Supporting Information

Light controlled protein release from a supramolecular hydrogel

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Experimental Section

Materials. 1,3-Dibromopropane, 4-phenylazophenol and sodium thiosulfate pentahydrate were obtained from Aldrich. β-cyclodextrin hydrate (β-CD) was supplied by Acros. Dextran (M_n = 20000, Pharmacia Fine Chemicals, Sweden) was dried in the vacuum oven for several days before use. 1,4-dioxane, ethanol and dimethyl sulfoxide (DMSO) were previously dried with molecular sieves. Water used in all experiments was purified through deionization and filtration with a Millipore purification apparatus. Preparation of maleimide functionalized dextran was described elsewhere.1

Methods. 1H NMR spectra were recorded on a Bruker AV-400 spectrometer operating at 400 MHz. The degree of substitution for AB-Dex and CD-Dex (DS; defined as the number of substituents per 100 AHG units) was calculated from the 1H NMR spectrum of Mal-Dex in D_2O based on the glucosidic protons of dextran (δ 3.2 - 4.0, 5.1 and 5.3) and the protons of the maleimides (δ 6.9). AB-Dex was isomerized from trans to cis by irradiation with a 100 W high intensity UV lamp (Blak-Ray) at the wavelength of 365 nm with a fixed distance of 30 cm between the sample cell and the lamp. Fluorescence measurements were performed using a luminescence spectrometer LS50B (Perkin Elmer). All spectra were obtained at room temperature using a quartz cuvette with a 1 cm path length. Each spectrum was measured with the excitation and emission slits of 5 nm. The excitation wavelength was 475 nm.

Synthesis of 3-[4-(Phenylazo)phenoxy]propane-1-thiol.2

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\begin{align*}
\text{[4-(3-Bromopropoxy)phenyl]phenylidiazene} \\
4-(Phenylazo)phenol (9.9 g, 50 mmol) and 1,3-dibromopropane (50.5 g, 250 mmol) were refluxed overnight in 150 mL of 1,4-dioxane together with 5 g of potassium
\end{align*}
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carbonate and 0.25 g of potassium iodide. After the reaction mixture was filtered while still hot and the solid was washed with chloroform, solvent and unreacted 1,3-dibromopropane were removed by evaporation. Purification by flash chromatography (Dichloromethane/petroleum ether = 1:1) afforded 9.7 g [4-[(3-bromopropoxy)phenyl] phenyldiazene with a yield of 60%. $^1$H NMR (400 MHz, CDCl$_3$): $\delta$ (ppm) 2.24 (m, 2H), 3.53 (t, $J = 6.4$, 2H), 4.05 (t, $J = 6.0$, 2H), 6.93 (m, 2H), 7.40 (m, 1H), 7.46 (m, 2H), 7.90 (m, 4H). $^{13}$C NMR (100 MHz, CDCl$_3$): $\delta$ (ppm) 29.7, 32.0, 65.4, 114.5, 122.45, 124.6, 128.9, 130.3, 146.9, 152.5, 161.0.

3-[4-(Phenylazo)phenoxy]propane-1-thiol

1.6 g (5 mmol) of [4-[(3-bromopropoxy)phenyl]phenyldiazene was dissolved in 10 mL of ethanol and 3 mL of sodium thiosulfate pentahydrate (1.3 g) aqueous solution was added. The mixture was refluxed for 2 h. After cooling, the precipitate (the Bunte salt) was collected. To a mixture of 30 mL of chloroform and 30 mL of 1M HCl degassed with nitrogen, 1.6 g of the Bunte salt was added and refluxed for 2 h. After cooling to room temperature, the organic layer was collected and the aqueous layer was extracted with chloroform. The organic fraction was washed with water for 3 times and dried over anhydrous MgSO$_4$. The product was obtained after removing the solvent by evaporation; Yield: 1.0 g (74%). $^1$H NMR (CDCl$_3$): $\delta$ (ppm) 1.42 (t, $J = 8.0$, 1H), 2.15 (m, 2H), 2.70 (m, 2H), 4.07 (t, $J = 6.0$, 2H), 6.97 (m, 2H), 7.45 (m, 3H), 7.89 (m, 4H). $^{13}$C NMR (100 MHz, CDCl$_3$): $\delta$ (ppm) 21.1, 33.1, 65.8, 114.6, 122.5, 124.7, 128.9, 130.3, 146.8, 152.6, 161.2.

Azobenzene modified dextran (AB-Dex). Maleimide modified dextran (DS = 6, 0.48 g) was dissolved in 15 mL of DMSO and 3-[4-(Phenylazo)phenoxy]propane-1-thiol (0.1 g, 2.2 eq to the maleimide) in 15 mL of DMSO was added slowly. The reaction mixture was stirred overnight at room temperature. After ultrafiltration (MWCO 3500) against DMSO and water, the product was obtained by lyophilization; Yield: 0.50 g, 96%. $^1$H NMR (400 MHz, DMSO-$d_6$): $\delta$ 2.1 (m, 2H), 3.1-3.8 (m, overlaps with HDO), 4.2-5.0 (m, 70H), 7.2 (d, $J = 8.4$, 2H), 7.6 (m, 3H), 7.9 (m, 4H).
Preparation of the mono-thiol β-cyclodextrin.

Scheme S2. Synthesis of mono-thiol β-cyclodextrin

Mono-thiol β-cyclodextrin was obtained by a two step reaction according to literature.\textsuperscript{3} \textsuperscript{1}H NMR (400 MHz, DMSO-d6): \(\delta\) 2.1 (t, SH), 2.7–3.2 (m, 2H, H-6a), 3.2–3.5 (m, overlapping with HDO, H-2, H-4), 3.5–3.8 (m, 26H, H-3, H-5, H-6b), 4.8 (br d, 7H, H-1), 5.7 (br, OH).

β-Cyclodextrin modified dextran (CD-Dex). Maleimide modified dextran (DS = 5, 0.60 g) was dissolved in 15 mL of water, 15 mL of mono-6-thio-β-cyclodextrin (0.4 g, 2 eq to the maleimide) aqueous solution was added slowly, the reaction mixture was stirred for 4 hours at room temperature. After purification by ultrafiltration (MWCO 3500) against water, the final product was collected by lyophilization. Yield: 0.76 g, 95%. \textsuperscript{1}H NMR (400 MHz, D\textsubscript{2}O): \(\delta\) 3.5 - 4.0 (m, 190H), 5.0 (s, 25H), 5.1 (s, 7H).

Hydrogel Preparation. Hydrogels were prepared by mixing the AB-Dex and CD-Dex. The molar ratio between CD and AB was kept at 1:1. Typically, AB-Dex (20 mg) and CD-Dex (30 mg) were dissolved into 200 µL phosphate buffered saline solution (PBS) and vortexed until a clear hydrogel was observed.

Photoresponse of the hydrogel. PBS solution containing AB-Dex (67 mg/mL) and CD-Dex (100 mg/mL) was prepared as mentioned above. After mixing it was a gel and after 3 hours of irradiation with UV light it showed fluidic behavior. When the fluidic solution placed overnight under normal light, gel was re-formed.

NOESY. 2-Dimensional NOESY spectrum was recorded on a 600-MHz Bruker DMX-600 spectrometer (Bruker) with a mixing time of 200 ms at 25 °C. Sample was prepared by dissolving 20 mg of AB-Dex in 600 µL D\textsubscript{2}O and then 45 mg of β-CD was added.
**Release of GFP from the hydrogel.** AB-Dex (20 mg) and CD-Dex (30 mg) were dissolved into 200 µL PBS containing green fluorescent protein (GFP, 0.1µg/µL) and vortexed until a clear hydrogel was observed. The hydrogel (18 mg) was placed into a cuvette, centrifuged for 10 minutes and stored overnight to form a thin layer at the bottom of the cuvette. After gentle washing with 1 mL PBS, 2 mL of fresh PBS was added. The released amount of GFP was monitored through the fluorescence from the solution part. The emission spectra were recorded every 10 mins while the cuvette was shaking at 200 rpm. After the experiment, the cuvette was shaken vigorously to make a homogeneous solution and check the fluorescence intensity of 100 % release.

**Photoisomerization of Dex-AB.**
The photoisomerization of AB-Dex was investigated by \(^1\)H NMR (Fig. S1). Before irradiation with UV light (a), peaks at \(\delta 7.2, 7.6\) and 7.9 that were ascribable to the aromatic protons of \textit{trans} AB moiety were observed with a trace amount of peaks ascribable to \textit{cis} AB moiety. \textit{trans} AB moieties were transformed to \textit{cis} configuration after 4 hour irradiation with UV light, thus only the peaks at \(\delta 6.8, 7.2\) and 7.3 attributed to \textit{cis} AB moiety were observed (b). From ratios of the integrals in these spectra, fractions of the \textit{trans} configuration were determined to be 95% and 0, respectively.

![Fig S1. \(^1\)H NMR spectra of 15 mg/mL AB-Dex in DMSO before (a), and after irradiation with UV light (b).](image-url)
References

