Supporting Information

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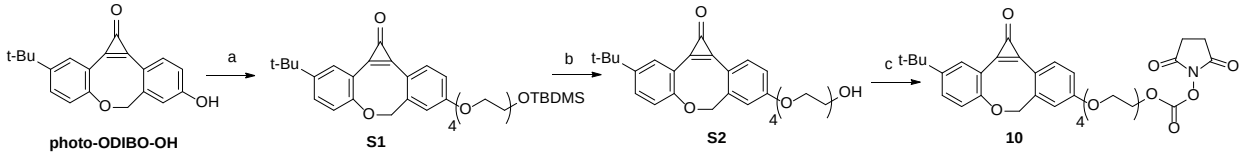
Title: Facile quenching and spatial patterning of cyclooctynes via strain-promoted alkyne–azide cycloaddition of inorganic azides.

General Methods. Flash chromatography was performed using 40-63 μm silica gel. All NMR spectra were recorded in $CDCl_3$ using 400 MHz instrument (unless otherwise noted). Electronic spectra and scanning kinetics were taken using a Cary 60 UV-Vis spectrometer. Rate measurements were performed using Carry-300 Bio UV-Vis spectrometer. Spectral changes in ODIBO-linked hydrogels were monitored by absorbance spectroscopy using a Thermo Scientific Nanodrop 2000 spectrophotometer. The gels were aligned in the 1mm gap between the quartz pedestals of the instrument, which were first wetted with buffer. Patterned gel imaging was performed using a Zeiss Axio Imager multichannel fluorescence microscope and Zeiss Axiovision imaging software to apply linear min/max thresholds to the images.

Materials. All organic solvents were dried and freshly distilled before use; Tetrahydrofuran was distilled from sodium/benzophenone ketyl and dichloromethane was distilled from CaH_2. Other reagents were obtained from Aldrich or VWR and used as received unless noted. DIBO-OH,[1] BCN (**4**)[2], ADIBO[3] and Rhodamine B azide[4] were prepared using previously reported procedures. 6-Carboxyfluorescein-TEG azide was obtained from Berry & Associates.

Preparation of photo-ODIBO-TEG-NHS (**10**)

Scheme S1



Reagents and conditions: a) $TBDMS(OCH_2CH_2)_4OH$, K_2CO_3, DMF, 58%; b) HF, CH_3CN, 85%; c) N,N'disuccinimidyl carbonate, Et_3N, CH_3CN, 92%

3-(tert-butyl)-9-((2,2,3,3-tetramethyl-4,7,10,13-tetraoxa-3-silapentadecan-15-yl)oxy)dibenzo[b,f]cyclopropa[d]oxocin-1(7H)-one (photo-ODIBO-TEG-TBS, **S1**).

TBDMS protected monotosyl tetra(ethyleneglycol) (2.49 g, 5.39 mmol) was added to a solution of photo-ODIBO-OH[5,6] (1.10 g, 3.59 mmol) in DMF (20 mL). Next, K_2CO_3 (0.50 g, 3.59 mmol) was added and the solution was stirred at 80 °C for 5 h. The reaction mixture was then diluted with

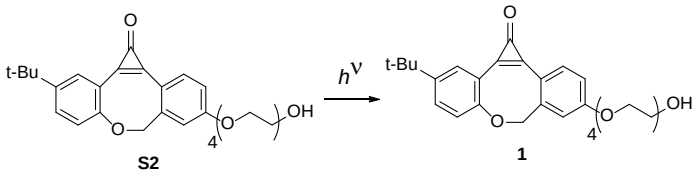
ethyl acetate (200 mL), washed with water (5 x 50 ml), brine (100 mL), and dried over $MgSO_4$. The organic layer was then filtered, concentrated in vacuum, and purified by flash chromatography (3:1 ethyl acetate: hexanes) to afford (1.24 g, 58%) photo-ODIBO-TEG-TBS (**S1**) as a colorless oil. ^{1}H-NMR: 7.92-7.94 (m, 2H), 7.48-7.51 (dd, J = 8.5, 2.5 Hz, 1H), 7.19-7.21 (d, J = 8.5 Hz, 1H), 7.02-7.06 (m, 2H), 5.24-5.27 (d, J = 12.1 Hz, 1H), 4.76-4.79 (d, J = 12.1 Hz, 1H), 4.21-4.23 (t, J = 4.7 Hz, 2H), 3.88-3.90 (t, J = 4.7 Hz, 2H), 3.72-3.77 (m, 4H), 3.65-3.69 (m, 6H), 3.53-3.56 (t, J = 5.4 Hz, 2H), 1.34 (s, 9H), 0.87 (s, 9H), 0.05 (s, 6H). ^{13}C-NMR: 162.10, 160.50, 152.79, 148.07, 144.10, 142.32, 140.73, 135.56, 131.17, 130.75, 122.09, 117.96, 117.48, 117.13, 114.72, 78.83, 72.82, 71.09, 70.89, 70.87, 70.81, 69.60, 68.06, 62.87, 34.71, 31.49, 26.10, 18.53, -5.08. ESI HRMS: calcd. ($M+H^+$): $C_{34}H_{49}O_7Si^+$ 597.3242, found 597.3241. IR: 1846 cm^{-1} ($\nu_{C=O}$).

3-(tert-butyl)-9-(2-(2-(2-(2-(hydroxyethoxy)ethoxy)ethoxy)ethoxy)dibenzo[b,f]cyclopropa [d]oxocin-1(7H)-one (photo-ODIBO-TEG-OH, **S2**). HF (0.123 g, 3.07 mmol, 50%) was added to a solution of photo-ODIBO-TBS (1.220 g, 2.04 mmol) in acetonitrile (11 mL). The solution was stirred for 30 minutes, diluted with ethyl acetate (300 mL) and water (100 mL). The organic layer was extracted, dried over MgSO4, filtered, concentrated in vacuum, and purified by flash chromatography (40:1 CH_2Cl_2: methanol) to afford photo-ODIBO-TEG-OH (0.835 g, 85 % yield) as a colorless oil. ^{1}H-NMR: 7.92-7.94 (m, 2H), 7.75-7.50 (dd, J = 8.5, 2.5 Hz, 1H), 7.18-7.20 (d, J = 8.4 Hz, 1H), 7.02-7.05 (m, 2H), 5.24-5.27 (d, J = 12.2 Hz, 1H), 4.74-4.77 (d, J = 12.1 Hz, 1H), 4.21-4.23 (t, J = 4.7 Hz, 2H), 3.86-3.89 (t, J = 4.7 Hz, 2H), 3.65-3.73 (m, 10H), 3.58-3.60 (t, J = 5.4 Hz, 2H), 2.91 (s, 1H), 1.33 (s, 9H). ^{13}C-NMR: 162.05, 160.48, 152.81, 148.05, 144.02, 142.23, 140.72, 135.56, 131.14, 130.46, 122.07, 117.91, 117.48, 117.07, 114.72, 78.80, 72.67, 70.98, 70.77, 70.68, 703.41, 69.57, 67.99, 61.81, 34.69, 31.46. $C_{28}H_{35}O_7^+$ 483.2377, found 483.2388. IR: 1846 cm^{-1} ($\nu_{C=O}$).

2-(2-(2-(2-((3-(tert-butyl)-1-oxo-1,7-dihydrodibenzo[b,f]cyclopropa[d]oxocin-9-yl)oxy) ethoxy)ethoxy)ethoxy)ethyl(2,5-dioxopyrrolidin-1-yl) carbonate (photo-ODIBO-TEG-NHS, **10**). N,N'disuccinimidyl carbonate (0.590 g, 2.07 mmol) was added to a solution of **1** (0.500 g, 1.31 mmol) and Et_3N (0.315 g, 3.11 mmol) in acetonitrile (11 mL). The reaction mixture was stirred for 16 hours, concentrated in vacuum, and purified by flash chromatography (1:1 acetone hexanes) to afford **S3** (0.593 g, 92% yield) as a colorless oil. ^{1}H-NMR: 7.93-7.95 (m, 2H), 7.49-7.52 (dd, J = 8.5, 2.5 Hz, 1H), 7.19-7.21 (d, J = 8.5 Hz, 1H), 7.04-7.07 (m, 2H), 5.25-5.28 (d, J = 12.2 Hz, 1H), 4.77-4.80 (d, J = 12.2 Hz, 1H), 4.44-4.46 (m, 2H), 4.23-4.25 (t, J = 4.8 Hz, 2H), 3.90-3.92 (t, J = 4.8 Hz, 2H), 3.73-79 (m, 4H), 3.65-3.71 (m, 6H), 2.82 (s, 4H), 1.35 (s, 9H). ^{13}C-NMR: 168.72, 162.19, 160.53, 152.81, 151.81, 148.13, 144.18, 142.35, 140.78, 135.61, 131.21, 130.80, 122.14, 117.99, 117.52, 117.47, 117.19, 114.82, 78.88, 71.09, 70.95, 70.81, 70.43, 69.62, 68.52, 68.14, 34.75, 31.52, 25.64. ESI HRMS: calcd. ($M+H^+$): $C_{33}H_{38}NO_{11}^+$ 624.2439, found 624.2442. IR: 1846 cm^{-1} ($\nu_{C=O}$).

Preparation of 2-tert-butyl-11,12-didehydro-8-(2-(2-(2-(2-hydroxyethoxy)ethoxy)ethoxy) ethoxy)-6H-dibenzo[b,f]oxocine (**ODIBO-TEG-OH, 1**).

Scheme S2



S2 **1**

A solution of photo-ODIBO-TEG-OH (**S2**, 0.412 g, 0.85 mmol) in methanol (400 mL) was irradiated for 25 min with 350 nm lamps in a Rayonet photoreactor. The photolysate was then concentrated and purified by flash chromatography (1:2 to 1:1 acetone: hexanes) to afford **1** (0.343 g, 88% yield) as a colorless oil. ^{1}H-NMR: 7.23-7.28 (m, 3H), 7.11-7.13 (m, 1H), 7.05 (d, J = 2.5 Hz, 1H), 6.91-6.94 (dd, J = 8.4, 2.5 Hz, 1H), 5.18-5.21 (d, J = 12 Hz, 1H), 4.54-4.57 (d, J = 12 Hz, 1H), 4.17-4.19 (t, J = 4.8 Hz, 2H), 3.87-3.90 (t, J = 4.8 Hz, 2H), 3.68-3.77 (m, 12H), 3.61-3.64 (m, 2H), 1.33 (s, 9H). ^{13}C-NMR: 167.32, 158.76, 149.12, 146.98, 126.92, 125.60, 123.74, 121.39, 118.36, 117.92, 117.69, 114.66, 114.21, 110.74, 78.07, 72.66, 71.07, 70.89, 70.82, 70.57, 69.83, 67.89, 61.98, 34.58, 31.59. $C_{27}H_{35}O_6^+$ 455.2428, found 455.2429.

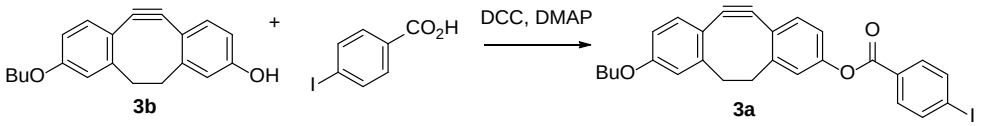
Reaction of ODIBO-TEG-OH (**1**) with sodium azide. A solution of sodium azide (0.037 g, 0.57 mmol) and ODIBO-TEG-OH (0.260 g, 0.57 mmol) in 50% aqueous methanol was incubated overnight at r.t. The reaction mixture was concentrated in vacuum and purified by flash chromatography to afford a single isomer of ODIBO triazole **8** (0.152 g, 53.4% yield) as a colorless oil and a vinyl methyl ether **9** (0.057 g, 20% yield) side product as a colorless oil.

ODIBO Triazole **8**: ^{1}H-NMR: 7.68 (s, 1H), 7.48-7.50 (d, J = 8.4 Hz, 1H), 7.24-7.27 (dd, J = 8.6 Hz, 2.5 Hz, 1H), 6.94-6.96 (d, J = 8.6 Hz, 1H), 6.85-6.87 (m, 2H), 5.07 (s, 2H), 4.08-4.10 (t, J = 4.8 Hz, 2H), 3.82-3.84 (m, 2H), 3.73-3.77 (m, 6H), 3.71 (s, 4H), 3.61-3.63 (m, 2H), 1.28 (s, 9H). ^{13}C-NMR: 159.17, 153.73, 145.04, 131.17, 131.25, 128.78, 127.32, 120.59, 115.38, 115.21, 72.92, 71.79, 70.86, 70.76, 70.74, 70.41, 69.81, 67.59, 61.81, 34.40, 31.56. ESI HRMS: calcd. ($M+H^+$): $C_{27}H_{36}N_3O_6^+$ 498.2599, found 498.2602.

Methyl Vinyl Ether **9**: ^{1}H-NMR: 7.49-7.51 (d, J = 8.4 Hz, 1H), 7.42 (s, 1H), 7.38 (d, 2.5 Hz, 1H), 7.28-7.29 (m, 1H), 6.87-6.92 (m, 3H), 4.65 (s, 2H), 4.17-4.20 (t, J = 8.6 Hz, 2H), 3.86-3.89 (m, 2H), 3.66-3.76 (m, 10H), 3.61-3.63 (m, 2H), 3.36 (s, 3H), 1.31 (s, 9H). ^{13}C-NMR Methyl Vinyl Ether: 158.83, 156.22, 142.70, 140.91, 133.27, 127.70, 127.53, 116.48, 115.19, 114.94, 114.10, 109.20, 93.34, 88.65, 74.09, 72.74, 71.02, 70.84, 70.77, 69.81, 67.74, 61.93, 57.48, 34.24, 31.63. ESI HRMS Vinyl Methyl Ether: calcd. ($M+Na^+$): $C_{28}H_{38}NaO_7^+$ 509.2515, found 509.2508.

Preparation of 9-butoxy-5,6-didehydro-11,12-dihydrodibenzo[a,e]-[8]annulen-2-yl 4-iodo-benzoate (**DIBO-IBA**, **3a**).

Scheme S3



DIBO-OH (**3b**, 0.418 g, 1.43 mmol) was added to a solution of DCC (0.295 g, 1.43 mol), 4-iodobenzoic acid (0.355 g, 1.43 mmol), and DMAP (cat.) in CH_2Cl_2 (15 mL). The reaction was stirred overnight, filtered, concentrated in vacuum, and purified via flash chromatography (2:1 hexanes: ethyl acetate) to afford **3a** (0.417 g, 56 % yield) as a colorless powder. M.P.= 154 - 155 °C. ^{1}H-NMR: 7.94 (s, 4H), 7.36-7.38 (d, J= 8.2 Hz, 1H), 7.29-7.30 (d, J = 4.7 Hz, 1H), 7.21 (s, 1H), 7.12-7.15 (dd, J = 8.2, 2.4 Hz, 1H), 6.94 (d, J = 2.7 Hz, 1H), 6.81-6.84 (dd, J = 8.4, 2.6 Hz, 1H), 4.01-4.04 (t, J = 6.4 Hz, 2H), 3.23-3.33 (m, 2H), 2.50-2.53 (d, J = 11.3 Hz, 2H), 1.79-1.86 (m, 2H), 1.50-1.59 (m, 2H), 1.01-1.05 (t, J = 7.3 Hz, 3H). ^{13}C-NMR: 164.93, 159.32, 155.45, 154.73, 149.90, 138.23, 131.73, 129.13, 127.25, 126.70, 123.08, 122.61, 119.80, 116.97, 115.51, 112.50, 112.23, 109.56, 101.93, 68.04, 36.71, 36.67, 31.51, 19.46, 14.06. ESI HRMS: calcd. ($M+H^+$): $C_{27}H_{24}IO_3^+$ 523.0764, found 523.0764.

Reaction of DIBO-IBA (**3a**) with sodium azide. A solution of sodium azide (0.012 g, 0.19 mmol) and **3a** (0.1 g, 0.19 mmol) in 50% aqueous methanol was incubated overnight at r.t. The reaction mixture was concentrated in vacuum and purified by flash chromatography to afford a single isomer of DIBO triazole **7a** (0.086 g, 79% yield) as a white powder and a single isomer of triazole **7b** (0.012 g, 19% yield) as a colorless oil.

DIBO-IBA Triazole **7a**: M.P. = 155 °C (decomp.); ^{1}H-NMR : 7.87 (s, 4H), 7.50-7.52 (d, J = 8.4 Hz, 1H), 7.32-7.34 (d, J = 8.3 Hz, 1H), 7.13-7.14 (d, J = 2.5 Hz, 1H), 7.05-7.08 (dd, 8.4, 2.4 Hz, 1H), 6.75-6.79 (m, 2H), 3.94-3.97 (t, J = 6.5 Hz, 2H), 3.15-3.22 (m, 4H), 1.72-1.79 (m, 2H), 1.44-1.53 (m, 2H), 0.95-0.99 (t, J = 7.4 Hz, 3H). ^{13}C-NMR: 164.82, 159.72, 151.01, 141.36, 140.79, 138.19, 132.19, 132.13, 131.70, 129.07, 123.21, 119.78, 116.19, 112.93, 101.93, 67.86, 34.94, 34.82, 31.48, 19.44, 14.06. ESI HRMS: calcd. ($M+H^+$): $C_{27}H_{25}IN_3O_3^+$ 566.0935, found 566.0933.

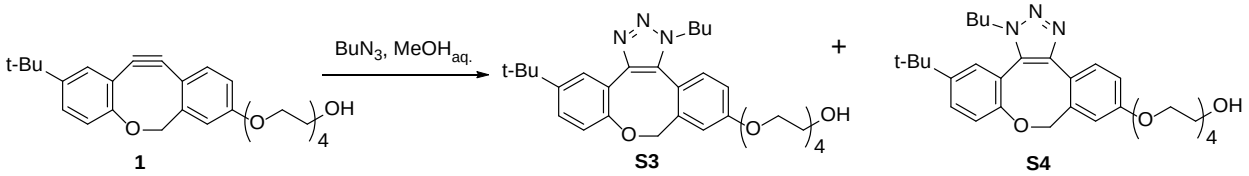
DIBO-OH Traizole **7b**: ^{1}H-NMR (DMSO-d_6, 400 MHz): 7.21-7.23 (d, J = 8.6 Hz, 1H), 7.11-7.13 (d, J = 8.5 Hz, 1H), 6.87-6.88 (d, J = 2.5 Hz, 1H), 6.78-6.81 (dd, J = 8.5, 2.6 Hz, 1H), 6.68-6.69 (d, J = 2.4 Hz, 1H), 6.61-6.64 (dd, J = 8.4, 2.5 Hz, 1H), 3.94-3.97 (t, J = 6.4 Hz, 2H), 3.00-3.04 (m, 4H), 1.64-1.71 (m, 2H), 1.37-1.46 (m, 2H), 0.90-0.94 (t, 3H). ^{13}C-NMR (DMSO-d_6, 125 MHz): 158.60, 157.42, 140.67, 140.54, 131.72, 131.62, 116.39, 115.52, 113.48, 112.52, 67.12, 34.25, 34.17, 30.78, 18.78, 13.74. ESI HRMS: calcd. ($M+H^+$): $C_{20}H_{22}N_3O_2^+$ 366.1706, found 366.1708.

ADIBO Triazole (**5**)**.** ESI HRMS: calcd. ($M+H^+$): $C_{18}H_{17}N_3O^+$ 320.1506, found 320.1509.

BCN Triaozle (**6**)**.** ESI HRMS: calcd. ($M+H^+$): $C_{10}H_{16}N_3O^+$ 194.1288, found 194.1290.

Reaction of ODIBO-TEG-OH (**1**) **with butyl azide.**

Scheme S4



Butyl azide (9 mg, 0.092 mmol) was added to a solution of ODIBO-TEG-OH (**1**,40 mg, 0.88 mmol) in MeOH (2 mL). The reaction mixture was stirred overnight, concentrated in vacuum, and purified via flash chromatography (1:1 hexanes: acetone) to afford two isomeric triazoles as colorless oils: 7 mg of triazole 1 (15%) and 21 mg (44%) of triazole 2. Triazole 1- ^{1}H-NMR: 7.60-7.61 (d, J = 2.5 Hz, 1H), 7.25 (d, J = 2.3 Hz, 1H), 7.18- 7.21 (dd, J = 8.7, 2.5 Hz, 1H), 7.10 (d, J = 2.5 Hz, 1H), 7.00-7.03 (dd, J = 8.5, 2.6 Hz, 1H), 6.83-6.85 (d, J = 8.7 Hz, 1H), 4.90-5.35 (d, J = 179.3 Hz, 2H), 4.33-4.35 (m, 2H), 4.17-4.20 (t, J = 4.6 Hz, 2H), 3.85-3.88 (t, J = 4.7 Hz, 2H), 3.65-4.74 (m, 10H), 3.58-3.60 (m, 2H), 1.82 (s, 2H), 1.27 (s, 11H), 0.84-0.87 (t, J = 7.4 Hz, 3H). ^{13}C-NMR: 159.85, 152.26, 145.17, 144.24, 138.51, 132.01, 130.03, 129.91, 127.06, 120.10, 119.98, 116.91, 116.71, 116.00, 72.69, 71.03, 70.86, 70.78, 70.51, 69.77, 69.70, 67.85, 61.93, 48.40, 34.31, 32.33, 31.56, 19.89, 13.63. ESI HRMS: calcd. (M+H$^+$): $C_{31}H_{44}N_3O_6^+$ 554.3225, found 554.3222.

Triazole 2- ^{1}H-NMR: 7.64 (d, J = 8.6 Hz, 1H), 7.34-7.36 (dd, J = 8.7, 2.5 Hz, 1H), 7.07-7.09 (d, J = 8.7 Hz, 1H), 7.05-7.06 (d, J = 2.4 Hz, 1H), 6.95-6.98 (dd, J = 8.6, 2.6 Hz, 1H), 6.87 (d, J = 2.6 Hz, 1H), 5.22 (s, 1H), 4.39-4.43 (t, J = 7.5 Hz, 2H), 4.14-4.16 (t, J = 4.8 Hz, 2H), 3.84-3.86 (t, J = 4.8 Hz, 2H), 3.66-3.74 (m, 10H), 3.59-3.62 (m, 2H), 1.85-2.62 (m, 1H), 1.89-1.97 (m, 2H), 1.33-1.37 (m, 2H), 1.28 (s, 9H) 0.87-0.91 (t, J = 7.4 Hz, 3H). ^{13}C-NMR: 158.82, 153.85, 145.71, 144.79, 136.86, 132.98, 132.06, 128.33, 127.12, 124.07, 121.51, 116.62, 115.09, 114.75, 73.77, 72.68, 71.03, 70.87, 70.81, 70.55, 69.88, 67.71, 61.95, 48.77, 34.50, 32.49, 31.54, 19.99, 13.64. ESI HRMS: calcd. (M+H$^+$): $C_{31}H_{44}N_3O_6^+$ 554.3225, found 554.3224.

Rate measurements of the reaction of sodium azide with ODIBO (**1**), ADIBO (**2**), or DIBO (**3a**) were performed at pH 7.4 (in PBS solution) or pH 1 (0.1 M $HClO_4$) at 25±0.1^{0}C. The reactions were conducted under pseudo-first order conditions with 10-fold or higher excess of sodium azide (0.05 mM of cyclooctyne **1, 2,** or **3a**; and 2,5 – 500 mM of sodium azide). The consumption of starting material was monitored by following the decay of the characteristic alkyne peak at 321 nm for ODIBO (**1**) and 309 nm for DIBO-OH (**3b**) and ADIBO (**2**). The measurements from each experiment at a given concentration of alkyne were fit to a single exponential equation to give the decay rate k_{obs}. Linear dependence of the observed pseudo-first order rate constants on azide concentration were analyzed by the least squares method to obtain the bimolecular rate constants.

BCN (**4**) lacks an absorbance band useful for monitoring its consumption during reaction. Therefore, we used a competition assay with DIBO-OH (**3b**) under pseudo-first order conditions to measure BCN reaction with azide ion or hydrazoic acid Thus, the rate of consumption of DIBO (**3b**, 0.5 mM) in the reaction with NaN_3 (0.05 mM) was measured in the presence of variable concentrations (0.25 – 2 mM) of BCN (**4**). Under these conditions, the concentration of azide as a function of time t is described by the equation

$$-\frac{dA(t)}{dt} = k_1 B(t)A(t) + k_2 D(t)A(t) \cong (k_1 B_0 + k_2 D_0)A(t)$$

where $A(t)$, $B(t)$, and $D(t)$ are the concentrations of azide, BCN, and DIBO at time t, respectively, with initial concentrations A_0, B_0, and D_0, and k_1 and k_2 are the second order reaction rates of BCN and DIBO with azide, respectively. To ensure pseudo-first order conditions, we have used $B_0, D_0 \gg A_0$ so $B(t)$ and $D(t)$ are approximately constant at B_0 and D_0, respectively. Solving for $A(t)$, we have that

$$A(t) \cong A_0 e^{-(k_1 B_0 + k_2 D_0)t}$$

However, we monitored changes in DIBO concentration instead, whose behavior should be similarly described by

$$-\frac{dD(t)}{dt} = k_1 D(t)A(t) \cong k_1 D_0 A(t)$$

Substituting the pseudo-first order solution for $A(t)$, we have that

$$D(t) \cong D_0 - (1 - e^{-(k_1 B_0 + k_2 D_0)t})K$$

where K is a constant. We used exponential regression to estimate $k_{obs} = k_1 B_0 + k_2 D_0$ from the exponential decay of the measured DIBO absorbance band at each BCN starting concentration, B_0. Note that $k_2 D_0$ is constant while B_0 varies, so that k_{obs} is a linear function of B_0, with slope k_1 and intercept $k_2 D_0$.

Thus we can estimate the second order rate constant for the reaction of BCN (**4**) with sodium azide at pH 7.4 (or with hydrazoic acid at pH 1) by determining the slope of the plot of the observed pseudo-first order rate constant k_{obs} (from the consumption of DIBO) versus the initial BCN concentration.

Amine-doped polyacrylamide hydrogels were prepared by radical polymerization of a solution consisting of 1.4 M acrylamide, 0.018 M N,N′-methylenebisacrylamide, 0.1 M sodium citrate, 0.01 M allylamine, 0.008 M N,N,N′,N′-Tetramethylethylenediamine, and 0.009 M ammonium persulfate, at pH 5.0. The latter two components were added immediately after vacuum degassing, and the solution pipetted into a mold.

SPAAC gradient generated by sodium azide quenching of ODIBO-TEG-hydrogel. An amine-doped gel was cast into a 1mm thick mold assembled from standard glass microscope slides. The

gel was washed with 0.5 M citrate buffer, pH 5, and then incubated overnight in 0.3 mM photo-ODIBO-TEG-NHS (**10**) allowing the succimidyl ester to react with the primary amine in the gel. The gel was washed in citrate and then assessed for **10** content and uniformity by UV absorbance spectroscopy. The gel was then irradiated with 350 nm bulbs to induce complete photochemical decarbonylation of its photo-ODIBO, yielding an ODIBO-labeled hydrogel. The extent of the conversion was confirmed by spectroscopy. Next a small stack of dry filter paper was applied to one edge of the ODIBO gel, and the paper wetted with 20 mM sodium azide in 0.1 M citrate buffer, pH 5. The next morning the paper was removed and the gel was washed by flooding the opposite edge with buffer and allowing it to run down the gel and drain away. Triazol formation in different regions of the gel was confirmed by spectroscopy. The gel was then flooded with a solution of 0.4 mM 6-carboxyfluorescein-TEG azide, incubated overnight, washed, fluorescein content confirmed by spectroscopy, and then imaged.

Patterning PhotoODIBO-TEG-hydrogel using light and sodium azide quenching. The image of an iris diaphragm mounted in the back plane of a 10X microscope objective was projected onto a photo-ODIBO-TEG-hydrogel (made as described above), generating a disc of ODIBO-labeled hydrogel surrounded by a uniform field of photo-ODIBO. The disc was then quenched by treating the gel briefly with 20 mM sodium azide and then washing. A second ODIBO disc, adjacent to the first, was generated by projecting a second 350 nm image of the diaphragm onto the photoODIBO-TEG-hydrogel. The hydrogel was then flooded with a solution of 0.15 mM Rhodamine B-azide, and then washed, creating a disc of Rhodamine B-labeled hydrogel. Any remaining ODIBO was then quenched by a sodium azide rinse. Finally, the entire photo-ODIBO-TEG-hydrogel was exposed to 350 nm light, inducing decarbonylation to ODIBO in the remainder of the gel which was then demonstrated reacting the gel with a solution of 0.7 mM 6-carboxyfluorescein-TEG-azide, washing, and imaging.

Stability of cyclopropenone in the presence of sodium azide

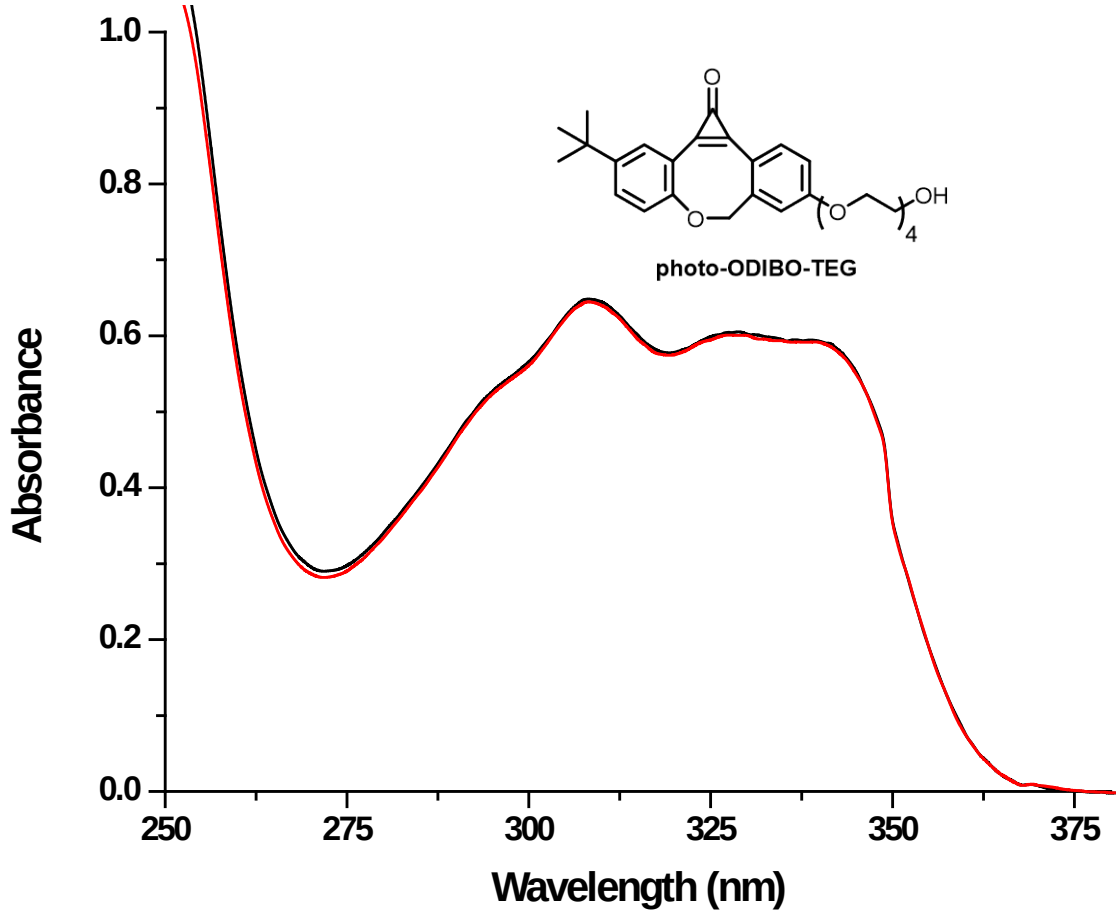


Figure S1. UV spectra of the PBS solution (5% of MeOH) of photo-ODIBO (0.05 mM) in the presence of 25 mM of NaN_3. Red line corresponds to initial spectrum, while the black line is spectrum recorded after 24 h incubation.

Stability of triazole 8 at pH=1

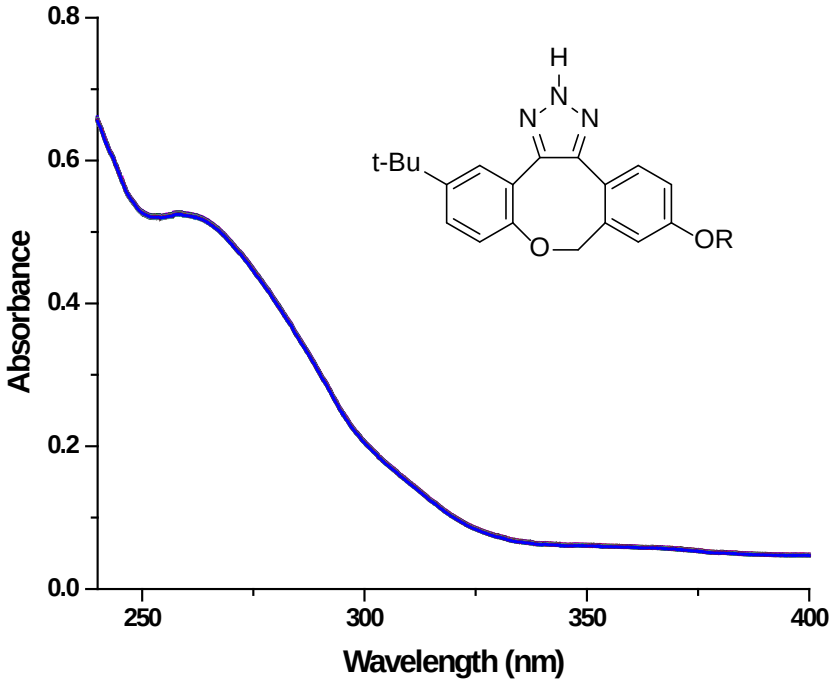


Figure S2. UV spectra of 0.05 mM solution of ODIBO-triazole in 1 M aqueous $HClO_4$. Spectra recorded every 30 min for 24 h.

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