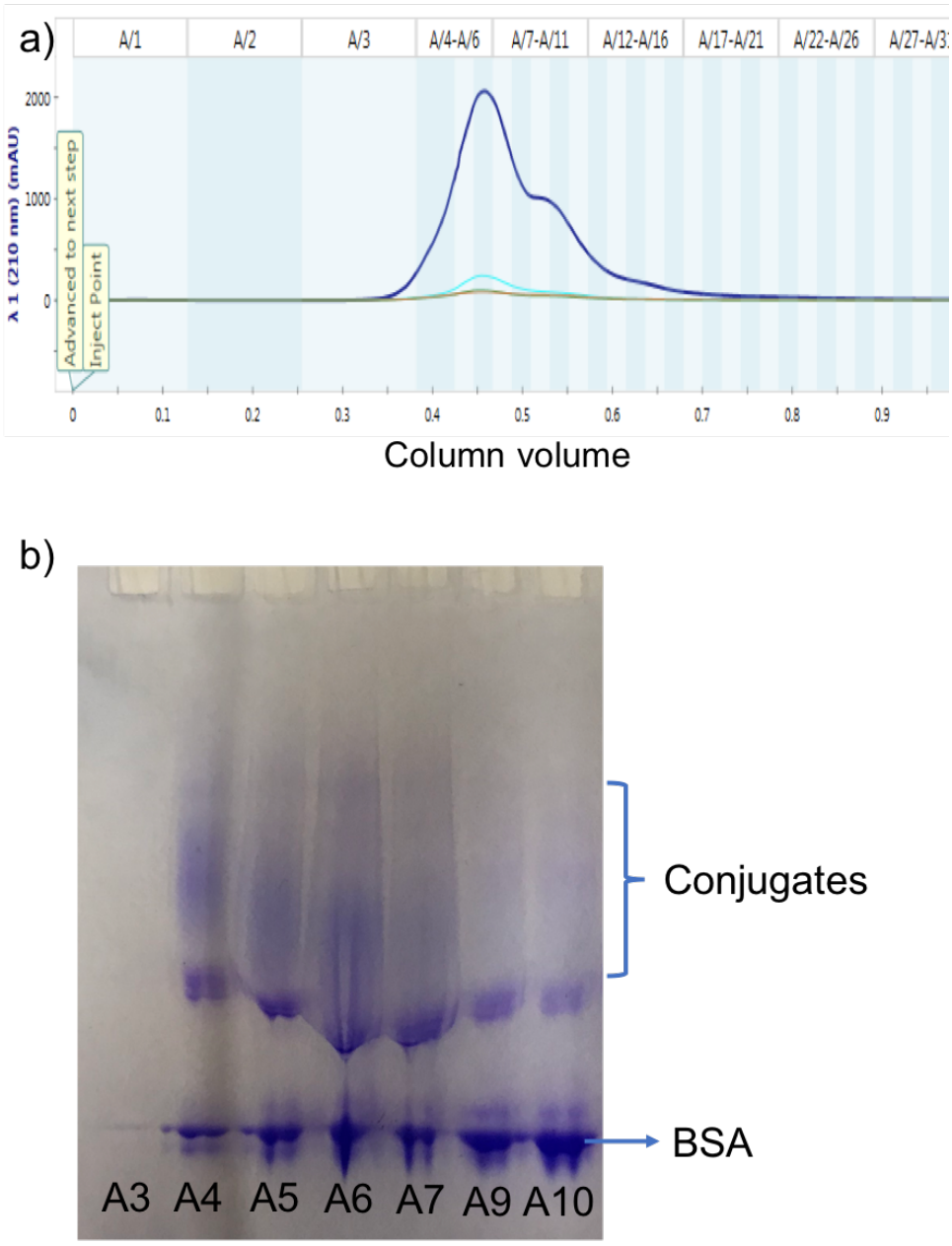


Figure S5. a) SEC trace for crude conjugates. b) SDS-PAGE gels for each of the fractions from (a). The SEC trace showed the difficulty in separation between polymer, protein, and conjugation. Further, SDS-PAGE gel showed the mixture of BSA and conjugates for different fractions, where the free polymer cannot be stained.

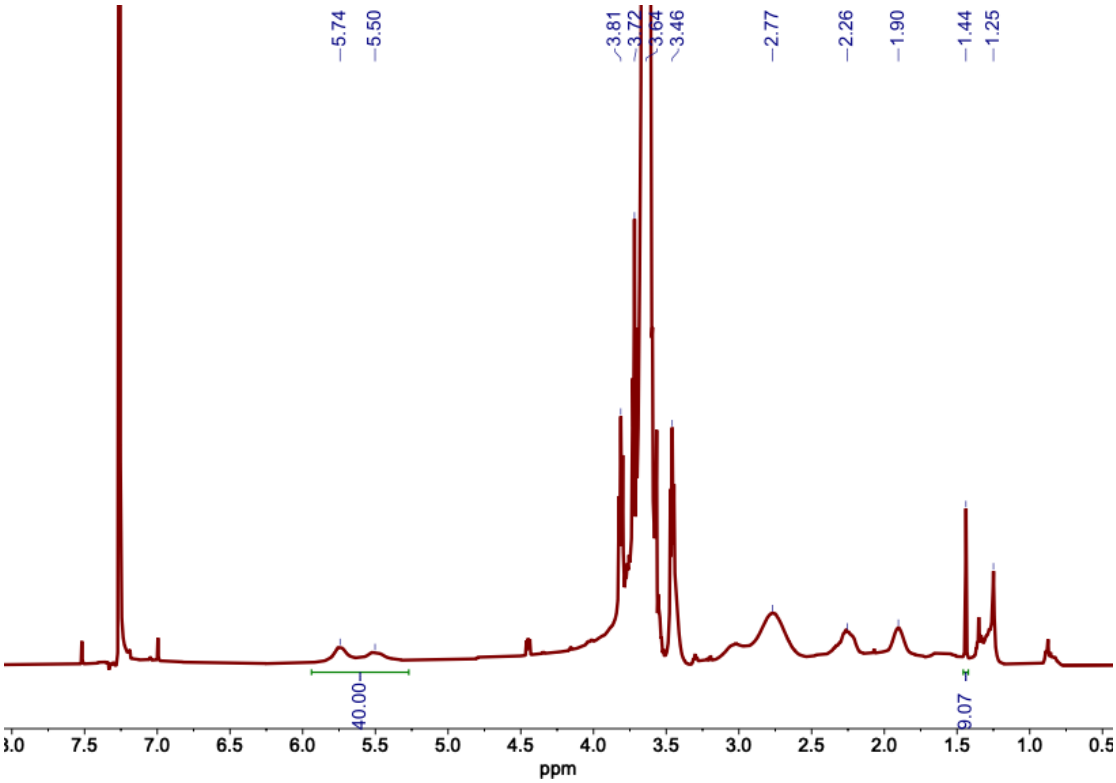


Figure S6. ^{1}H NMR spectra of **BP^{Cy3}-NHBoc**. The ratio of the integral of the backbone olefinic resonances to the Boc resonance is 40:9, which is consistent with the theoretical backbone degree of polymerization of 20.

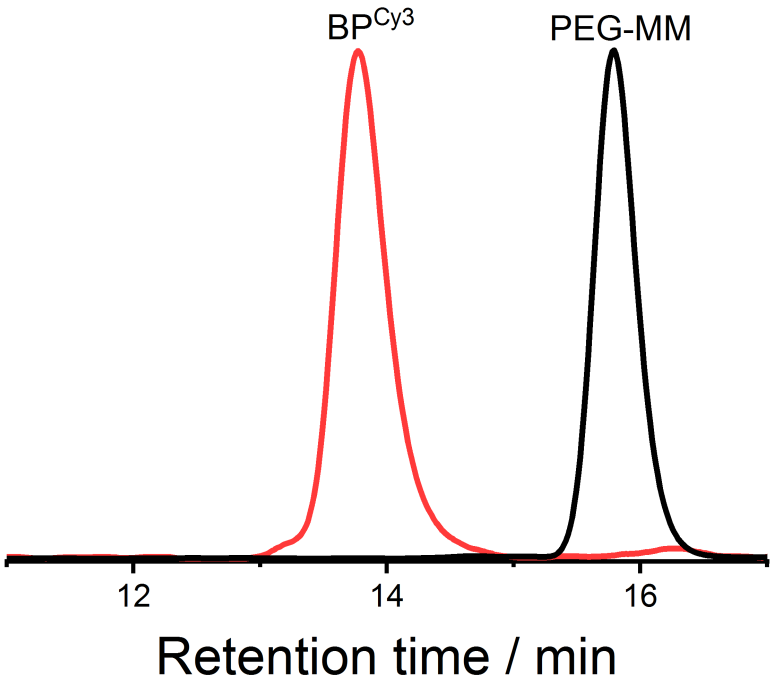


Figure S7. GPC traces of PEG-MM and BP^{Cy3}.

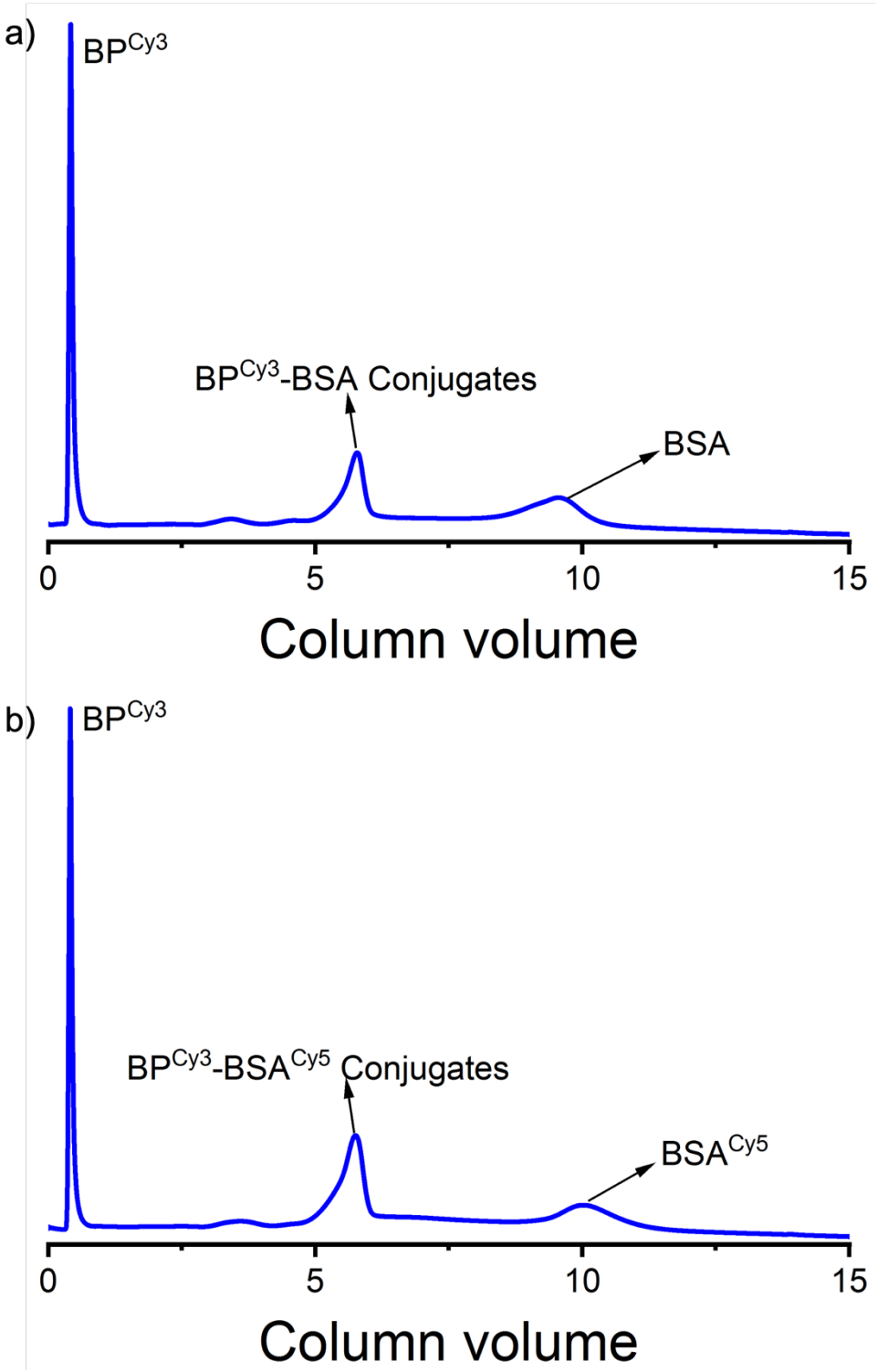


Figure S8. FPLC purification of conjugates from Cy3 dye-labeled BP. a) **BP^{Cy3}-BSA** conjugates FPLC trace. b) **BP^{Cy3}-BSA^{Cy5}** conjugates FPLC trace.

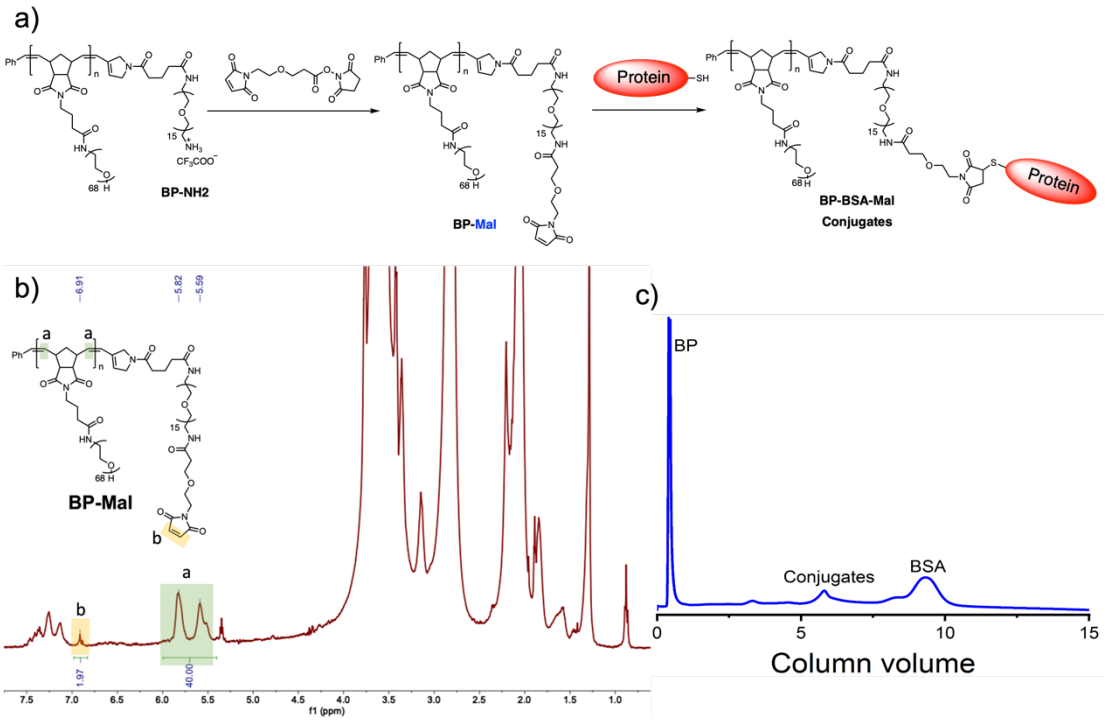


Figure S9. Synthesis of **BP-BSA-Mal**. a) The synthesis scheme for **BP-Mal** and **BP-BSA-Mal** from the thiol-maleimide reaction. b) ^{1}H NMR spectra of **BP-Mal**. The ratio of the integral of the backbone olefinic resonances to the maleimide resonance is 40:2, suggesting quantitative terminal functionalization of the BP with the maleimide group. c) FPLC trace for purification of **BP-BSA-Mal** conjugates.

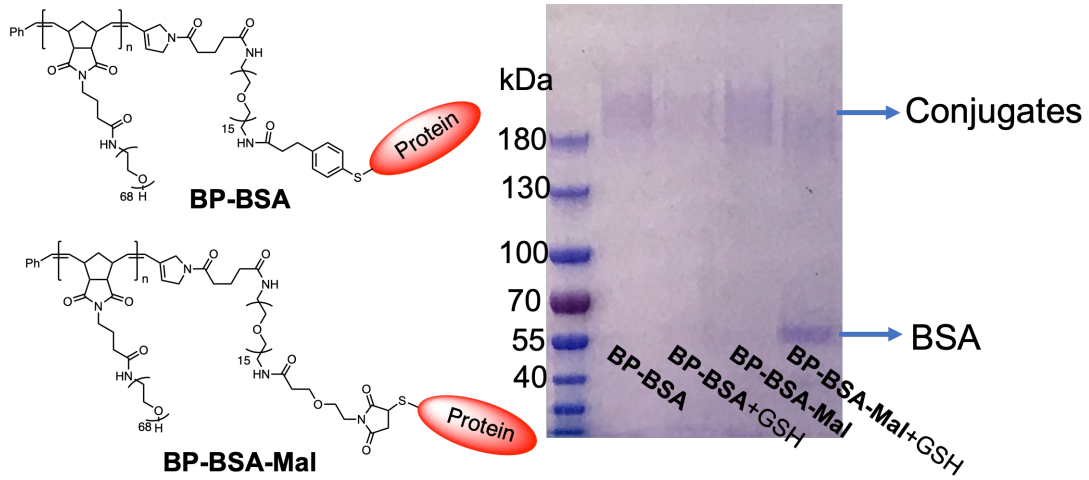


Figure S10. Stability study of the conjugates. Both conjugates were incubated with 10 mM GSH for 2 days.

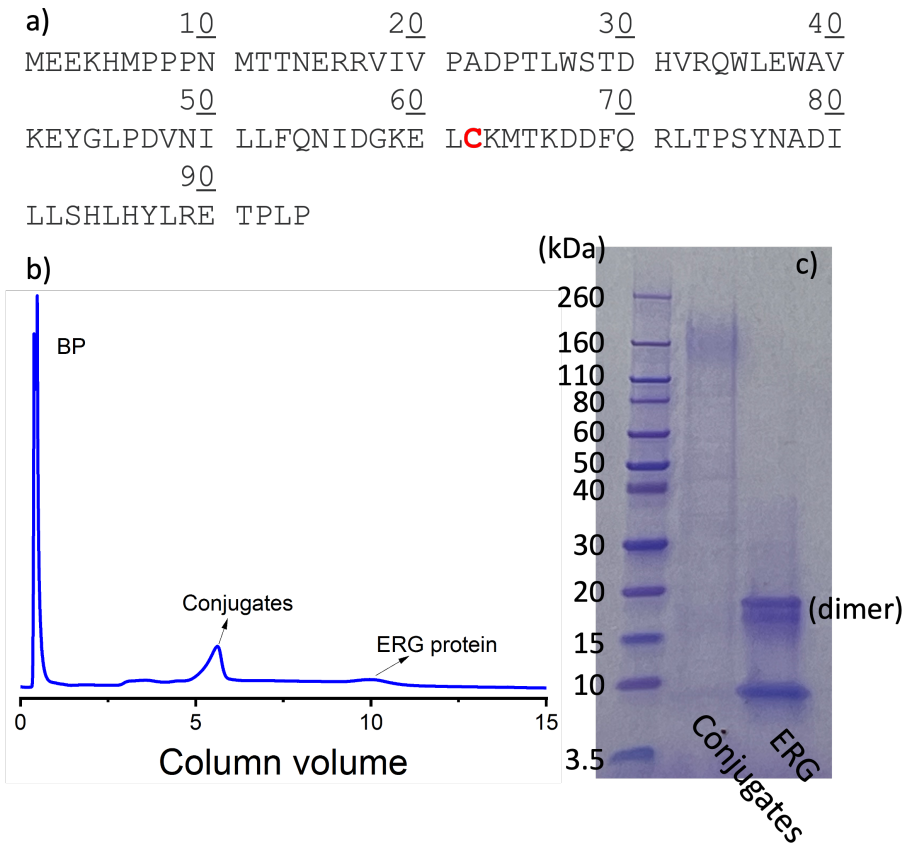


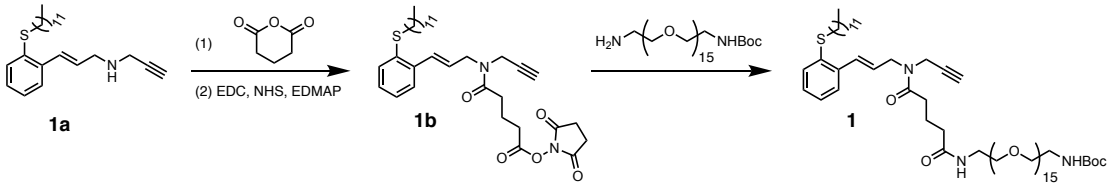
Figure S11. Characterizations of **BP-ERG** conjugates. a) Sequence of ERG protein. b) FPLC trace for **BP-ERG** conjugates separation under an anionic exchange column. c) SDS PAGE gel electrophoresis.

2. Experimental Section

Materials and instrumentation

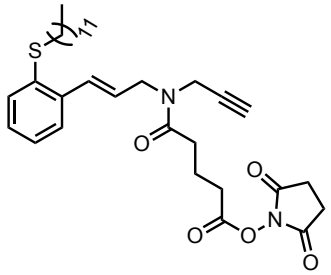
Unless mentioned, all chemicals and proteins were purchased from commercial suppliers and used without further purification unless stated otherwise. REG protein was chemically synthesized according to our reported method.[S1] ^{1}H nuclear magnetic resonance (^{1}H-NMR) and ^{13}C nuclear magnetic resonance (^{13}C-NMR) spectra were recorded on Bruker AVANCE-400 NMR spectrometer, or INOVA 500 MHz spectrometer. Gel permeation chromatography (GPC) measurements were performed on an Agilent 1260 LC system with two Shodex KD-806M GPC columns in series at 60 °C and a flow rate of 1 mL / min. Dimethyl formamide (DMF) with 0.1M LiBr was used as the eluent. A T-rEX refractive index detector (Wyatt) and a DAWN EOS 18 angle light scattering (MALS) detector (Wyatt) were used for polymer analysis. Circular dichroism (CD) spectra in 200 - 260 nm region were collected on an Aviv Model 202 Circular Dichroism Spectrometer. Protein and Polymer-protein conjugate samples were prepared in PBS buffer at 0.1 mg/mL concentration equivalent to the concentration of BSA in each sample. Spectra with PBS buffer only was collected and subtracted as blank. SDS-PAGE gel electrophoresis studies were performed on NuPAGE™ 3-8% Tri-acetate Gels (Purchased from Invitrogen) with a Mini Gel Tank (Purchased from Invitrogen). Fast protein liquid chromatography (FPLC) analyses were performed on a BioRad NGC Quest10 Plus system with a BioRad ENrich SEC 70 column at a flow rate of 1 mL/min, a GE HiTrap Butyl HP column (5 mL) at a flow rate of 5 mL/min, and a BioRad Nuvia Q anion exchange column (5 mL) at a flow rate of 5 mL/min attached separately, which utilize 1X PBS buffer and 0.1M Tris Base buffers as mobile phases.

Synthesis of the enyne compound 1



Scheme S1. Synthesis of functionalized enyne compound **1**.

*Synthesis of molecule **1b***



Enyne compound **1a** was synthesized according to a reported method. [S2]

1a (872 mg, 2.35 mmol, 1 eq.) was dissolved in 3 mL of dichloromethane. A small

crystal of 4-(dimethyamino)pyridine was added. Glutaric anhydride (321 mg, 2.82 mmol, 1.2 eq.) was added, and the solution was stirred at room temperature. The reaction was monitored by TLC (5% MeOH/DCM). Upon complete consumption of **1a**, the solution was evaporated under reduced pressured. The crude compound was used directly for EDC coupling to prepare compound **1b** as following.

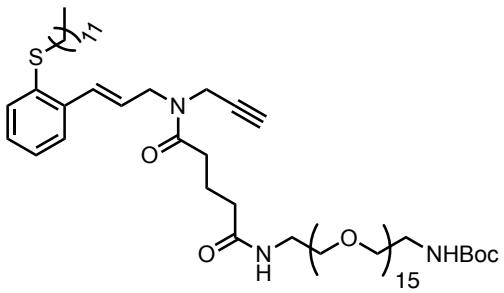
Crude compound (256 mg, 0.53 mmol, 1 eq.), N-hydroxysuccinimide (132 mg, 1.15 mmol, 2.2 eq), and a small crystal of DMAP were dissolved in 2 mL of anhydrous dichloromethane. The flask was then evacuated and back-filled with nitrogen 3x. In a separate vial, 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide (EDC) hydrochloride (204 mg, 1.06 mmol, 2 eq.) was suspended in 5 mL of anhydrous dichloromethane. The suspension was slowly injected into the reaction flask over 5 minutes with a syringe fitted with a thick needle. The reaction was monitored by thin layer silica gel chromatography (EtOAc/Hexanes). Upon complete consumption of 4, 10 mL of water was added to the reaction and stirred for 10 minutes. The heterogeneous mixture was transferred to a separatory funnel, and the aqueous layer discarded. The organics were washed with 2 x 10 mL water followed by 1x10mL brine. The solution was dried with anhydrous Na_2SO_4, decanted, and evaporated. The residue was purified by silica gel column chromatography (EtOAc/Hexanes) to afford compound **1b** (201 mg, 65% yield).

^{1}H NMR (500 MHz, $CDCl_3$) (δ ppm): 7.47 – 7.39 (m, 1H), 7.36 – 7.30 (m, 1H), 7.25 – 7.12 (m, 2H), 7.10 – 6.97 (m, 1H), 6.08 – 5.99 (m, 1H), 4.35 – 4.02 (m, 4H), 2.88 – 2.68 (m, 8H), 2.66 – 2.56 (m, 2H), 2.33 – 2.20 (m, 1H), 2.15 (h, J = 6.9 Hz, 2H), 1.61 (p, J = 7.2 Hz, 2H), 1.40 (p, J = 7.1 Hz, 2H), 1.26 (s, 16H), 0.88 (t, J = 7.2 Hz, 3H).

^{13}C NMR (126 MHz, $CDCl_3$) (δ ppm): 171.44, 169.21, 169.15, 168.59, 137.37, 136.93, 135.79, 135.34, 131.46, 130.81, 130.06, 129.55, 128.34, 128.14, 126.63, 126.46, 126.26, 125.70, 79.31, 78.72, 72.79, 71.90, 49.04, 47.82, 36.45, 34.41, 34.29, 34.08, 32.06, 31.57, 31.42, 30.45, 30.39, 29.80, 29.78, 29.74, 29.66, 29.49, 29.33, 29.24, 29.07, 29.03, 25.72, 22.83, 20.30, 20.18, 14.27.

LRMS-ESI: Calcd for $C_{33}H_{46}N_2O_6S$: m/z = 583.31 $[M + H]^+$; Found: 583.29 $[M +H]^+$.

*Synthesis of molecule **1***



1b (53 mg, 0.092 mmol, 1.5 eq.) and diisopropylethylamine (32 µL, 0.184 mmol, 3 eq.) were dissolved in 1 mL of dichloromethane at room temperature. H_2N-PEG_{15}-NHBoc (50 mg, 0.061 mmol, 1 eq.) was dissolved in a minimal amount of dichloromethane and was added to the above stirred solution. The reaction was allowed to stir overnight at room temperature. The solution was then evaporated under reduced pressure, and the residue was purified by silica gel chromatography (MeOH/DCM) to

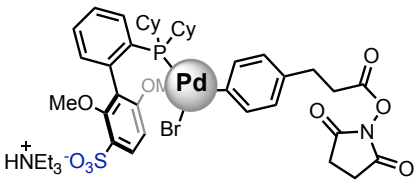
afford **1** (57 mg, 72% yield) as a slightly yellow gummy solid.

^{1}H NMR (500 MHz, $CDCl_3$) (δ ppm): 7.42 (dd, J = 11.2, 7.5 Hz, 1H), 7.32 (d, J = 7.7 Hz, 1H), 7.25 – 7.11 (m, 2H), 7.03 (dd, J = 15.7, 11.7 Hz, 1H), 6.35 (s, 1H), 6.02 (dt, J = 16.0, 6.1 Hz, 1H), 5.05 (s, 1H), 4.35 – 4.01 (m, 4H), 4.01 – 3.56 (m, 54H), 3.57 – 3.49 (m, 4H), 3.43 (dq, J = 15.6, 5.2 Hz, 2H), 3.32 – 3.27 (m, 2H), 2.84 (t, J = 7.4 Hz, 2H), 2.61 – 2.44 (m, 2H), 2.36 – 2.25 (m, 2H), 2.23 (t, J = 2.4 Hz, 1H), 2.06 – 1.94 (m, 2H), 1.60 (p, J = 7.5 Hz, 2H), 1.44 (s, 9H), 1.39 (p, J = 7.2 Hz, 2H), 1.34 – 1.18 (m, 16H), 0.87 (t, J = 6.8 Hz, 3H).

^{13}C NMR (126 MHz, $CDCl_3$) (δ ppm): 172.80, 172.36, 156.14, 137.29, 136.86, 135.68, 135.33, 131.33, 130.85, 129.93, 129.61, 128.37, 128.14, 126.60, 126.58, 126.41, 126.31, 126.28, 125.72, 79.38, 79.27, 78.84, 72.76, 71.85, 70.70, 70.37, 69.99, 49.14, 47.60, 40.51, 39.32, 36.56, 35.57, 35.54, 34.22, 34.08, 32.50, 32.28, 32.04, 29.78, 29.75, 29.72, 29.64, 29.47, 29.32, 29.23, 29.05, 29.02, 28.57, 25.54, 22.81, 21.28, 14.25.

LRMS-ESI: Calcd for $C_{66}H_{117}N_3O_{19}S$: m/z = 1310.79 $[M + Na]^+$; Found: 1310.80 $[M +Na]^+$.

Synthesis of Pd OACs compound 2



Compound **2** was synthesized according to our previous work. [S3]

ROMP of PEG-MM for BP-NHBoc

PEG-MM was synthesized according to our previous work. [S4]

In a nitrogen filled glovebox, 100 mg PEG-MM was dissolved in 0.4 mL THF in a 2 mL vial containing a stir bar. To this solution was added Grubbs III catalyst solution in THF (44 μL, 25 mg/mL, 1.53 μmol) to give MM : Grubbs III ratio of 20 : 1. The polymerization reaction was stirred at room temperature for 1 h. Then, ~3 mg of enyne compound **1** in THF (50 μL) was added to terminate the polymerization and simultaneously functionalize the termini of PEG BP. The resulting mixtures were stirred at 25 °C for additional 2 hrs. The functionalized BP (BP-NHBoc) was purified by precipitation in cold ethyl ether.

Deprotection of the Boc group on the end termini of BP for BP-NH2

90 mg of the BP-NHBoc was dissolved in 2 mL DCM under stirring. Then, 1 mL of TFA was added dropwise. The reaction was performed under room temperature for 2 hrs. The solvent was removed and the resulting BP-NH2 was purified by precipitation in cold ethyl ether.

Pd OACs functionalization for BP-[Pd]

25 mg of BP-NH2 was dissolved in 0.5 mL PBS buffer under stirring. 2 mg of compound **2** in 80 μL THF was added to the solution dropwise. The reaction was performed at room temperature overnight. Then, the crude solution was filtered through a 0.45 μm filter and further purified by ultrafiltration using Amicon® Ultra Centrifugal Filters (MWCO = 10 kDa). Finally, the brush solution was flash frozen using liquid nitrogen and lyophilized to afford white solid.

Synthesis of Maleimide functionalized bottlebrush polymer BP-Mal

25 mg of BP-NH2 was dissolved in 0.5 mL PBS buffer under stirring. 1 mg of Mal-PEG_1-NHS compound (as shown In Figure S8a) in 80 μL THF was added to the solution dropwise. The reaction was performed at room temperature overnight. Then, the crude solution was filtered through a 0.45 μm filter and further purified by ultrafiltration using Amicon® Ultra Centrifugal Filters (MWCO = 10 kDa). Finally, the brush solution was flash-frozen using liquid nitrogen and lyophilized to afford a white solid.

Synthesis of BP^{Cy3}-NHBoc from ROMP of PEG-MM and PEG^{Cy3}-MM and the corresponding BP^{Cy3}-NH2 and BP^{Cy3}-[Pd]

In a nitrogen filled glovebox, 94 mg PEG-MM and 7.5 mg PEG^{Cy3}-MM were dissolved in 0.4 mL THF in a 2 mL vial containing a stir bar. To this solution was added Grubbs III catalyst solution in THF (44 μL, 25 mg/mL, 1.53 μmol) to give MM : Grubbs III ratio of 20 : 1. The polymerization reaction was stirred at room temperature for 1 h. Then, ~3 mg of enyne compound **1** in THF (50 μL) was added to terminate the polymerization and simultaneously functionalize the termini of PEG BP. The resulting mixtures were stirred at 25 °C for additional 2 hrs. The functionalized BP (BP^{Cy3}-NHBoc) was purified by precipitation in cold ethyl ether.

The procedures for the synthesis of BP^{Cy3}-NH2 and BP^{Cy3}-[Pd] were the same as BP-

NH2 and BP-[Pd].

Synthesis of bottlebrush polymer-protein conjugates

The conjugation was performed by mixing the **BP-Pd, BP^{Cy3}-Pd** or **BP-Mal** aqueous solution and BSA (or BSA^{Cy5}) aqueous solution together with a Pd to BSA molar ratio of 5:1 overnight. Then, the conjugation was monitored by SDS-PAGE gel and the mixture was purified by FPLC. The conjugation procedure between the ERG protein and **BP-Pd** was similar to BSA conjugation with a Pd to ERG molar ratio of 5:1 overnight.

3. NMR spectra

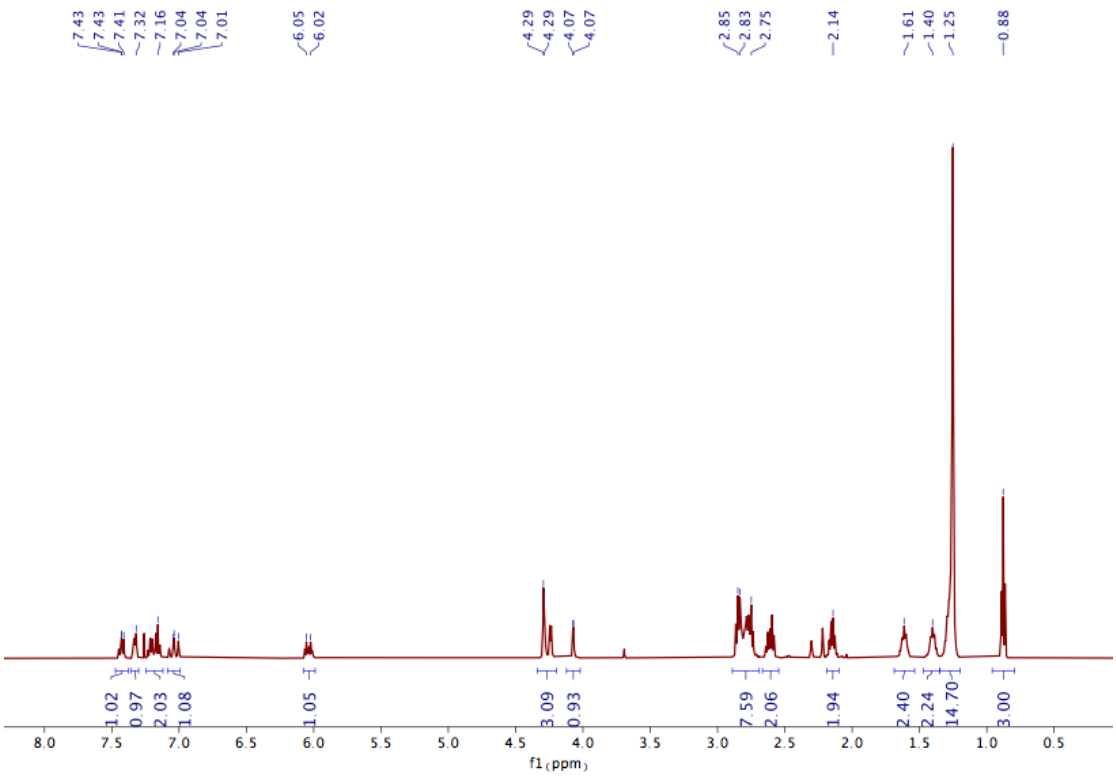


Figure S12. ^{1}H NMR spectrum of **1b**.

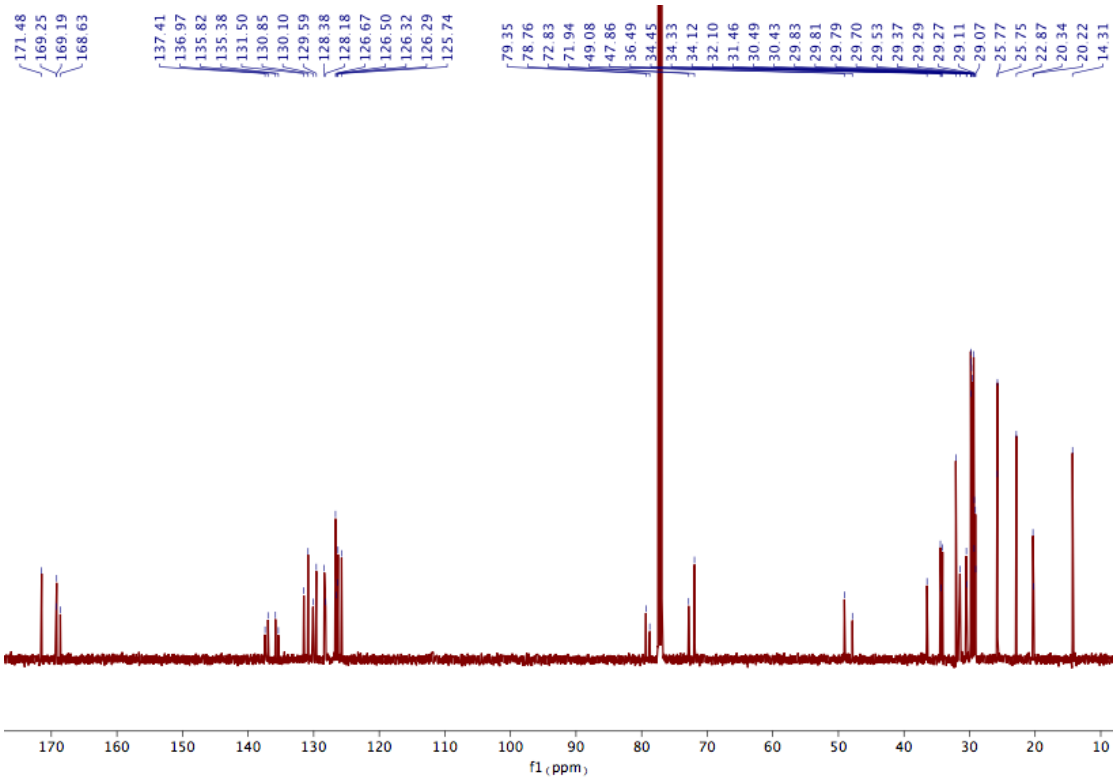


Figure S13.^{13}C NMR spectrum of **1b**.

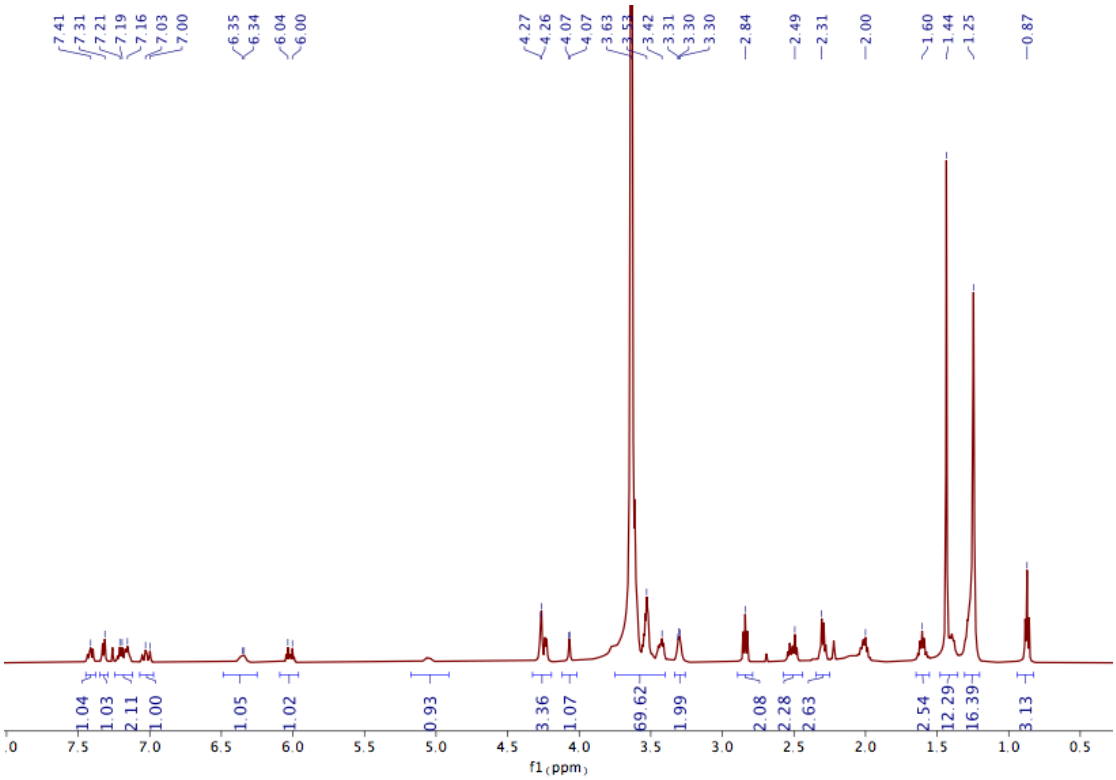


Figure S14. ^{1}H NMR spectrum of **1**.

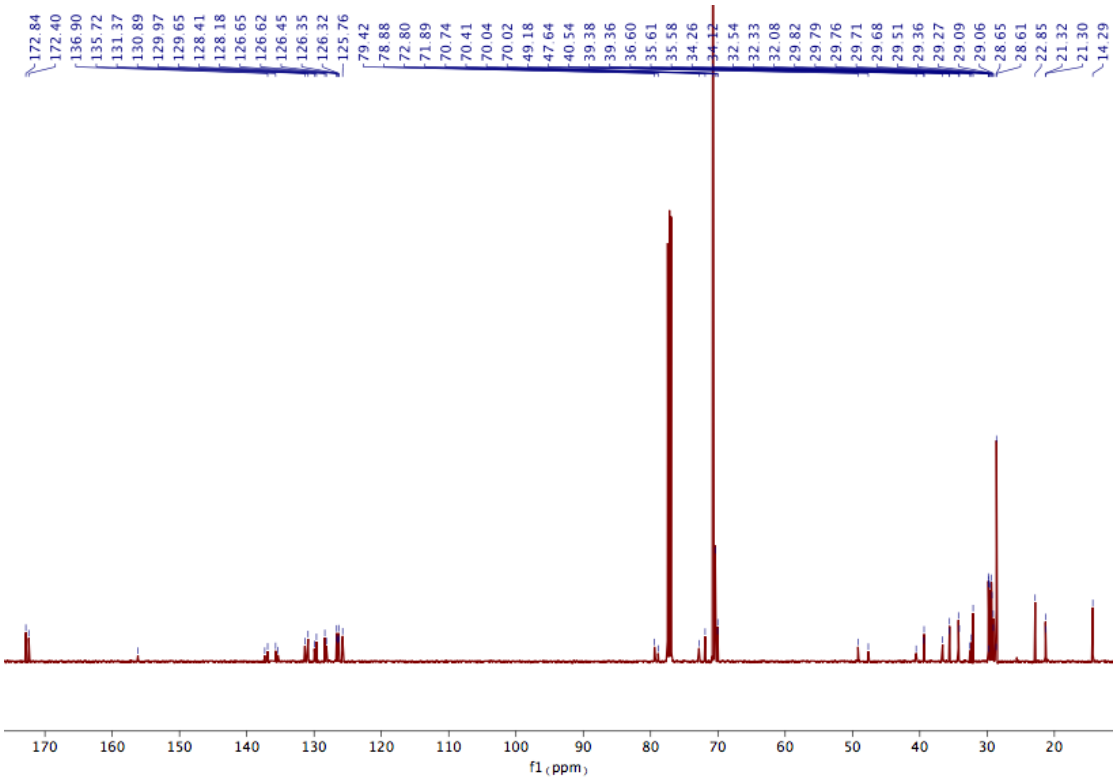


Figure S15. ^{13}C NMR spectrum of **1**.

4. References

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