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Supporting Information

Light-Sensitive Phenacyl Crosslinked Dextran Hydrogels for Controlled Delivery

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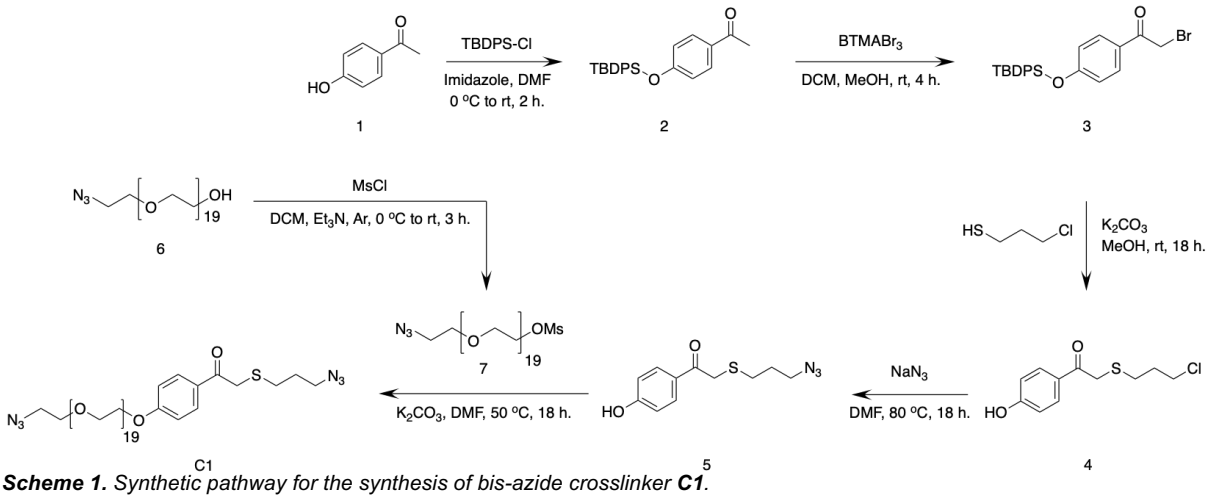
1. Materials and methods

All solvents and chemicals were purchased from Sigma Aldrich or Apollo scientific and used without further purification. ^{1}H-NMR and ^{13}C-NMR were recorded on an Agilent-400 MR DD2 (399.67 MHz for 1H and 100.5 MHz for 13C) at 298 K. The chemical shifts are given with respect to solvent residual signals as reported by Fulmer et al.[1] The rheological measurements were performed using a rheometer (AR G2, TA instruments) equipped with a steel plate-and-plate geometry of 40 mm in diameter and equipped with hexadecane trap. UV-Vis spectroscopic measurements were recorded on an Analytik Jena Specord 250 photospectrometer, using quartz cuvettes (pathlength = 1 cm) at RT. Osram Puritec HNS 8W G5 OFR UV-C lamps (2 units) were used for all 254 nm light irradiation experiments. The irradiance at 254 nm was determined using a UVC-254SD meter (Lutron). All γ-irradiation experiments were conducted using a Nordion GC220 ^{60}Co source.

2. Experimental methods

2.1 Synthesis of phenacyl photo-labile crosslinker **C1**



Scheme 1. *Synthetic pathway for the synthesis of bis-azide crosslinker* ***C1****.*

2.1.1 1-(4-((tert-butyldiphenylsilyl)oxy)phenyl)ethan-1-one (**2**)

4′-hydroxy-acetophenone (**1**) (6.0 g, 44 mmol) and imidazole (7.52 g, 110 mmol) were dissolved in DMF (30 ml) and cooled to 0 ºC using an ice bath. Tert-butyl(chloro)diphenylsilane (17.4 mL, 66.9 mmol) was slowly added and the reaction mixture was stirred for 2 hours at RT. The reaction was quenched by the addition of H_2O (100 mL) and the product was extracted to EtOAc (3 x 100 mL). The combined organic layers were dried over $MgSO_4$, filtered, concentrated *in vacuo* and purified over silica (EtOAc/PE 5% - 25%). This afforded the product pure as a white powder (**2**) (13.4 g, 35.7 mmol, 81%). ^{1}H-NMR (400 MHz, $CDCl_3$) δ = 7.75 – 7.69 (m, CH_{arom}, 6H), 7.46 – 7.36 (m, CH_{arom}, 6H), 6.80 (d, J = 8.60 Hz, CH_{arom}, 2H), 2.48 (s, CH_3, 3H), 1.11 (s, CH_3, 9H). ^{13}C-NMR (100 MHz, $CDCl_3$) δ = 197.05, 160.16, 135.53, 134.95, 132.28, 130.80, 130.46, 130.30, 129.75, 128.07, 127.84, 119.77, 26.54, 19.5. ^{1}H- and ^{13}C-NMR data match reported values.[2]

2.1.2 2-bromo-1-(4-((tert-butyldiphenylsilyl)oxy)phenyl)ethan-1-one (**3**)

Compound **2** (8,47 g, 22.6 mmol) was dissolved in MeOH/DCM (2:5, 28 mL). Benzyltrimethylammonnium tribromide ($BTMABr_3$) (9.15 g, 39.8 mmol) was slowly added and the reaction mixture was stirred 4 hours at RT. TLC analysis (EtOAc/PE, 2:8) showed a single product spot (rf: 0.66) and subsequently H_2O (50 ml) was added to the reaction mixture. The aqueous layer was extracted with Et_2O (3 x 50 ml) and the combined organic layers were dried over $MgSO_4$, concentrated *in vacuo* and purified over silica (EtOAc/PE 5% - 25%). This afforded the product pure as a white powder (yield: 8.26 g, 18.2 mmol, 81%). ^{1}H-NMR (400 MHz, $CDCl_3$), δ = 7.78 (d, J = 8.84 Hz, CH_{arom}, 2H), 7.69 (m, CH_{arom}, 4H), 7.47 – 7.37 (m, CH_{arom}, 6H), 6.82 (d, J = 8.88 Hz, CH_{arom}, 2H), 4.33 (s, J = 8 Hz, CH_2, 2H), 1.11 (s, CH_3, 9H). ^{1}H-NMR data match reported values.[3]

2.1.3 2-((3-chloropropyl)thio)-1-(4-hydroxyphenyl)ethan-1-one (**4**)

Compound **3** (1.70 g, 3.76 mmol) and KOH (211 mg, 3.76 mmol) were dissolved in MeOH (20 mL). Then 3-chloro-1-propanethiol (403 µL, 4.14 mmol) was added and the reaction mixture was left to stir overnight at RT. Subsequently H_2O (100 ml) was added to reaction mixture and the aqueous layer was extracted with EtOAc (3 x 50 ml). The combined organic layers were washed with brine (20 mL), dried over $MgSO_4$, concentrated *in vacuo* and purified over silica (EtOAc/PE 10% to 25%). This afforded the product as a colorless oil. (427 mg, 1.75 mmol, 47%). ^{1}H-NMR (400 MHz, $CDCl_3$), δ = 7.92 (d, J = 8.76 Hz, CH_{arom}, 2H), 6.96 (d, J = 8.76 Hz, CH_{arom}, 2H), 3.79 (s, CH_2, 2H), 3.58 (t, J = 6.32, CH_2, 2H), 2.72 (t, J = 7.00, CH_2, 2H), 2.03 (q, J = 6.80, CH_2, 2H).

2.1.4 2-((3-azidopropyl)thio)-1-(4-hydroxyphenyl)ethan-1-one (**5**)

Compound **4** (20.4 g, 83.4 mmol) and NaN_3 (54.2 g, 833 mmol) were dissolved in DMF (200 ml) and stirred overnight at 80 ºC. H_2O was added to the reaction mixture and extracted with EtOAc (3 x 150 ml). The combined organic layer was dried over $MgSO_4$, filtered and concentrated *in vacuo*. Residual DMF was removed using a high vacuum oil pump connected to the rotavap (40 ºC). This afforded the product as a colorless oil (20.9 g, 83 mmol, quantitative). ^{1}H-NMR (400 MHz, $CDCl_3$), δ = 7.93 (d, J = 8.80 Hz, CH_{arom}, 2H), 6.93 (d, J = 8.76 Hz, CH_{arom}, 2H), 3.76 (s, CH_2, 2H), 3.37 (t, J = 6.60 Hz, CH_2, 2H), 2.65 (t, J = 7.16, CH_2, 2H), 1.85 (q, J = 6.92 Hz, CH_2, 2H). ^{13}C-NMR (100 MHz, $CDCl_3$) δ = 194.09, 161.03, 131.69, 127.99, 115.75, 50.08, 37.02, 29.46, 28.29. Mass calc.: 251.07 [M], mass found: 273.90 $[M+Na]^+$

2.1.5 1-(4-((1-azido-nonadeca-ethylene glycol)phenyl)-2-((3-azidopropyl)thio)ethan-1-one (**C1**)

Compound **5** (175.8 mg, 0.696 mmol), mesylated PEG **7** (540 mg, 0.539 mmol) and K_2CO_3 (164.4 mg, 1.19 mmol) were dissolved in DMF (10 ml) and stirred overnight at 50 ºC. TLC analysis (1% MeOH in DCM) was used to determine the progress of the reaction and revealed a single UV (254 nm) active product spot (rf: 0.3). Water (50 ml) was added to the reaction mixture and was then extracted with EtOAc (3 x 50 ml). The combined organic layers were dried over $MgSO_4$ and concentrated *in vacuo* (residual DMF traces were removed using a high vacuum oil pump). The concentrate was then re-

dissolved in DCM (2-4 ml) and purified over silica (0% MeOH to 5% MeOH in DCM) which afforded the title compound as a white paste (383 mg, 0.331 mmol, 61%). ^{1}H-NMR (400 MHz, $CDCl_3$), δ = 7.92 (d, J = 8.40 Hz, C*H*$_{arom}$, 2H), 7.92 (d, J = 8.44 Hz, C*H*$_{arom}$, 2H), 4.17 (t, J = 4.44 Hz, O-C*H*2, 2H), 3.85 (t, J = 4.60 Hz, O-C*H*2, 2H), 3.72 – 3.68 (m, 4H), 3.61 (m, -(OCH_2CH_2O)$_{19}$-, 74H), 3.37 – 3.34 (m, 4H), 2.62 (t, J = 7.26 Hz, N_3-C*H*2, 2H), 1.84 (q, J = 6.84 Hz, CH_2CH_2CH_2, 2H). ^{13}C-NMR (100 MHz, $CDCl_3$) δ = 193.16, 163.01, 131.07, 128.09, 114.45, 70.88, 70.55, 70.03, 69.47, 67.65, 50.67, 49.94, 36.79, 29.21, 28.11. Mass calc.: 1156.60 [M], mass found: 1157.42 $[M+H]^+$

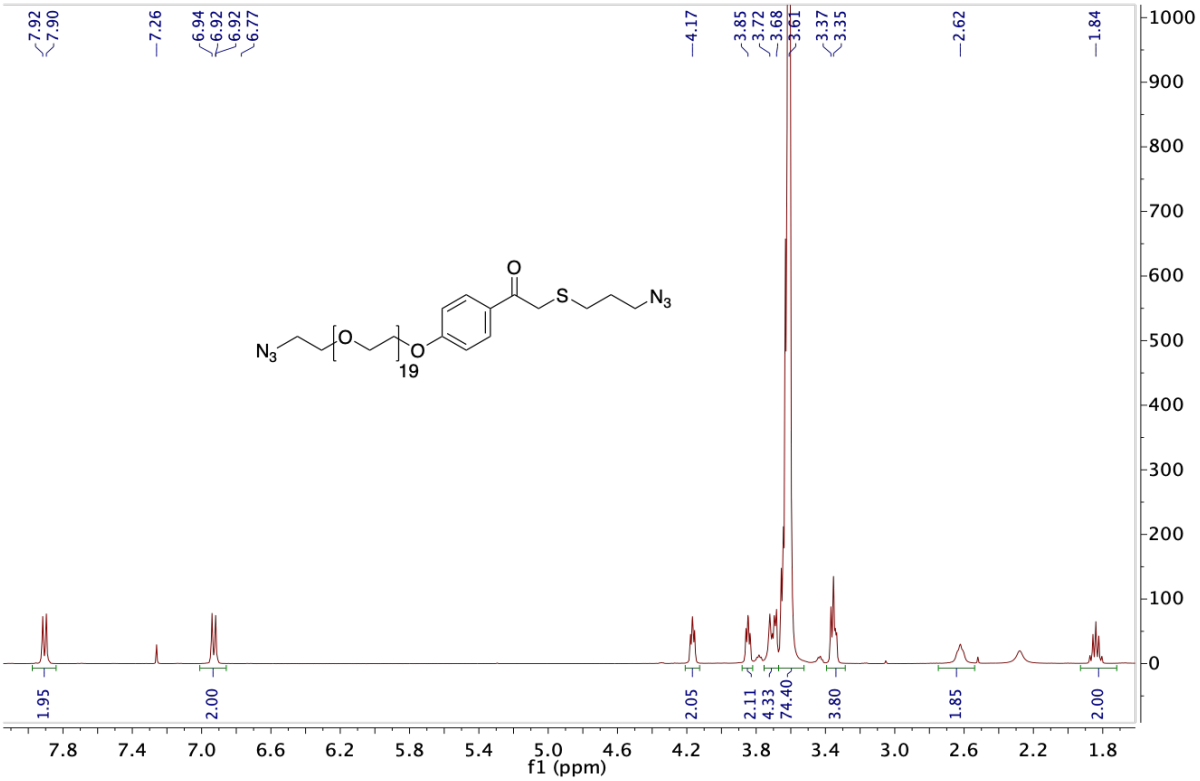
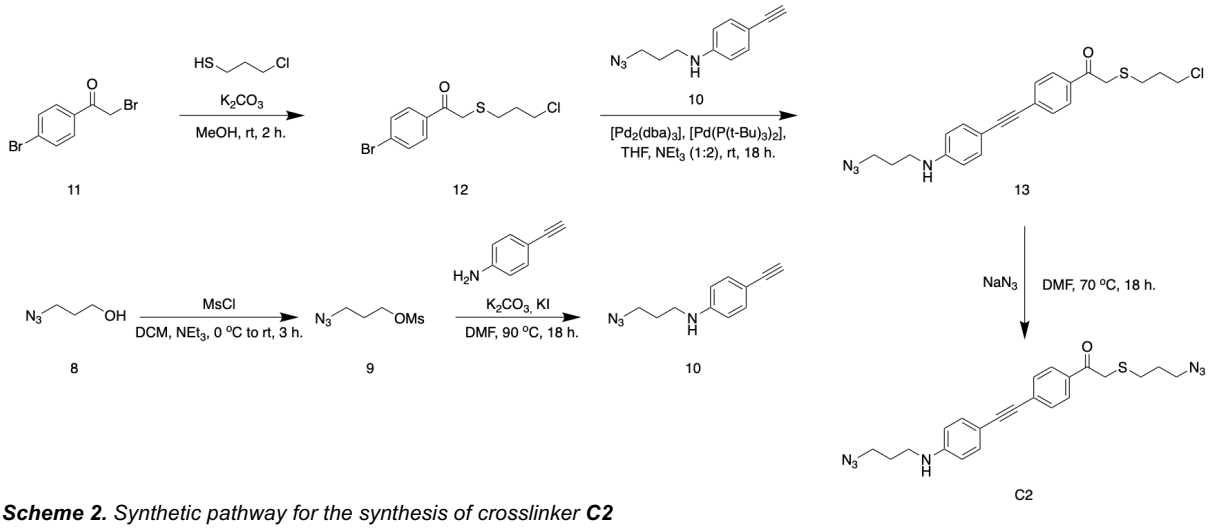


Figure S1. *^{1}H-NMR spectrum recorded for crosslinker **C1** in $CDCl_3$.*

2.1.6 1-azido-nonadeca-ethylene glycol methanesulfonate (**7**)[4]

O-(2-Azidoethyl)nonadeca-ethylene glycol (**6**) (250 mg, 0.258 mmol) and Et_3N (57 µL, 0.409 mmol) were dissolved in DCM (2 mL) and cooled using an ice bath. MsCl (23 µL, 0.295 mmol) was dissolved in DCM (2 mL) and added to the reaction mixture over the course of 1 hour using a syringe pump. The ice batch was removed and the reaction mixture was allowed to stir an additional 2 hours. Subsequently HCl (3%, 10 mL) was added to the reaction mixture and stirred for 5 minutes. The aqueous layer was extracted with DCM (3 x 25 mL). The combined organic layers were dried over $MgSO_4$ and concentrated *in vacuo*. This afforded the product as a white paste (yield: 230.2 mg, 85%). ^{1}H-NMR (400 MHz, $CDCl_3$) δ = 4.32 (t, J = 2.84 Hz, CH_2, 2H), 3.71 (t, J = 2.88 Hz, CH_2, 2H), 3.58 (m, -(OCH_2CH_2O)$_{19}$-, 76H), 3.33 (t, J = 4.28 Hz, CH_2, 2H), 3.03 (s, CH_3, 3H). ^{13}C-NMR (100 MHz, $CDCl_3$) δ = 70.53, 69.99, 69.30, 68.98, 50.64, 37.69. Mass calc.: 1001.52 [M], mass found: 1040.90 $[M+K]^+$.

2.2 Synthesis of extended phenacyl photolabile bis-azide crosslinker **C2**



Scheme 2. *Synthetic pathway for the synthesis of crosslinker* ***C2***

2.2.1 3-azido-1-mesyl propane (**9**)

3-azido-propanol (**8**) (2.64 ml, 28.7 mmol) and Et_3N (4.8 ml, 34.4 mmol) were dissolved in DCM (5 mL) and cooled using an ice bath. MsCl (2.66 ml, 34.4 mmol) was dissolved in DCM (4 ml) and added to the reaction mixture over the course of 1 hour using a syringe pump. The ice batch was removed and the reaction mixture was allowed to stir an additional 2 hours. Subsequently water (30 ml) was added to the reaction mixture and stirred for 5 minutes. The aqueous layer was extracted with Et_2O (3 x 50 ml). The combined organic layers were dried over $MgSO_4$ and concentrated *in vacuo*. This afforded the product as a transparent oil (yield: 5.01 g, 28.4 mmol, 99%). ^{1}H-NMR (400 MHz, $CDCl_3$) δ = 4.29 (t, J = 6.04 Hz, OC*H*$_2$, 2H), 3.46 (t, J = 6.48 Hz, N_3C*H*$_2$, 2H), 3.01 (s, C*H*$_3$, 3H), 1.98 (q, J = 6.44 Hz, CH_2C*H*$_2$$CH_2$, 2H). ^{13}C-NMR (100 MHz, $CDCl_3$) δ = 66.50, 47.22, 37.19, 28.55. ^{1}H- and ^{13}C-NMR data match reported values.[5]

2.2.2 N-(3-azidopropyl)-4-ethynylaniline (**10**)

4-ethynylaniline (3.3 g, 28 mmol), K_2CO_3 (11.8 g, 85.2 mmol) and KI (0.94 g, 5.68 mmol) were dissolved in DMF and stirred at 90 ºC for 5 minutes. After all reactants dissolved, 3-azido-1-mesyl propane (**9**) (5.01 g, 28.4 mmol) was added and the reaction mixtures was stirred overnight. The reaction progress was evaluated using TLC analysis (25% EtOAc in PE) and showed the product spot (rf: 0.66). Subsequently the reaction was quenched by the addition of NH_4Cl (sat.) (100 ml) and the product was extracted to EtOAc (3 x 50 ml). The combined organic layers were washed with H_2O (3 x 50 ml), dried over $MgSO_4$, concentrated *in vacuo* and purified over silica (10% EtOAc to 20% EtOAc in PE). This afforded the title compound as a red oil (yield: 1.08 g, 5.41 mmol, 19%). ^{1}H-NMR (400 MHz, $CDCl_3$) δ = 7.32 (d, J = 8.32 Hz, C*H*$_{2arom}$, 2H), 6.52 (d, J = 8.36 Hz, C*H*$_{2arom}$, 2H), 3.91 (s (broad), N*H*, 1H), 3.43 (t, J = 6.48 Hz, N_3C*H*$_2$, 2H), 3.25 (t, J = 6.4 Hz, NHC*H*$_2$, 2H), 2.96 (s, CC*H*, 1H), 1.88 (q, J = 6.6 Hz, CH_2C*H*$_2$$CH_2$, 2H). ^{13}C-NMR (100 MHz, $CDCl_3$) δ = 148.21 (*C*$_{arom}$), 133.49 (*C*H$_{arom}$), 112.22 (*C*H$_{arom}$), 110.17 (*C*$_{arom}$), 84.57 (*C*$_{alkyne}$), 74.79 (*C*H$_{alkyne}$), 49.23 (N_3*C*H_2), 40.85 (NH*C*H_2), 28.51 (CH_2*C*$H_2$$CH_2$). Mass calc.: 200.11 [M], mass found: 201.05 $[M+H]^+$.

2.2.3 4-bromophenacyl-3-chloropropyl-thio-1-ethanone (**12**)

4-bromophenacyl bromide (2.0 g, 7.2 mmol) and K_2CO_3 (1.0 g, 7.2 mmol) were dissolved in MeOH (80 ml) and stirred at RT for 5 minutes. Then 3-chloropropanethiol (840 µL, 863 mmol) was added and the reaction mixture stirred 2 hours at RT. TLC analysis (5% EtOAc in PE) showed all starting compound had reacted and revealed a single product spot (rf = 0.3). MeOH was removed *in vacuo* and the residue was re-dissolved in EtOAc (100 mL) and washed with H_2O (3 x 100 mL). The organic layer was dried over $MgSO_4$, filtered and concentrated *in vacuo*. This afforded the title compound as a yellowish oil (yield: 2.1 g, 6.8 mmol, 95%). ^{1}H-NMR (400 MHz, $CDCl_3$) δ = 7.84 (d, J = 8.56 Hz, CH_{2arom}, 2H), 7.61 (d, J = 8.60 Hz, CH_{2arom}, 2H), 3.75 (s, CH_2, 2H), 3.60 (t, J = 6.32 Hz, CH_2, 2H), 2.69 (t, J = 7.00 Hz, CH_2, 2H), 2.05 (q, J = 6.64 Hz, CH_2, 2H). ^{13}C-NMR (100 MHz, $CDCl_3$) δ = 193.46 ($C_{carbonyl}$), 133.86 (C_{arom}), 132.18 (CH_{arom}), 130.44 (CH_{arom}), 128.85 (C_{arom}), 43.32 (CH_2), 36.98 (CH_2), 31.52 (CH_2), 29.36 (CH_2).

2.2.4 1-(4-((4-((3-azidopropyl)amino)phenyl)ethynyl)phenyl)-2-((3-chloropropyl)thio)ethan-1-one (**13**)

Compound **10** (1.0 g, 5.0 mmol), compound **12** (1.5 g, 4.9 mmol), $[Pd_2(dba)_3]$ (34.3 mg, 0.037 mmol), and $[Pd(P(t\text{-}Bu)_3)_2]$ (38.3 mg, 0.075 mmol) were charged in a round bottom flask which was evacuated and purged with Argon (3 cycles). Next, using standard Slenck techniques, triethylamine (4 ml, anhydrous) and THF (2 ml, anhydrous) were added and the reaction was stirred overnight at RT. TLC analysis (25% EtOAc in PE) revealed a 366 nm UV active product spot (rf = 0.1). The reaction mixture was filtered over a Whatman (0.45 µm) filter and the residue was washed with EtOAc (3 x 5 mL). The filtrate was concentrated in vacuo and purified over silica (10% EtOAc to 30% EtOAc in PE), which afforded the product as a yellow powder (yield: 928.8 mg, 2.19 mmol, 44%). ^{1}H-NMR (400 MHz, $CDCl_3$) δ = 7.93 (d, J = 8.52 Hz, CH_{2arom}, 2H), 7.56 (d, J = 8.48 Hz, CH_{2arom}, 2H), 7.36 (d, J = 8.68 Hz, CH_{2arom}, 2H), 6.56 (d, J = 8.72 Hz, CH_{2arom}, 2H), 3.78 (s, CH_2, 2H), 3.63 (t, J = 6.32 Hz, CH_2, 2H), 3.45 (t, J = 6.44 Hz, CH_2, 2H), 3.30 (t, J = 6.76 Hz, CH_2, 2H), 2.74 (t, J = 7.00 Hz, CH_2, 2H), 2.06 (q, J = 6.76 Hz, CH_2, 2H), 1.90 (q, J = 6.60 Hz, CH_2, 2H). ^{13}C-NMR (100 MHz, $CDCl_3$) δ = 193.61 ($C_{carbonyl}$), 133.30 (CH_{arom}), 131.32 (CH_{arom}), 128.70 (CH_{arom}), 112.35 (CH_{arom}), 49.25 (CH_2), 43.22 (CH_2), 40.85 (CH_2), 36.97 (CH_2), 31.48 (CH_2), 29.28 (CH_2), 28.52 (CH_2). Mass calc.: 426.13 [M], mass found: 427.20 $[M+H]^+$.

2.2.5 1-(4-((4-((3-azidopropyl)amino)phenyl)ethynyl)phenyl)-2-((3-azidopropyl)thio)ethan-1-one (**C2**)

Compound **13** (928.8 mg, 2.19 mmol) and NaN_3 (1.5 g, 25 mmol) were dissolved in DMF (50 ml) and stirred overnight at 70 °C. Subsequently, H_2O (150 ml) was added and the product extracted to EtOAc (3 x 100 ml). The combined organic layers were dried over $MgSO_4$, filtered, concentrated *in vacuo* and purified over silica (10% EtOAc to 30% EtOAc in PE). This afforded photo cleavable crosslinker **C2** as a yellow/orange powder (yield: 777.9 mg, 1.794 mmol, 70%). ^{1}H-NMR (400 MHz, $CDCl_3$) δ = 7.93 (d, J

= 8.48 Hz, CH_{2arom}, 2H), 7.56 (d, J = 8.48 Hz, CH_{2arom}, 2H), 7.39 (d, J = 8.68 Hz, CH_{2arom}, 2H), 6.59 (d, J = 8.68 Hz, CH_{2arom}, 2H), 4.22 (s_{broad}, N*H*, 1H), 3.78 (s, CH_2, 2H), 3.43 (t, J = 6.44 Hz, CH_2, 2H), 3.39 (t, J = 6.68 Hz, CH_2, 2H), 3.30 (t, J = 6.72 Hz, CH_2, 2H), 2.67 (t, J = 7.08 Hz, CH_2, 2H), 1.94 – 1.84 (m, CH_2, 4H). ^{13}C-NMR (100 MHz, $CDCl_3$) δ = 193.70 ($C_{carbonyl}$), 148.54 (C_{arom}), 133.57 (C_{arom}), 133.47 (CH_{arom}), 131.48 (CH_{arom}), 129.71 (C_{arom}), 128.86 (CH_{arom}), 112.52 (CH_{arom}), 110.77 (C_{arom}), 94.82 (C_{alkyne}), 87.11 (C_{alkyne}), 50.10 (CH_2), 49.41 (CH_2), 41.01 (CH_2), 37.11 (CH_2), 29.39 (CH_2), 28.68 (CH_2), 28.27 (CH_2). Mass calc.: 433.17 [M], mass found: 434.14 $[M+H]^+$.

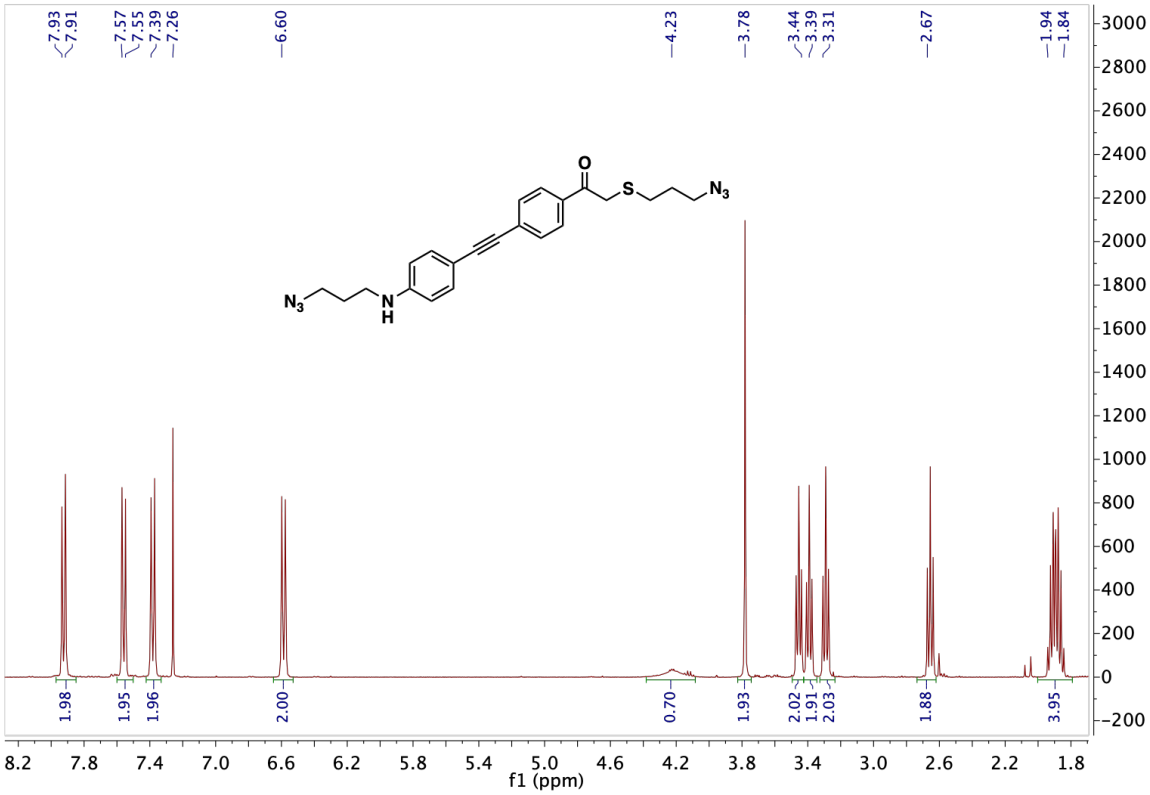


Figure S2. *^{1}H-NMR spectrum recorded for crosslinker* ***C2*** *in $CDCl_3$.*

2.3 Dextran-alkyne synthesis

Dextran (500 kDa, 3.4 g, 0.0068 mmol) was dissolved in a NaOH solution (30 ml, 0.1 M) and heated to 35 ºC. Glycidyl propargyl ether (5 ml, 0.046 mmol) was added and the solution was stirred overnight. After cooling the reaction mixture to RT, the solution was poured in isopropyl alcohol (IPA) (500 ml) to precipitate alkyne modified dextran. The IPA was decantated and the precipitate re-dissolved in demineralized water and dialyzed (MWCO = 3.5 kDa) against demineralized water for 72 hours (4 x 2 L), after which it was freeze dried to obtain pure alkyne modified dextran as a white fluffy powder. The degree of substitution determined by ^{1}H-NMR is 36% (figure **S3**).[6] Yield: 3.1 g. ^{1}H-NMR (400 MHz, D_2O) δ = 5.36, 5.19, 5.03, 4.85 (anomeric C*H*, 1H), 4.14 (O-CH_2, HO-C*H*, 3H, s) 3.88-3.30 (9H, m), 2.82 (alkyne CC*H*, 1H, s). ^{13}C-NMR (101 MHz, D_2O) δ = 97.66, 79.50, 76.13, 73.33, 71.34, 70.70, 70.11, 69.44, 68.80, 65.45, 58.24

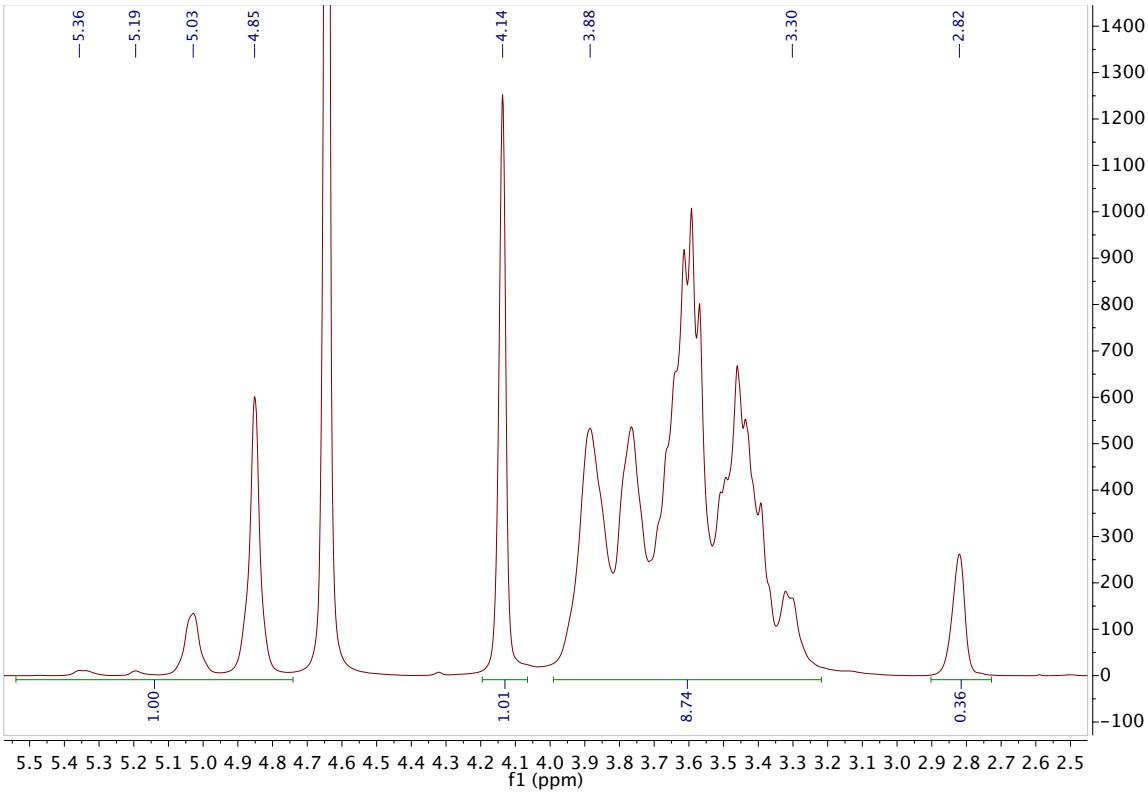


Figure S3. *^{1}H-NMR spectrum recorded for alkyne modified dextran (500 kDa) in D_2O. 5.03 ppm resonance belonging to anomeric proton of alkyne modified glucose units, 4.85 ppm resonance belonging to anomeric proton of unmodified glucose units and 2.82 ppm resonance belonging to alkyne proton. Degree of substitution (DS) = 36% (ratio between alkyne resonance (2.82 ppm) and the sum of anomeric proton resonances (5.36 to 4.85 ppm)).*

2.4 Protocol for dextran hydrogel preparation

2.4.1 Dextran hydrogel preparation using bis-azide crosslinker **C1**

A 10 wt% dex-alkyne solution was prepared by dissolving dex-alkyne in H_2O (300 µL), this solution was shaken for 1 hour. Crosslinker **C1** (4.0 mg, 0.004 mmol) was dissolved in a Cu-click stock solution (200 µL, ($CuSO_4$ (0.59 mM), sodium ascorbate (3.79 mM) and THTPA (0.29 mM))), which was shortly shaken to dissolve and mix all components. Next, the dex-alkyne and the crosslinker **C1** solution were mixed and shortly shaken, after which the solution was transferred in a cubic mold and left overnight to gel. After gelation was complete, the hydrogel was removed from the mold and placed in a phosphate buffer (PB) (25 mL, 100 mM, pH 7.4) solution or demineralized water to remove Cu-click components, unreacted dex-alkyne and crosslinker **C1**. This washing step was repeated 3 times and afforded transparent self-supporting cubic hydrogels.

2.4.2 Dextran hydrogel preparation using bis-azide crosslinker **C2**

A 10 wt% dex-alkyne solution was prepared by dissolving dex-alkyne in H_2O (300 µL), this solution was shaken for 1 hour. Crosslinker **C2** was dissolved in a CuI stock solution (200 µL, 3.15 mM CuI and 8.6 µM DIPEA in DMSO), which was shortly shaken to dissolve and mix all components. Next, the dex-

alkyne and crosslinker **C2** solution were mixed and shortly shaken, after which the solution was transferred in a cubic quartz cuvette (4 x 4 x 4 cm) and left overnight to gel. After gelation was complete, phosphate buffer (PB) (25 mL, 100 mM, pH 7.4) solution was carefully placed on the hydrogel patch to remove Cu-click components, unreacted dex-alkyne and crosslinker **C2**. This washing step was repeated 3 times and afforded yellow transparent self-supporting hydrogel patches.

3. Photochemical characteristics of bis-azide crosslinkers C1 and C2

3.1.1 Photocleavage reaction of bis-azide crosslinker **C1**

Crosslinker **C1** was dissolved in demineralized H_2O (40 µM) and 0.5 ml was added to a quartz cuvette. The crosslinker **C1** solution was then irradiated with a UVC lamp (17 cm distance to sample) under continuous stirring and protected from other light sources. UV/Vis spectra were recorded at selected timepoints (Figure **2A**) and the absorbance at 283 nm was plotted against time (Figure **2B**).

3.1.2 Photocleavage reaction of bis-azide crosslinker **C2** monitored by UV/Vis analysis

Crosslinker **C2** was dissolved in DMSO (40 µM) and 20 ml was added to a 4 x 4 x 4 cm quartz cuvette. The crosslinker **C2** solution was then irradiated with a 375 nm LED lamp (M375L4 (Thorlabs), 1.2 A, maximal power setting, distance to cuvette 4 cm, photon flux = $2.93 \pm 0.08 * 10^{15}$ s^{-1}) under continuous stirring and protected from other light sources. UV/Vis spectra were recorded at selected timepoints (Figure **3B**) and the absorbance at 383 nm was plotted against time (Figure **3C**).

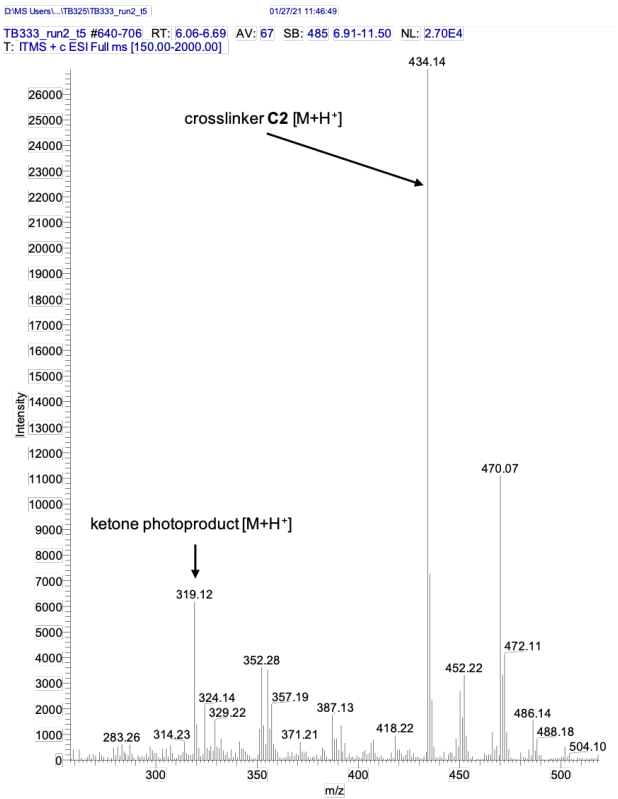


Figure S4 *ESI-MS spectrum recorded at t = 5 min during 375 nm light irradiation experiment. The mass of crosslinker **C2** ([M+H⁺]) and the mass of the ketone photoproduct ([M+H⁺]) is detected, indicating photocleavage of **C2**.*

3.1.3 Photocleavage reaction of bis-azide crosslinker **C2** monitored by ^{1}H-NMR spectrocopy

Crosslinker **C2** (2.99 mg, 6.9 μmol) was placed in a quartz cuvette (4 x 4 x 4 cm) and dissolved in DMSO-d6 (6 ml). Isopropyl alcohol (IPA, 1 μl, 13 μmol) was added as internal standard. The solution was irradiated with 375 nm light (M375L4 (Thorlabs), 1.2 A, maximal power setting, distance to cuvette 4 cm, photon flux = 2.93 ± 0.08 * 10^{15} s^{-1}) under continuous stirring and protected from other light sources. During irradiation aliquots were collected at selected timepoints and analyzed by ^{1}H-NMR (Figure **S5**).

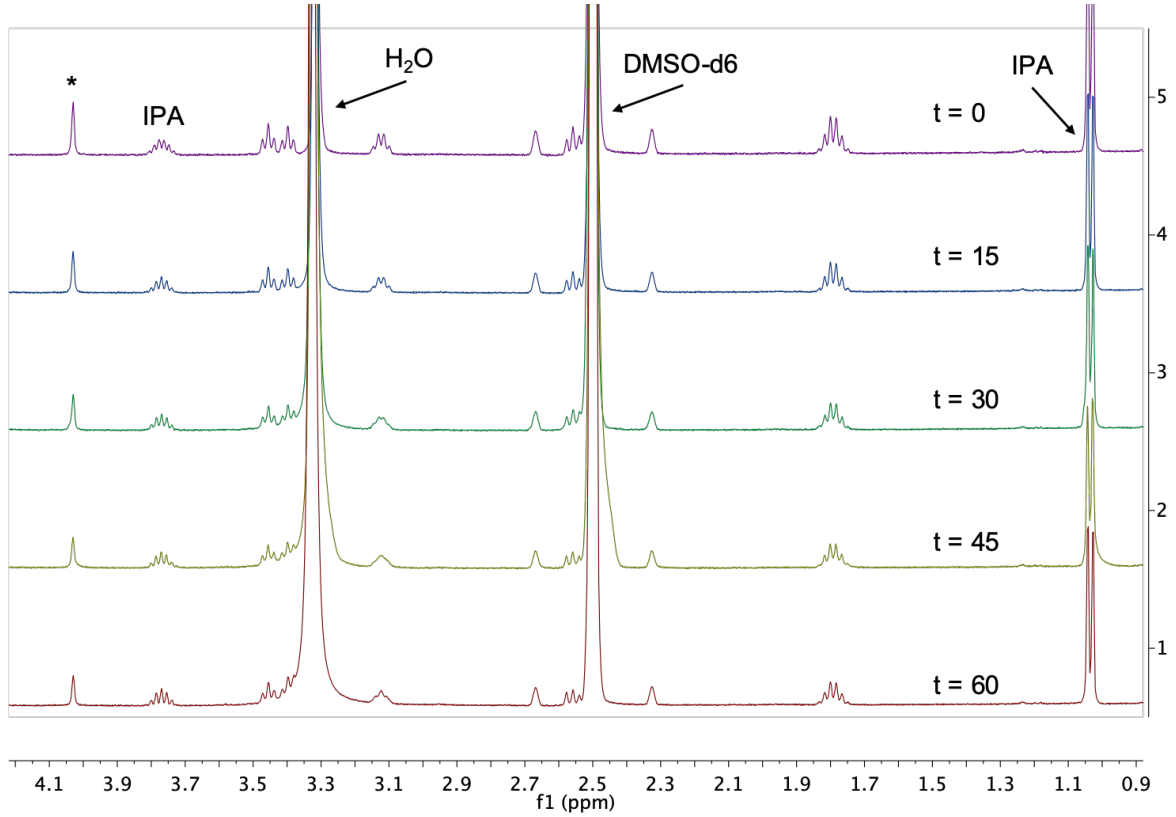


Figure S5 *Stacked ^{1}H-NMR spectra recorded during the 375 nm light irradiation of crosslinker **C2** (13.8 μM). CH_2 resonance at 4.03 ppm (indicated with asterisk) was monitored to determine the **C2** concentration over time relative to internal standard isopropyl alcohol (IPA). Spectra where recorded in DMSO-d6.*

3.1.4 Molar extinction coefficient determination of bis-azide crosslinker **C2**

A stock solution of crosslinker **C2** (6.42 mg in 50 ml DMSO) was prepared in DMSO using volumetric glassware. Subsequently a dilution series (DMSO) ranging from 21 µM to 59 µM was prepared and its absorbance was spectrophotometrically determined (Figure **S6A**). The absorbance at 383 nm was plotted against the concentration from which the molar extinction coefficient was determined (Figure **S6B**). ε = 27210 M^{-1} cm^{-1}.

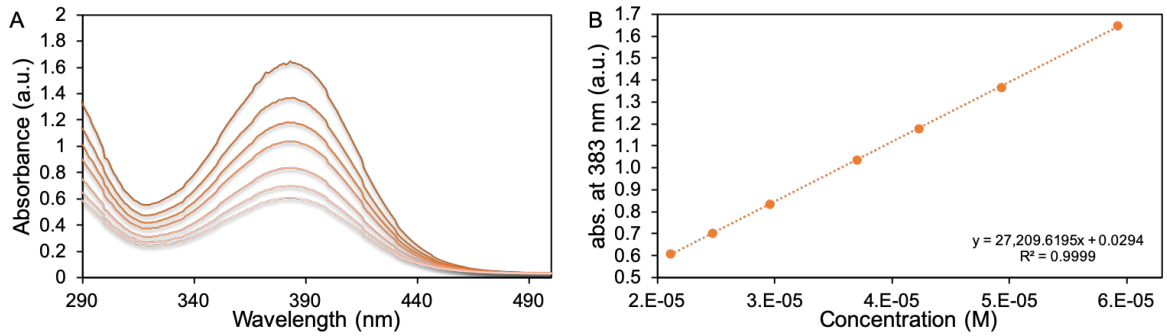


Figure S6 A) *UV/Vis absorbance spectra recorded for crosslinker* ***C2*** *(concentration range = 21 μM to 59 μM).* ***B)*** *absorbance (383 nm) plotted vs crosslinker* ***C2*** *concentration. Molar extinction coefficient (ε_{383}) = 27210 M^{-1} cm^{-1}.*

3.1.5 Photon flux determination of 375 nm LED using ferrioxalate actinometry

The incident photon flux (I_0) of a 375 nm LED light (Thorlabs ML375) driven by Thorlabs T-cube LED driver (1.2 A and maximum current) was determined by irradiation of the potassium ferrioxalate actinometer. In brief, the potassium ferrioxalate solution (0.006 M in H_2SO_4 (0.05 M)) was placed in a 4 x 4 x 4 cm quartz cuvette which was irradiated with the 375 nm LED lamp (distance to cuvette 4 cm). At fixed timepoints, aliquots (100 µL) were taken and 1,10-phenanthroline (140 µL, 5 mM), acetic acid (sodium salt, 1.15 ml, 1.0 M) and sulfuric acid (750 µL, 0.5 M) were added and kept in the dark for 30 minutes. The collected aliquots were spectrophotometrically analyzed for their absorbance at 510 nm and plotted against time. The experiment was repeated three times yielding an average slope of absorbance change of 3.41 ± 0.09 * 10^{-3} s^{-1}. The incident photon flux (I_0) of the 375 nm LED lamp is: 2.93 ± 0.08 * 10^{15} s^{-1} calculated using equation **1**.[7]

$$\text{incident photon flux} = \left(\frac{dA_{510}}{dt}\right)\frac{N_A * V}{\phi_{365} * \varepsilon_{510} * l} \qquad (\mathbf{1})$$

Where,

dA_{510} = 3.41 * 10^{-3} s^{-1}

N_A = 6.02214 * 10^{23} mol^{-1}

V = 0.002 dm^3

ϕ_{365} = 1.26

ε_{510} = 11.1 *10^3 mol^{-1} dm^3 cm^{-1}

l = 1.000 cm

3.1.6 Quantum yield determination of the photodegradation of crosslinker **C2**

The quantum yield (ϕ) of the 375 nm light driven photodegradation process of crosslinker **C2** was determined using equation **2**. The photodegradation process follows a linear trend and equals 6.2 * 10^{-3} s^{-1} (first 30 minutes UV/Vis data) (Figure **3B**). Number of photons absorbed is 2.93 * 10^{15} s^{-1}. By substituting these values in equation **2** the ϕ is calculated and equals: 0.14 (14%).

$$\phi = \frac{\text{number of molecules } \mathbf{C2} \text{ degraded}}{\text{number of photons absorbed}} \quad (\mathbf{2})$$

$$number\ of\ molecules\ \boldsymbol{C2}\ degraded = \frac{dA_{383} * V * N_A}{\varepsilon_{383} * l} = 4.1 * 10^{14}\ s^{-1}$$

Where,

dA_{383} = 6.2 * 10^{-3}

N_A = 6.02214 * 10^{23} mol^{-1}

V = 0.003 dm^3

ε_{383} = 27.2 *10^3 mol^{-1} dm^3 cm^{-1}

l = 1.000 cm

4. Light triggered release studies

Dextran hydrogels were prepared according the protocol described in section 2.4, with minor modifications in order to load doxorubicin, BSA or human IgG antibody.

4.1.1 Light triggered doxorubicin release

A set of cubic transparent self-supporting hydrogels were prepared and washed according the protocol described in section 2.4.1 (Figure **S7A**). A doxorubicin stock solution was prepared in DMSO (100 mg/ml) which was diluted in demineralized water (30 µl doxorubicin stock in 30 ml H_2O). Then 25 ml of the diluted aqueous doxorubicin solution was added to the dextran hydrogels (Figure **S7B**) which were stored in the dark for 148 hours (Figure **S7C**). The doxorubicin loaded hydrogels were removed from the doxorubicin loading solution (Figure **S7D**) and placed in H_2O (25 ml) for 24 hours to remove excess doxorubicin (Figure **S7E**). The washed doxorubicin loaded hydrogels were then placed in fresh H_2O (25 ml) of which one was irradiated with UVC light (1.02 mW/cm^2, irradiance determined at the gel location)

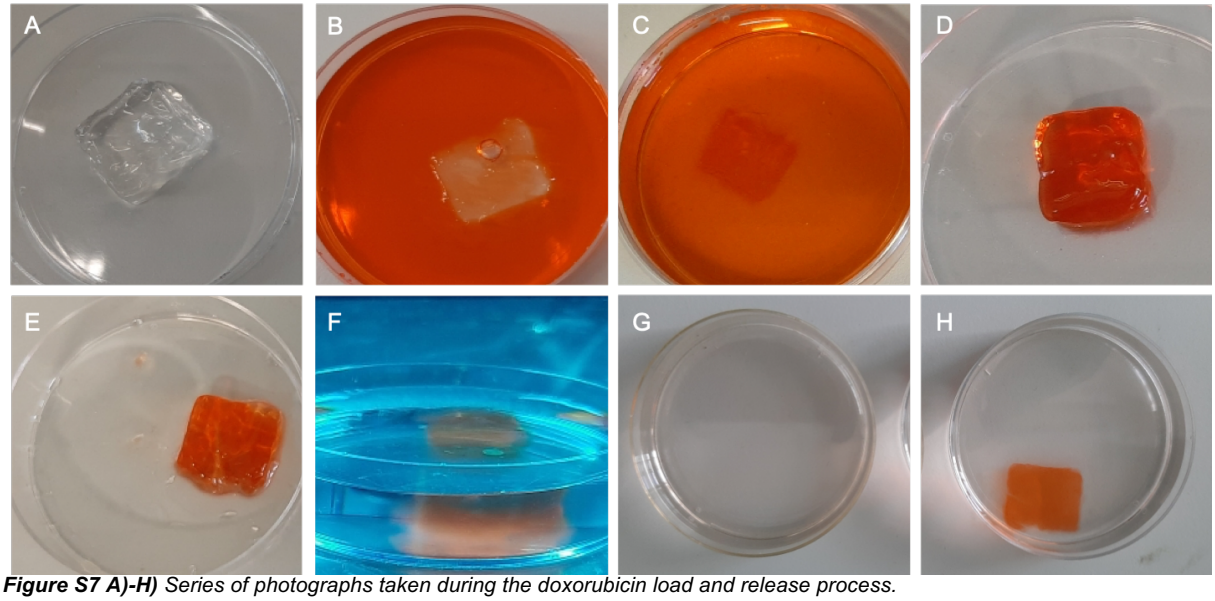


Figure S7 A)-H) *Series of photographs taken during the doxorubicin load and release process.*

for 24 hours (Figure **S7F**) while the other was kept in the dark as a control. The UVC irradiated hydrogel completely degraded (Figure **S7G**) in contrast to the non-irradiated hydrogels which stayed intact and retained the loaded doxorubicin inside the hydrogel matrix (Figure **S7H**). Aliquots of the surrounding solution of both samples were collected at fixed timepoints and analyzed by UV/Vis spectrophotometry. The absorbance at 283 nm and 480 nm (absorption maximum of doxorubicin) was plotted against time and shows that the absorbance at both wavelengths increased over time, in contrast to the control sample were the absorbances remain constant over time (Figure **S8A** and **S8B**). The measured absorbance characteristic for doxorubicin (480 nm) is low (0.05 to 0.07), while the absorbance at 283 nm is approximately 5-fold higher. Therefore, we conclude that the majority of the loaded and released doxorubicin is degraded as a result of the UVC light dose.

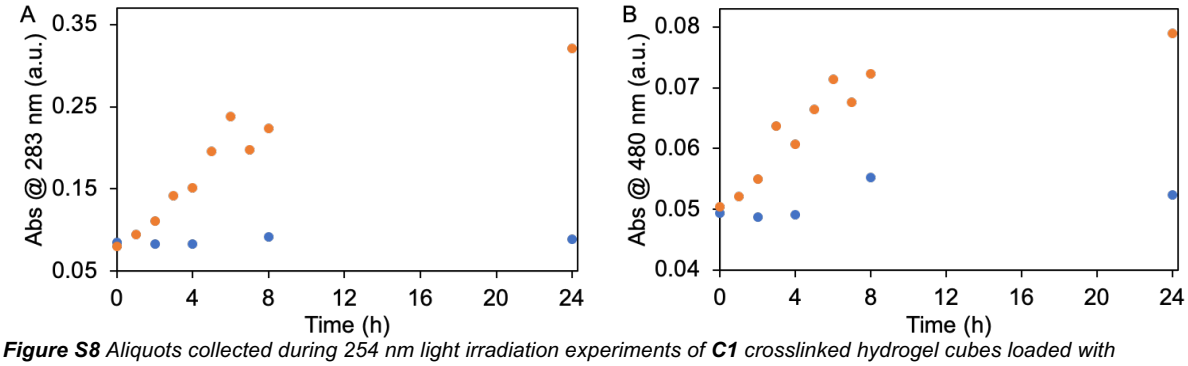


Figure S8 *Aliquots collected during 254 nm light irradiation experiments of* ***C1*** *crosslinked hydrogel cubes loaded with doxorubicin.* ***A)*** *Absorbance at 282 nm.* ***B)*** *Absorbance at 480 nm.*

The light degradation process of doxorubicin in the hydrogels aqueous supernatant was confirmed by fluorescence analysis (excitation: 470 nm) (Figure **S9**). At t = 15 minutes (orange data) the characteristic emission spectrum of doxorubicin is detected (emission max 595 nm). However, when light irradiation is prolonged the emission spectrum significantly changes (blue and green data) and reduces in intensity, indication doxorubicin's degradation.

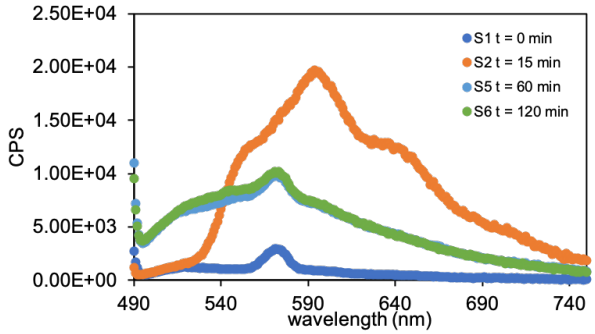


Figure S9 *Fluorescence spectra recorded during light irradiation experiments of doxorubicin loaded dextran hydrogels.*

4.1.2 Light triggered protein release

Hydrogel patches were prepared according the procedure described in section 2.4.2 and were loaded with bovine serum albumin (BSA) or immunoglobulin G (IgG) from human serum. In case of BSA loading, 60 µl of a BSA stock solution (0.63 mg/ml in phosphate buffer (PB) (100 mM, pH 7.4)) was added to the liquid dextran (540 µl) phase prior to gelation. In case of IgG loading, 100 µl of an IgG stock solution (50 mg/ml in phosphate buffer (100 mM, pH 7.4)) was added to the liquid dextran (500

µl) phase prior to gelation. After gelation and washing, the protein loaded hydrogel patches were irradiated with 375 nm light and aliquots (40 µl) were collected at selected timepoints. Each of the released protein fractions was labeled with 20x excess of NHS-Cy5 in PBS at room temperature for 4 hours. The BSA protein content was determined with SDS-PAGE gel analysis and fluorescence analysis (Typhoon, GE Healthcare). The IgG protein content was determined by the Bradford assay (n = 4 experiments for irradiated samples, n = 2 experiments for non-irradiation samples).[8] A concentration plot (0 mg/ml to 1.00 mg/ml) was prepared with n = 3 experiments (Figure **S10**).

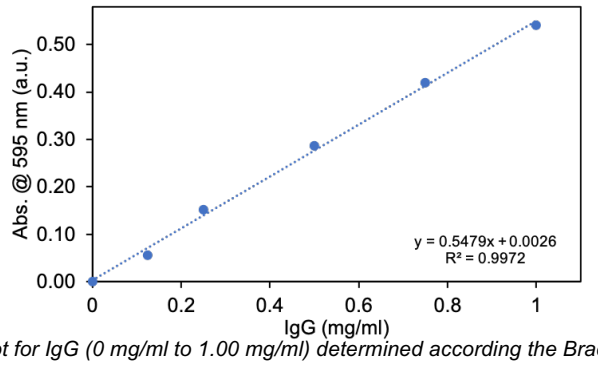


Figure S10 *Concentration plot for IgG (0 mg/ml to 1.00 mg/ml) determined according the Bradford assay.*

4.1.3 Cerenkov luminescence triggered protein release

Cubic hydrogels were prepared as described in section 2.4.1 with one minor modification for loading the protein solution. 30 µl of a BSA stock solution (0.63 mg/ml in phosphate buffer (PB) (100 mM, pH 7.4)) was added to the liquid dextran (270 µl) phase prior to gelation. The hydrogels were then placed in glass vials and irradiated with γ-rays (0.6 kGy/h) delivered by a ^{60}Co-source. At selected timepoints the aliquots (30 µl) were taken and analyzed for their protein content using UV/Vis (Nanodrop) analysis and standard SDS-Page gel analysis.

5. Rheology analysis

5.1.1 Rheological measurements of **C1** crosslinked hydrogels

Hydrogels were prepared as described in section 2.4.1. After mixing, 0.5 ml gel solution was placed on the rheometer plate (40 mm diameter steel plate-and-plate geometry, equipped with a hexadecane trap). After 90 minutes the gel point is reached, as the storage modulus surpasses the loss modulus, indicating a hydrogel has formed (Figure **S11A**). Time sweep measurements were performed at a fixed strain (γ = 1%) and frequency (ω =6.28 rad/s = 1 Hz). The frequency sweep measurements were performed from 0.1 to 100 rad/s at a fixed strain (γ = 1%), using a 40 mm steel plate-and-plate geometry (Figure **S11B**).

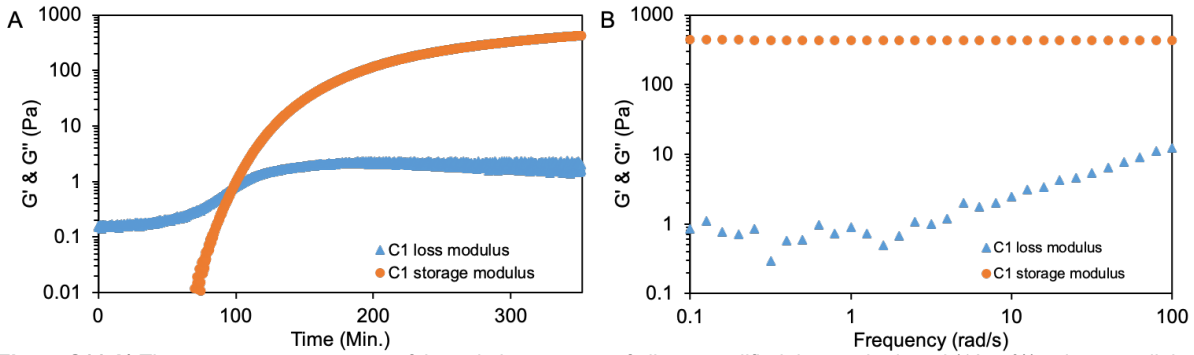


Figure S11 A) Time sweep measurement of the gelation process of alkyne modified dextran hydrogel (10 wt%) using crosslinker **C1** (γ = 1%, ω = 1 Hz, 25 °C). The gel point is reached after approximately 90 minutes. **B)** Frequency sweep measurements of **C1** crosslinked dextran-based hydrogels.

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