Supplementary Information

Synthesis and Functionalization of dendritic Polyglycerol-based Nanogels: Application in T Cell Activation

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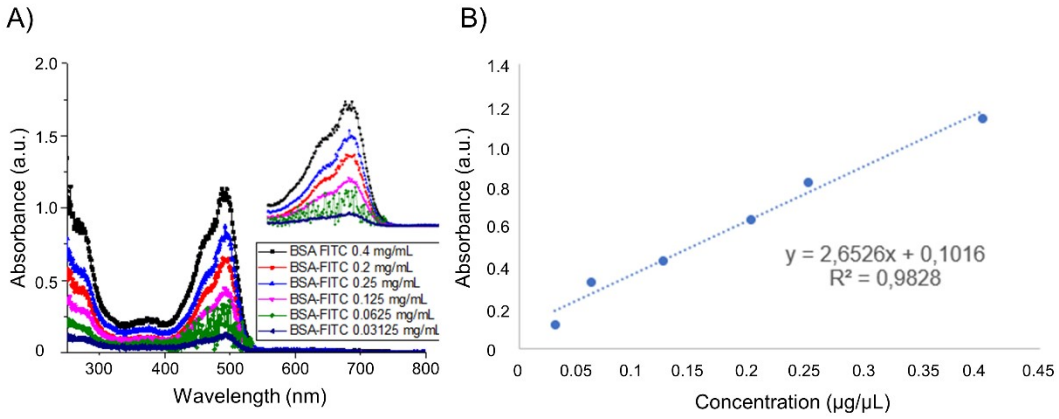

Figure S1: UV/Vis measurements of BSA-FITC at different concentrations (A) and derived calibration curve at 495 nm (B).

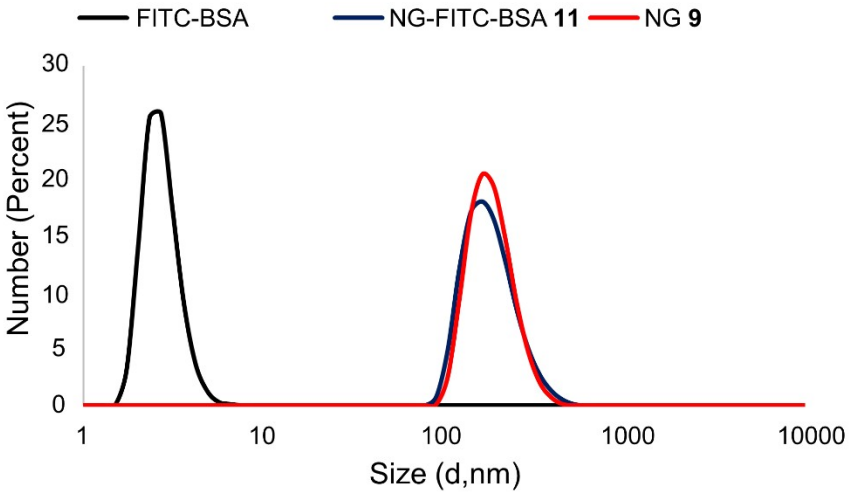

*Figure S2: Number average size distribution obtained by DLS of the NG 9, free FITC-BSA and their conjugate **11***

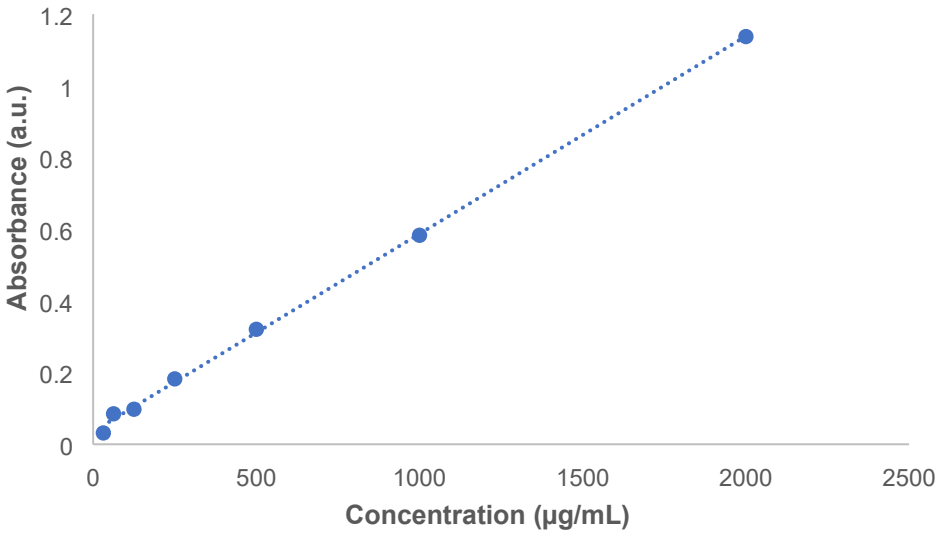

Figure S3: Exemplary BCA calibration of Avidin at 562 nm.

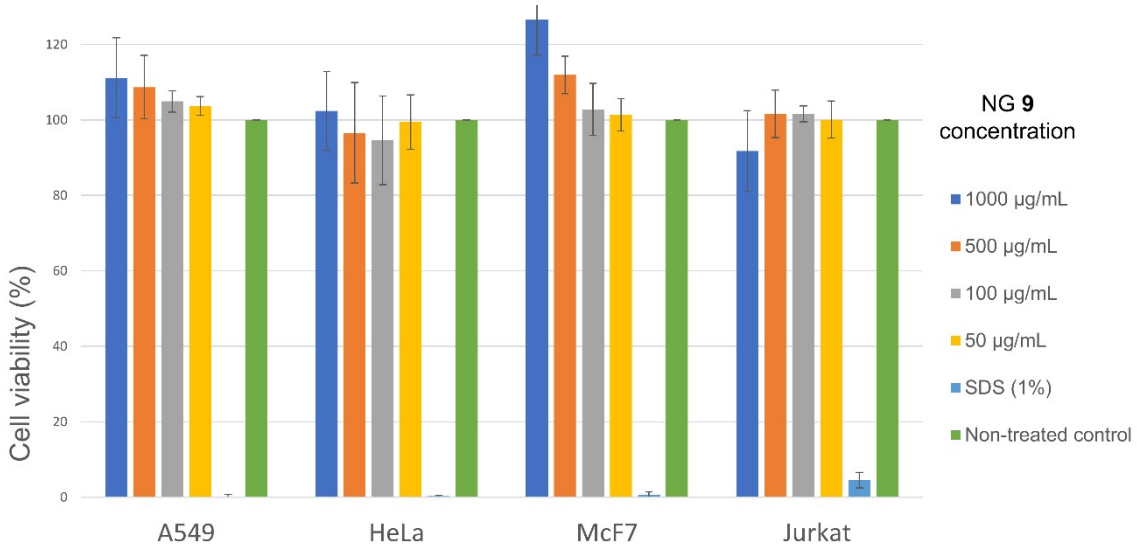

*Figure S4: Relative cell viability of A549, HeLa, McF7, and Jurkat JE6.1 NFκB-eGFP T reporter cells after treatment with NG **9** at different concentrations.*

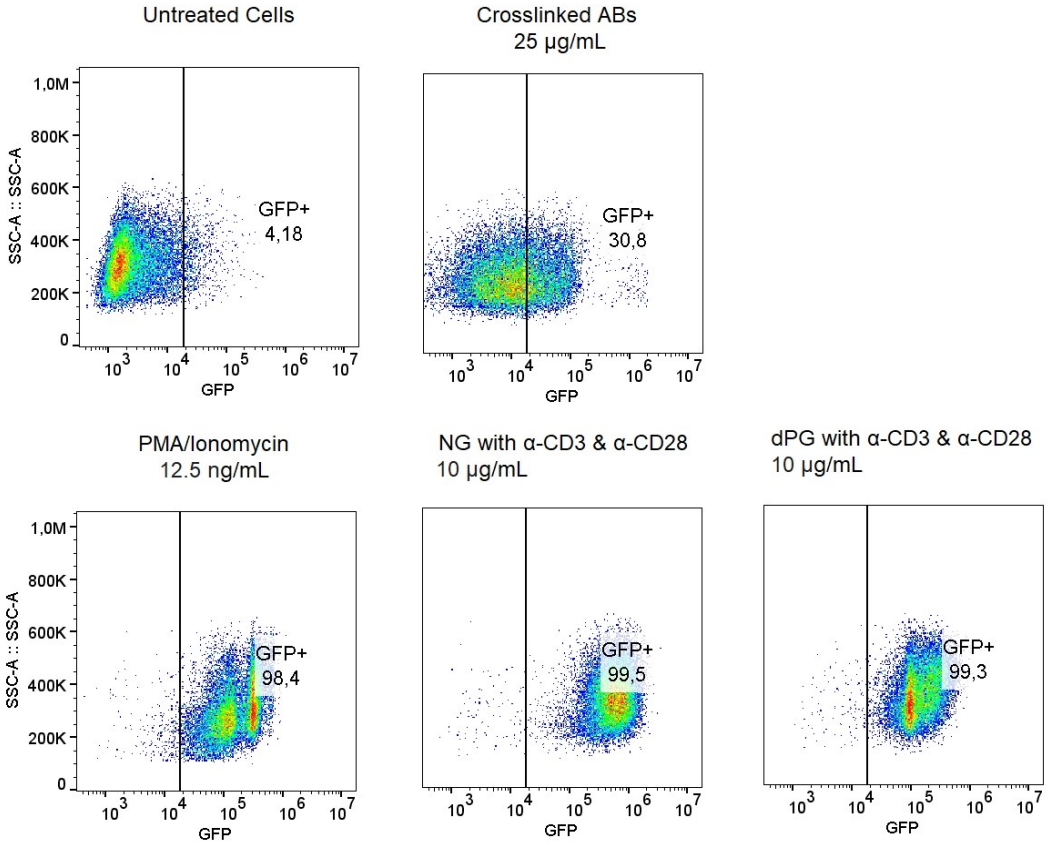

Figure S5: Flow Cytometry of Jurkat cells treated with NG and dPG conjugates compared to free antibodies and PMA/Ionomycin. Dotplots showing the gating of the populations for the quantification of GFP-expressing cells.

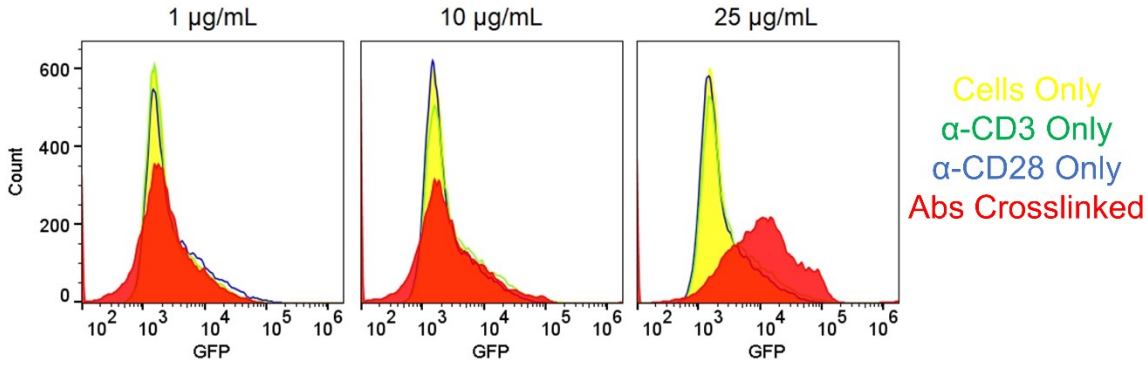

Figure S6: Flow Cytometry of Jurkat cells treated with free or Fc-crosslinked antibodies at different concentrations. Histograms of the fluorescence of treated or untreated cell populations on the GFP channel.

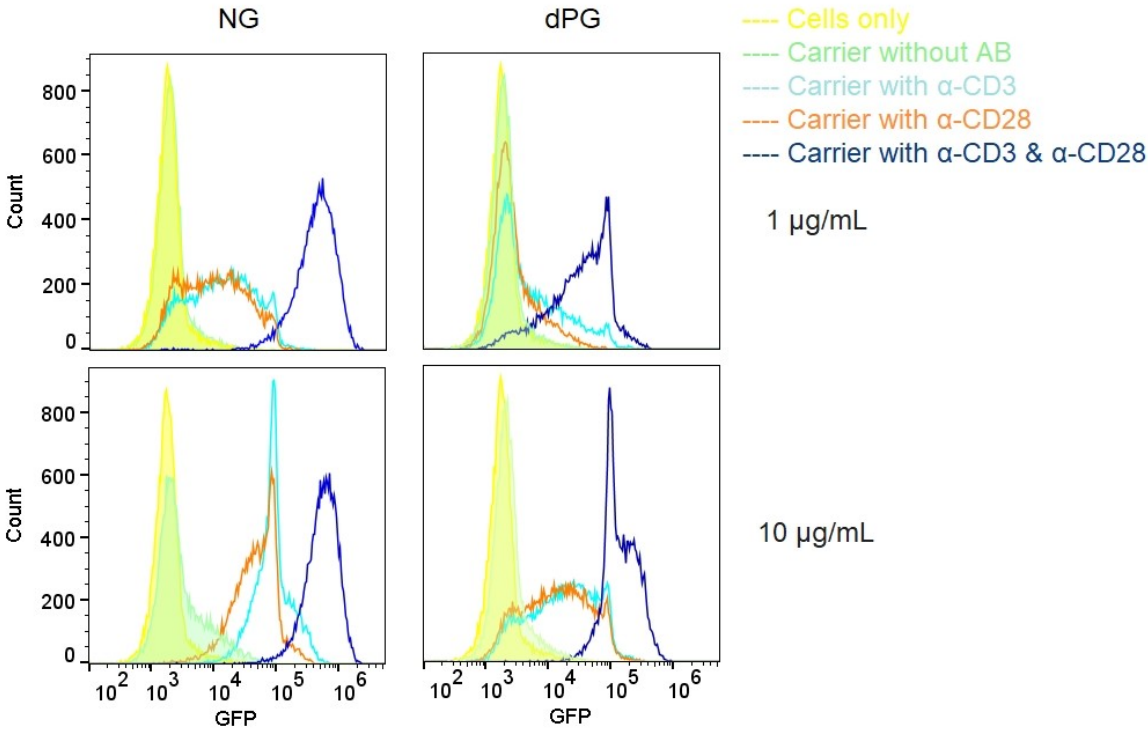

Figure S7: Flow cytometry of Jurkat cells of NG or dPG conjugated with single mAbs or the combination of both. Histograms of the fluorescence intensity of the cell populations in the GFP channel.

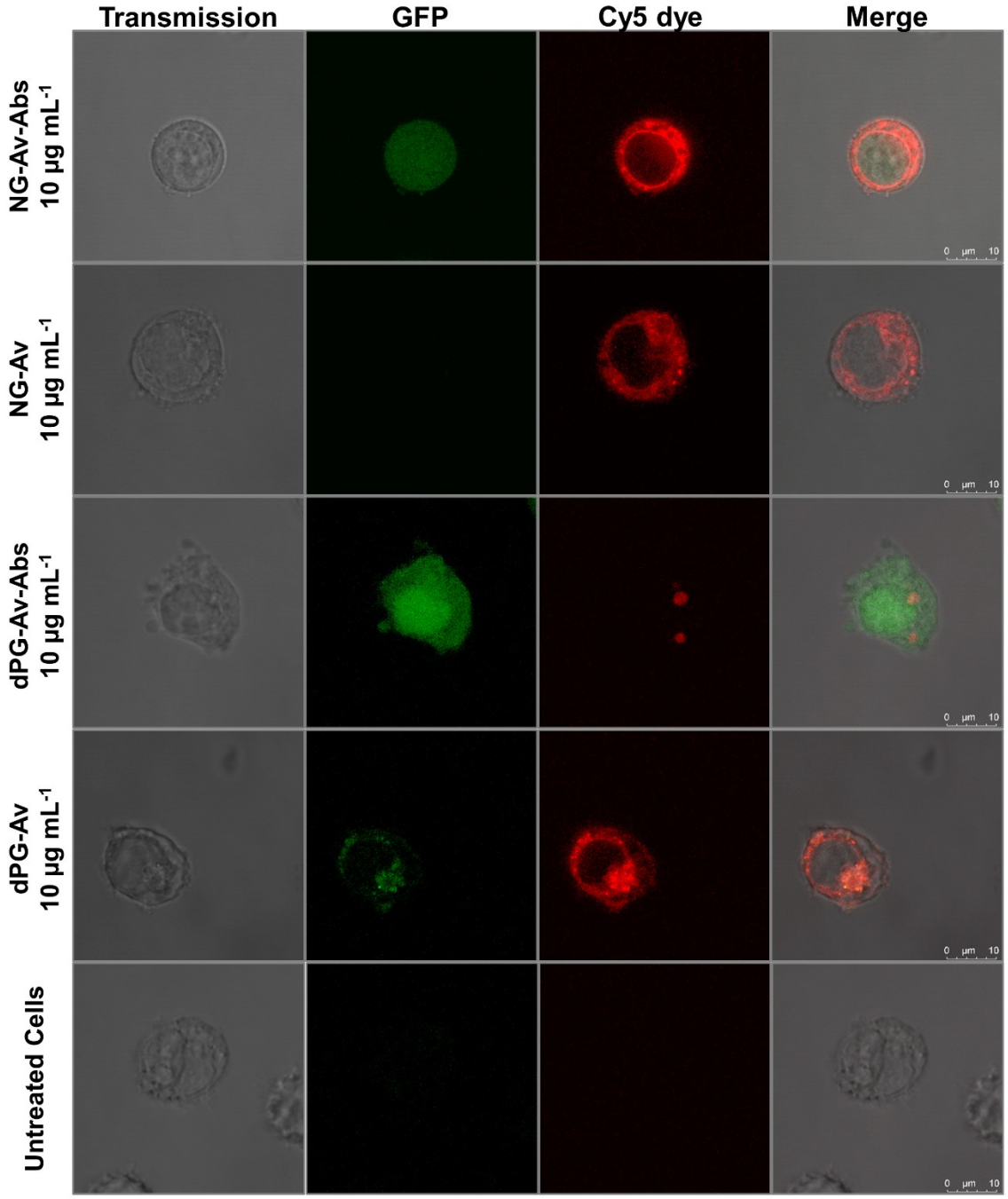

Figure S8: Confocal microscope images of Jurkat-GFP reporter cells treated for 6 h with Cy5-labeled nanocarriers: NG-Av-Abs, dPG-Av-Abs, NG-Av, dPG-Av, and untreated cells, respectively (4× Zoom). Green: GFP; red: Cy5.

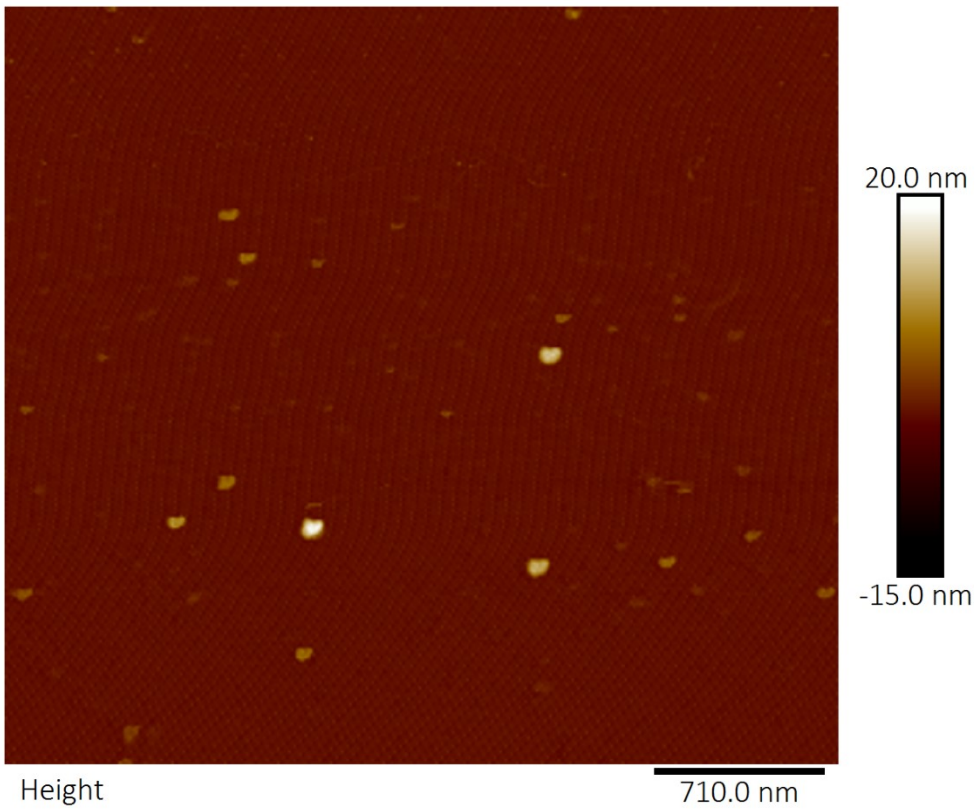

*Figure S9: Full AFM image of NGs **9***

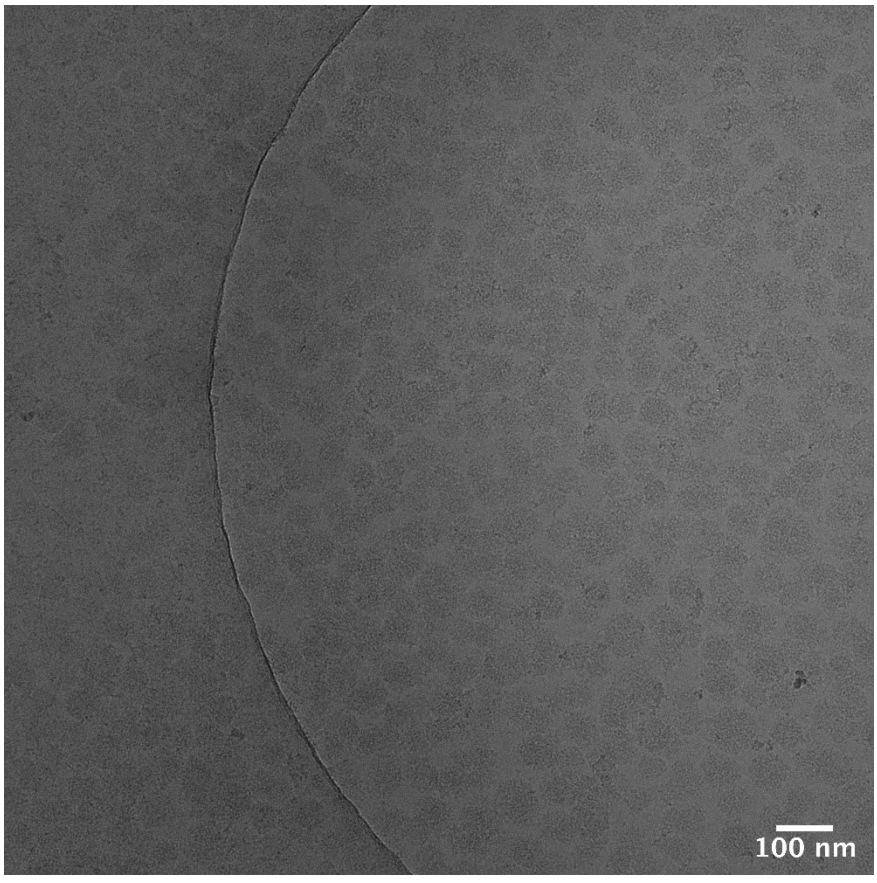

*Figure S10: Cryo-TEM image of NG-Av **12***

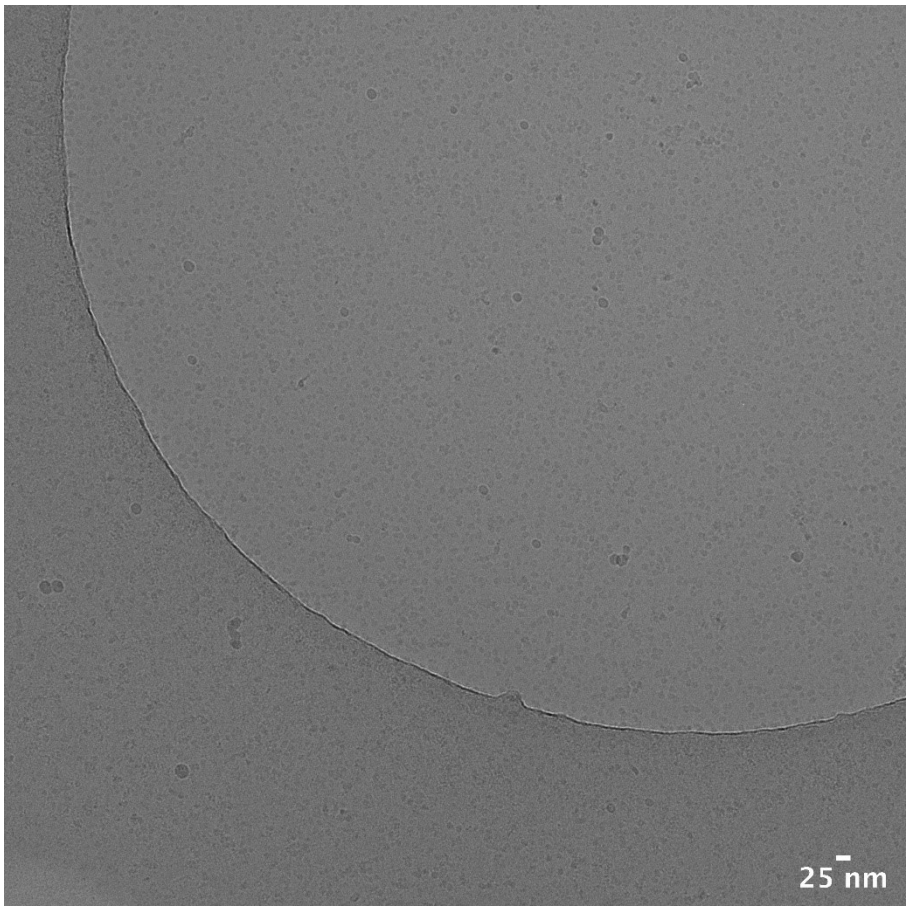

Figure S11: Cryo-TEM image of dPG-Av

Synthetic Procedures:

Synthesis of dPG-azide-linker 6

The carboxylic acid linker **3** was synthesized according to a published procedure by Cai and coworkers.[1] Briefly, in a three step reaction, (2,2‘-oxybis(ethan-1-ol) was converted with propargyl bromide, tert-butyl acrylate and subsequently deprotected with trifluoroacetic acid.

^{1}H NMR (400 MHz, Chloroform-d) δ 8.15 (s, -COOH, 1H), δ 4.19 (d, *J* = 2.4 Hz, CH_2-CCH, 2H), 3.75 (t, *J* = 6.3 Hz, CH_2-OEG, 2H), 3.70 – 3.59 (m, OEG, 8H), 2.63 (t, *J* = 6.3 Hz, HOOC-CH_2, 2H), 2.42 (t, *J* = 2.4 Hz, CCH, 1H).

Subsequently, **3** was coupled to dPG-azide according to a modified procedure by Rostovtsev *et al*.[2] Briefly, to a solution of dPG-$(N_3)_{10\%}$ **5** (196 mg, 19.3 µmol, 1 equiv.) in a THF/H_2O solution (3 mL/1 mL), 3-(2-(2-prop-2-yn-1-yloxy)ethoxyethoxy)propanoic acid **3** (21.3 mg, 98.4 µmol, 4×1.275 equiv.) was added and the mixture was degassed meticulously. Sodium ascorbate (7.65 mg, 38.6 µmol, 2 equiv.) and $CuSO_4$ (0.96 mg, 3.86 µmol, 0.2 equiv.) were dissolved in H_2O separately (1 mL each), unified and added dropwise to the solution. The reaction mixture was stirred for 2d at room temperature and dialyzed against a saturated EDTA-solution and Milli-Q

water (1d each). The product (212 mg, 19.22 µmol, quant. conversion, yield 99%) was stored in aqueous solution. ^{1}H NMR (500 MHz, Deuterium Oxide) δ 8.11 (s, triazole, 4H), 4.10 – 3.33 (m, PG backbone), 2.67 (s, C$\underline{H_2}$-COOH, 8H), 0.93 (s, PG starter, 3H). dPG$_{1.4MDa}$-(N$_3$)$_{7\%}$ was proceeded analogously, aiming for conversion of 200 groups.

Synthesis of dPG-BCN 8

BCN was synthesized according to a published procedure[3] and subsequently coupled to dPG-amine according to a modified procedure.[4] To a solution of dPG-(NH$_2$)$_{10\%}$ **7** (140 mg, 0.19 mmol NH$_2$-groups, 1 equiv.) in anhydrous DMF (15 mL) and triethyl amine (0.19 mmol, 1 equiv.), Bicyclo[6.1.0]non-4-yn-9-ylmethyl (4-nitrophenyl) carbonate (88 mg, 0.28 mmol, 1.5 equiv.) was added. The reaction mixture was stirred for 2 h at room temperature and the solvent was evaporated *in vacuo*. The crude product was dialyzed against MeOH for 1 d (MWCO 2 kDa) and the dialysate was changed regularly. The product (130 mg, 9.9 mmol, quant. conversion, yield 68%) was stored as a solution in MeOH. ^{1}H NMR (500 MHz, Deuterium Oxide) δ 3.94 – 3.44 (m, PG-backbone, 141H), 2.67 – 0.22 (m, BCN moiety, 28H).

Nanoprecipitation procedure:

dPG-azide-linker **6** (20 mg) and dPG-BCN **8** (30 mg) were dissolved separately in Milli-Q water (5 mL each). The solutions were pooled, vortexed and injected into acetone (800 mL) *via* syringe under vigorous stirring (1100 rpm). The reaction was quenched after 45 min by additon of excess 3-azidopropanol, again under vigorous stirring. After 24 h, Milli-Q water (50 mL) was added, the solvent was reduced *in vacuo* and the nanogels were purified *via* dialysis against water for 3d (MWCO 50 kDa). Nanoprecipitations were performed with batch sizes of 5 mg, 25 mg and 50 mg macromolecule and produced comparable results. Size (Intensity) = 143±2; ξ = −19 mV ^{1}H NMR (700 MHz, Deuterium Oxide) δ 8.30 – 7.92 (m, triazole of compound **6**), 4.08 – 3.46 (m, dPG backbone), 3.26 – 3.13 (m, C$\underline{H_2}$-COOH of compound **6**), 0.65 (m, BCN moieties of compound **8**).

General Procedure for the NHS ester formation

To a solution of dPG-azide-linker **6** (16 mg, 5.33 µmol COOH groups, 1 equiv.) in anhydrous DMF (8 mL), DIPEA (1.4 µL, 8 µmol, 1.5 equiv.) and HSTU (2.9 mg, 8 µmol, 1.5 equiv.) were added. The reaction mixture was stirred for 1 d and the solvent removed *in vacuo* and the ester purified by tripple precipitation in diethyl ether. Since only the NHS ester formation was studied, no yield was recorded. ^{1}H NMR (500 MHz, DMSO-d6) δ 8.00 (s, triazole, 4H), 3.92 – 3.28 (m, PG backbone), 2.93 (t, J = 5.9 Hz, C$\underline{H_2}$-COONHS, 8H), 2.82 (s, NHS, 16H), 0.84 (s,

PG starter, 3H). After confirmation of NHS ester formation by ^{1}H NMR, the formation was performed *in situ* and directly used for further protein coupling. For NGs, the amount of dPG-azide-linker incorporated was used for calculation based on the nanoprecipitation feed (40 wt%) and for dPG1.4MDa-$(N_3)_{6\%}$-(COOH), 200 COOH groups per dPG were assumed.

Av and dye coupling for $dPG_{1.4MDa}$-$(N_3)_{7\%}$-COOH

First, the corresponding active ester of NG **9** was formed in situ as described above and used without further purification. NG-NHS **10** (10 mg, 0.33 µmol of **6**), was cooled down to 0 °C and Avidin (2.2 mg, 0.03 µmol, 0.1 eq), dissolved in PBS (1.1 mL) was added under stirring. After 2 h, Cy5-NH_2 (20 µg, 0.03 µmol, 0.1 eq) in DMSO (20 µL) was added and the reaction was stirred overnight, diluted with PBS pH 7.4 (2 mL) and purified *via* dialysis against PB (pH 7.4, MWCO 1000 kDa).

$dPG_{1.4MDa}$-$(N_3)_{7\%}$-COONHS was formed *in situ* as described above without purification. Subsequently, the active ester (10 mg, 7.14 nmol) was cooled down to 0°C with an ice bath and a solution of Avidin (0.48 mg, 7.14 nmol, 1.0 equiv.) in PBS pH 7.4 (0.24 mL) was added and the reaction was stirred at rt. After 2 h, Cy5-NH_2 (5 µg; 7.14 nmol, 1 equiv.) dissolved in DMSO (5 µL) was added and the reaction mixture was stirred overnight.

The coupling efficiency was determined by BCA Protein Quantification Kit and varied between 30% and 41%.

Av and dye coupling for the nanogels

First, the corresponding active ester of NG-COOH was formed *in situ* as described above without purification. NG conjugates were prepared as described for $dPG_{1.4MDa}$-$(N_3)_{7\%}$-COOH. The only exception is that here, 0.1 equiv. Cy5-NH_2 was used.

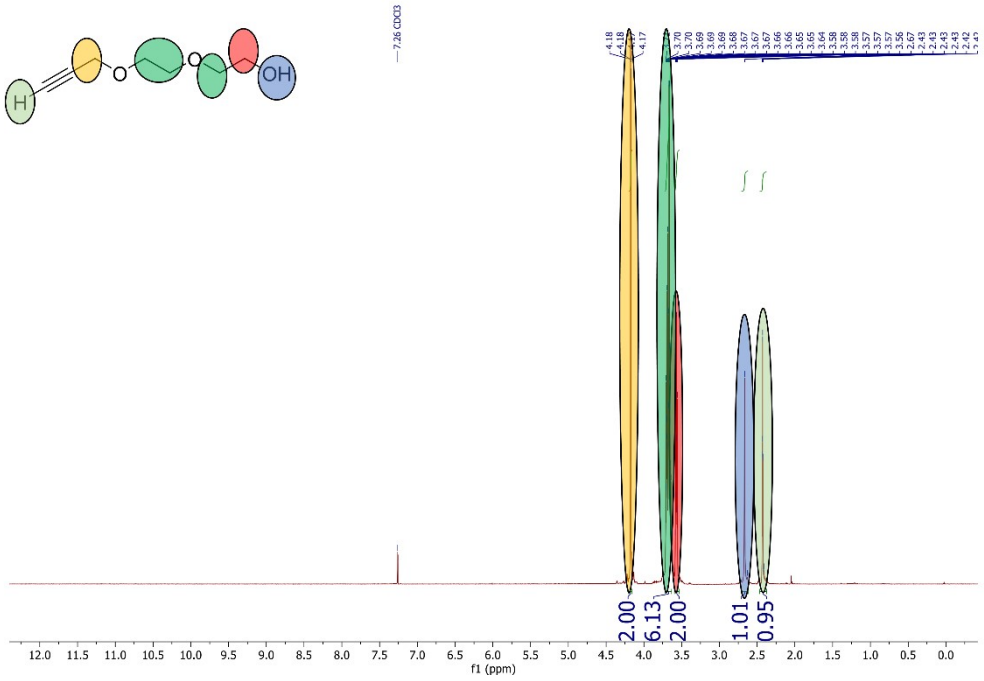

*Figure S12: ^{1}H NMR spectrum of **1***

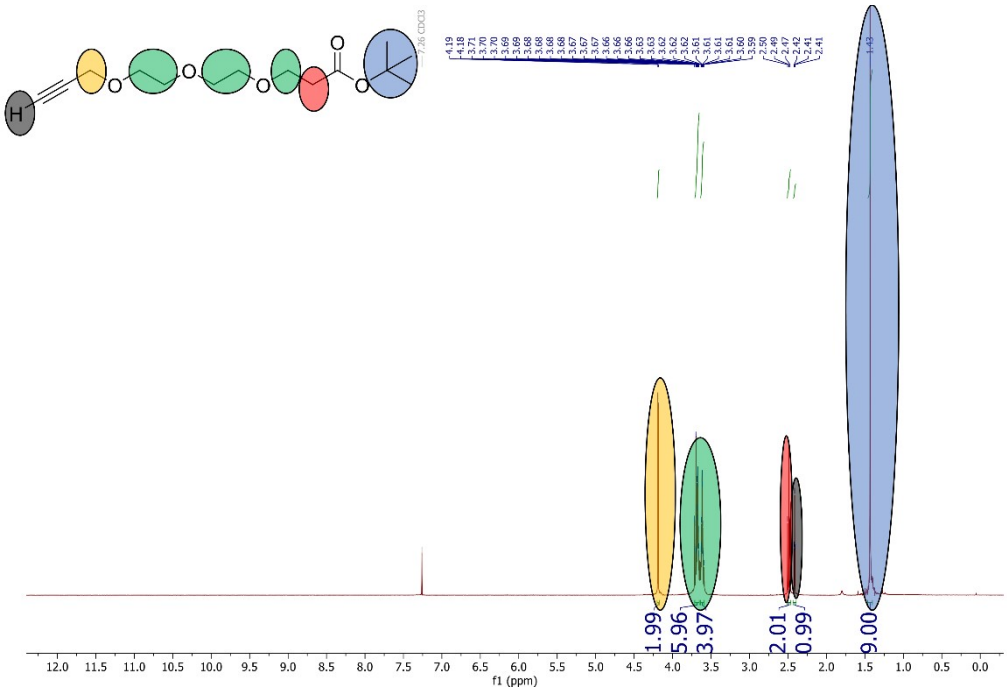

*Figure S13: ^{1}H NMR spectrum of **2***

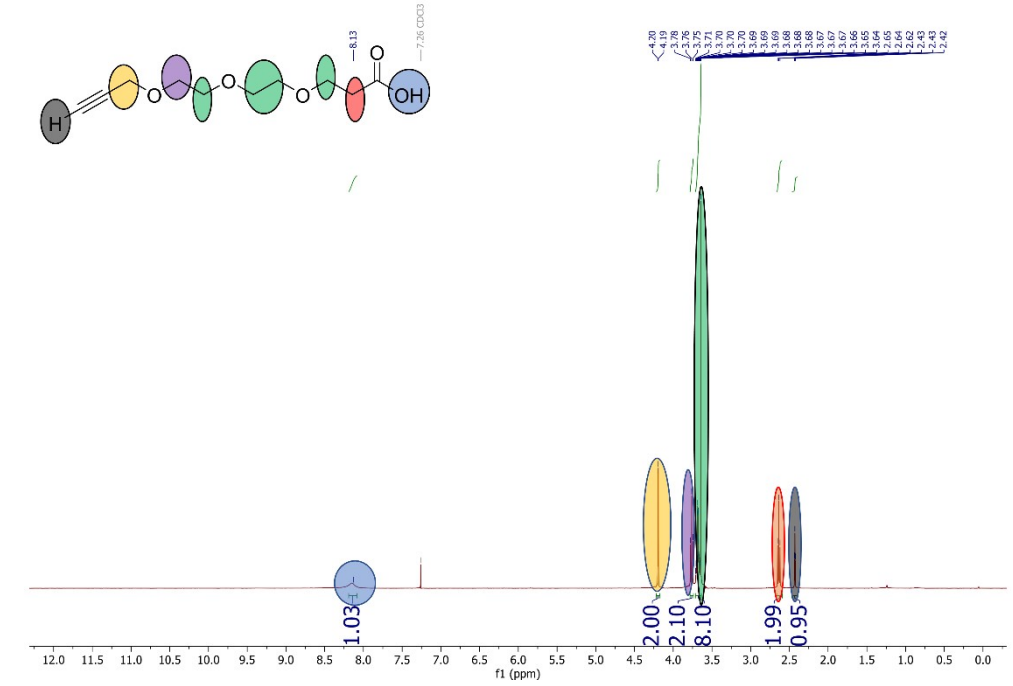

*Figure S14: ^{1}H NMR spectrum of **3***

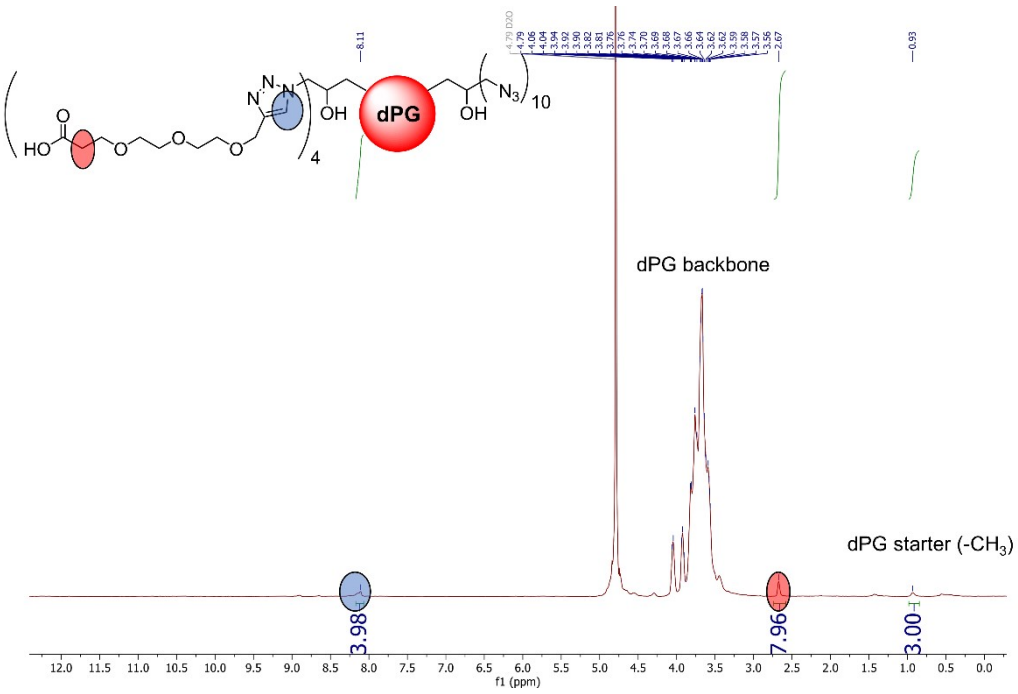

*Figure S15: ^{1}H NMR spectrum of **6***

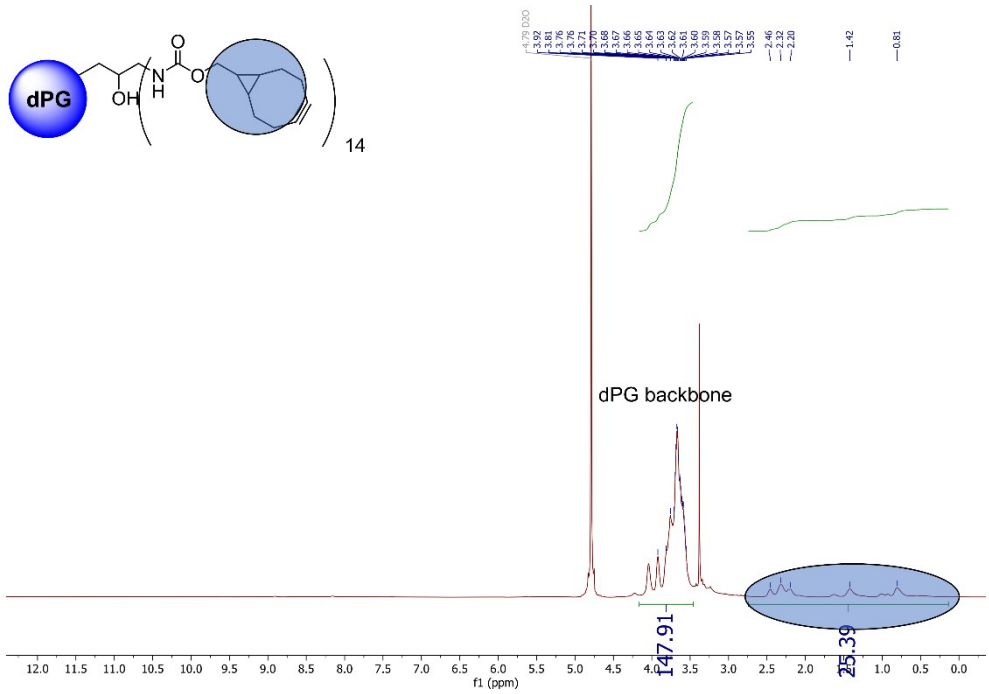

*Figure S16: [1]H NMR spectrum of **8***

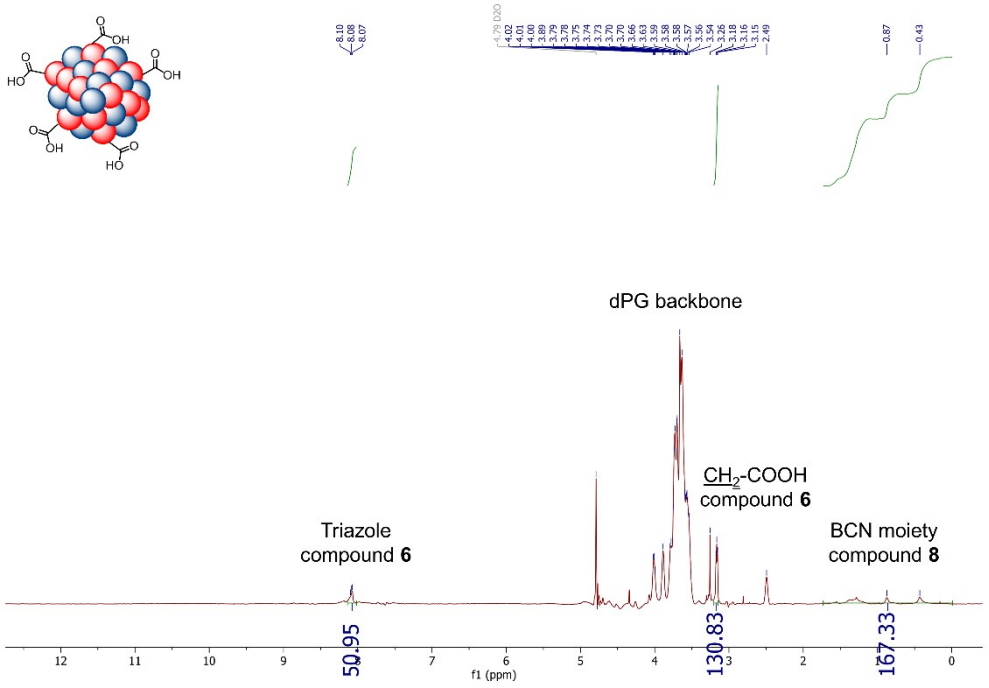

*Figure S17: [1]H NMR spectrum of NG **9***

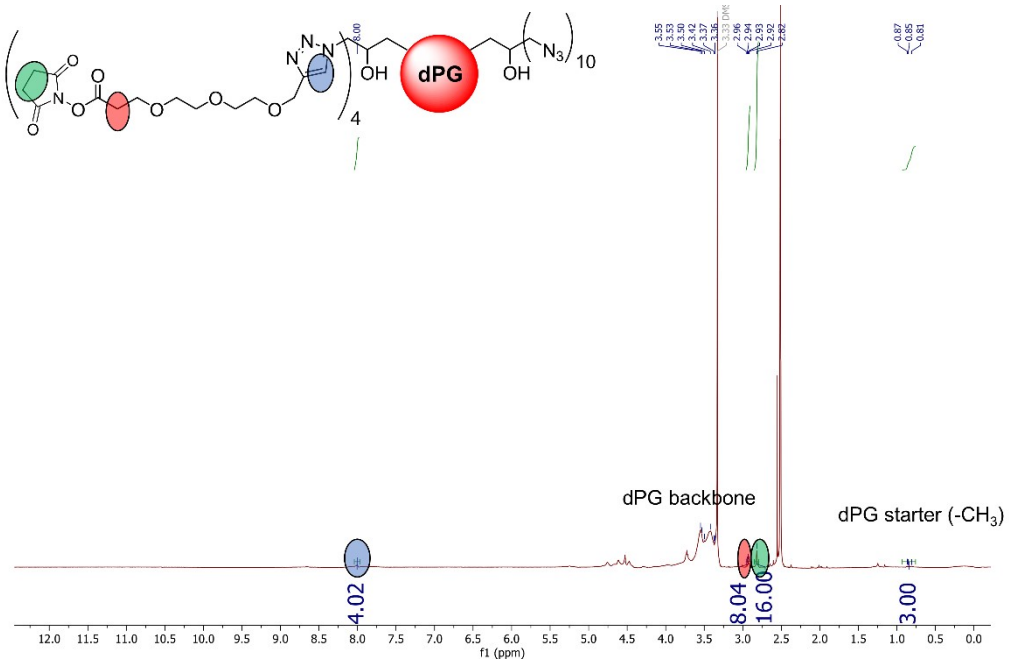

*Figure S18: ^{1}H NMR spectrum of NHS ester of **6***

1. A. Kumar, U. J. Erasquin, G. Qin, K. Li and C. Cai, *Chemical Communications*, 2010, **46**, 5746-5748.
2. V. V. Rostovtsev, L. G. Green, V. V. Fokin and K. B. Sharpless, *Angewandte Chemie International Edition*, 2002, **41**, 2596-2599.
3. J. Dommerholt, S. Schmidt, R. Temming, L. J. A. Hendriks, F. P. J. T. Rutjes, J. C. M. van Hest, D. J. Lefeber, P. Friedl and F. L. van Delft, *Angewandte Chemie International Edition*, 2010, **49**, 9422-9425.
4. D. Steinhilber, T. Rossow, S. Wedepohl, F. Paulus, S. Seiffert and R. Haag, *Angewandte Chemie International Edition*, 2013, **52**, 13538-13543.