Electronic supplementary information

Tuning photosensitized singlet oxygen production from microgels synthesized by polymerization in aqueous dispersed media

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1. Synthesis of VB-RB

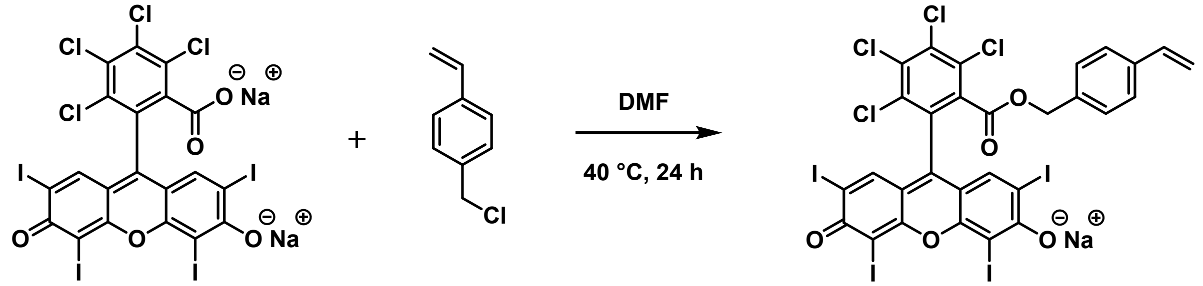

Figure S1: Scheme of the synthesis of VB-RB

RB (1.831 g, 1.8 mmol) was added to a solution of VBC (0.533 mL, 3.78 mmol) in DMF (40 mL) in a 100 mL round bottom flask dried with a heat gun under argon. The reaction mixture was degassed with a gentle flow of Nitrogen for 30 min and was then stirred at 40 °C for 24 h. After cooling down to room temperature, 35 mL of DMF were removed by cryogenic distillation and the remaining 5 mL were poured into diethyl ether (150 mL) under vigorous stirring. The resulting purple precipitate was filtered and washed 5 times with diethyl ether, then dried under vacuum to give the compound RB-Sty as a purple solid (1.917 g, yield 96 %); $R_f = 0.40$ (SiO_2, Acetone/Methanol/Chloroform = 10/10/80).

^{1}H-NMR (400 MHz, $(CD_3)_2SO$, 25°C, **Fig. SI-2**) δ = 7.45 (2H, s, -ICCH-), 7.23 (2H, d, *J* = 8 Hz, ar. CH), 6.80 (2H, d, *J* = 7.6 Hz, ar. CH), 6.67 (1H, dd, *J* = 10.8 Hz, $-CHCH_2$), 5.81 (1H, d, *J* = 17.6 Hz, $-CHCH_2$), 5.26 (1H, d, *J* = 10.8 Hz, $-CHCH_2$), 5.02 (2H, s, $-OCH_2-$); **^{13}C-NMR** (100 MHz, $(CD_3)_2SO$, 25°C, **Fig. SI-3**) δ = 171.8, 163.1, 157.0, 139.0, 137.0, 136.2, 136.0, 135.0, 134.4, 133.6, 133.3, 131.9, 129.9, 128.9, 127.7, 126.1, 114.9, 110.3, 97.5, 76.1, 67.8, 64.9.

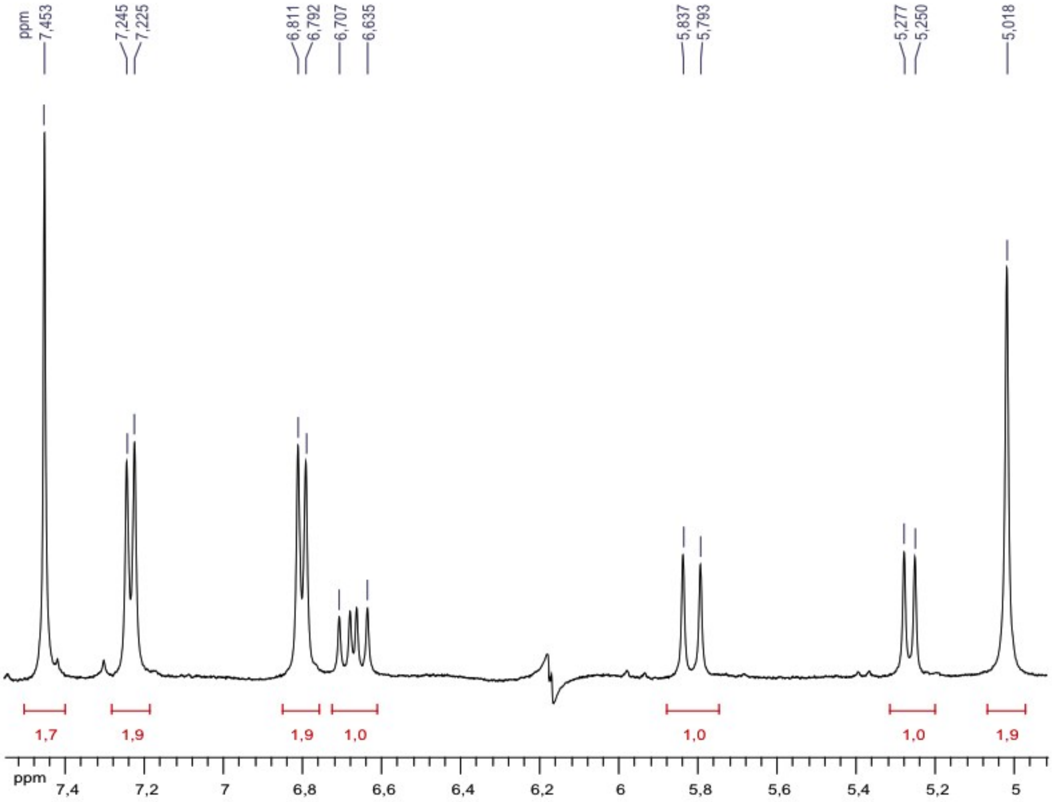

Figure S2: ^{1}H-NMR spectrum of VBRB (400 MHz, $(CD_3)_2SO$, 25°C)

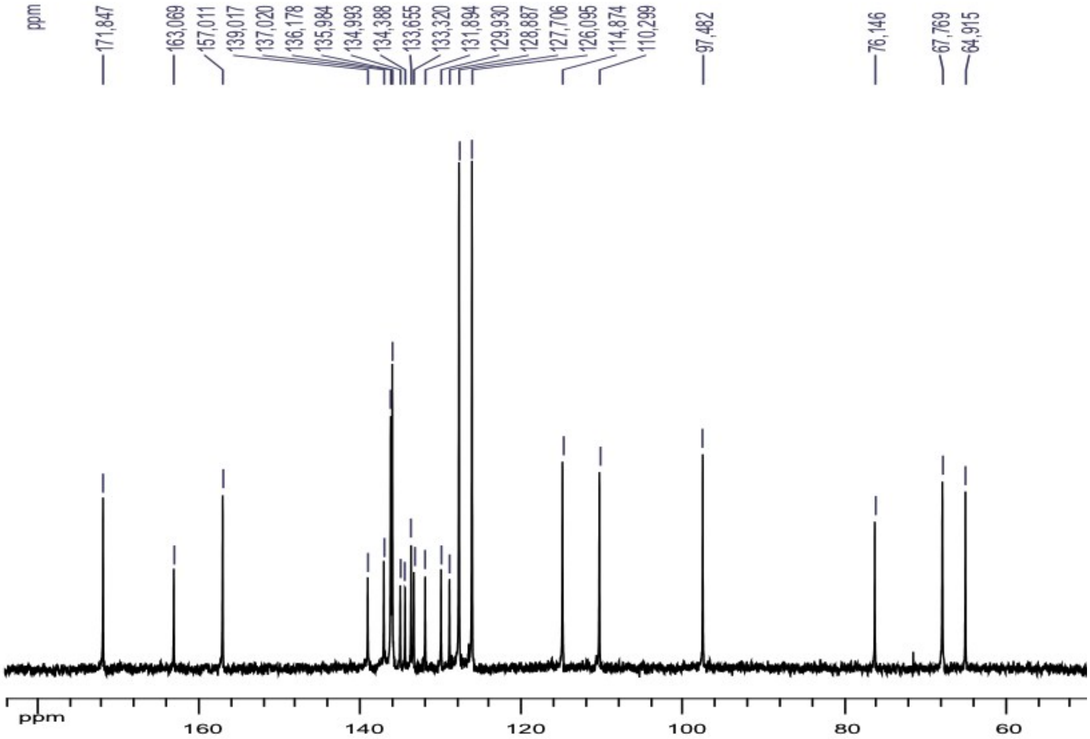

Figure S3: ^{13}C-NMR of VBRB (100 MHz, $(CD_3)_2SO$, 25°C)

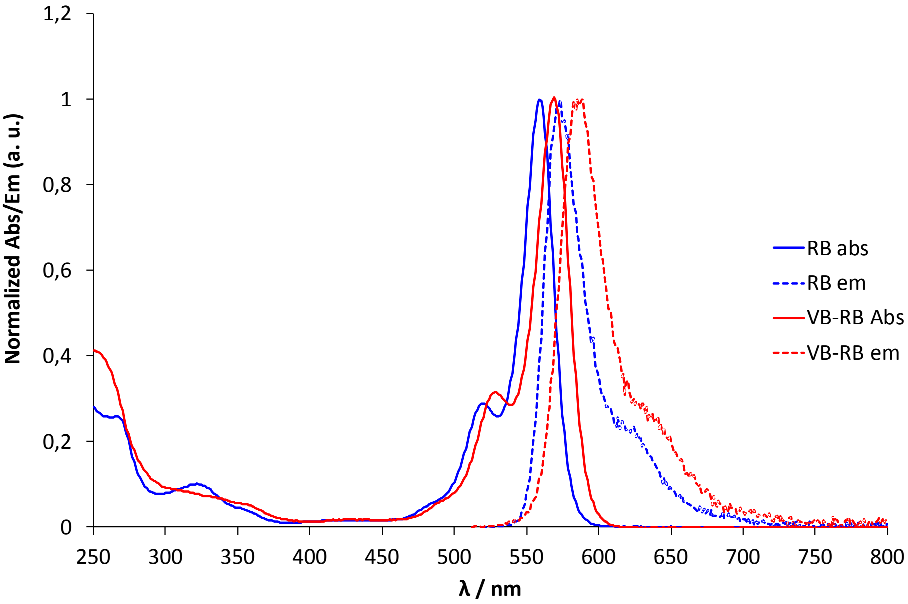

Figure S4: Absorption and emission spectra of RB (λ_{ex} 514 nm) and VBRB (λ_{ex} 507 nm) [1μM] in EtOH

2. Synthesis of microgels

Table S1: Experimental conditions for the miniemulsion copolymerization of VAc and VCL performed at 65 °C with 10 wt% of initial solids content in Water

Sample	**VBRB@P**	**MG-8**	**VBRB@MG-8**	**MG-14**	**VBRB@MG-14**
VAc (g)			1.74	1.51	1.77
VCL (g)			2.80	2.31	2.82
VBRB (g)			0.36	-	0.34
AIBN (g)			0.26	0.27	0.26
HD (g)			0.10	0.09	0.11
DVA (g)			0.74	1.15	1.52
Trioxane (g)			0.37	0.36	0.37
NaHCO3 (g)			0.05	0.06	0.05
SDS (g)			0.10	0.11	0.10
DW (g)	44	48	48.55	45.13	55.62
Mol-% crosslinker	0	8	8	14	14

[1] Mol-% crosslinker = $n_{DVA}/(n_{DVA} + n_{VCL} + n_{VAc})$

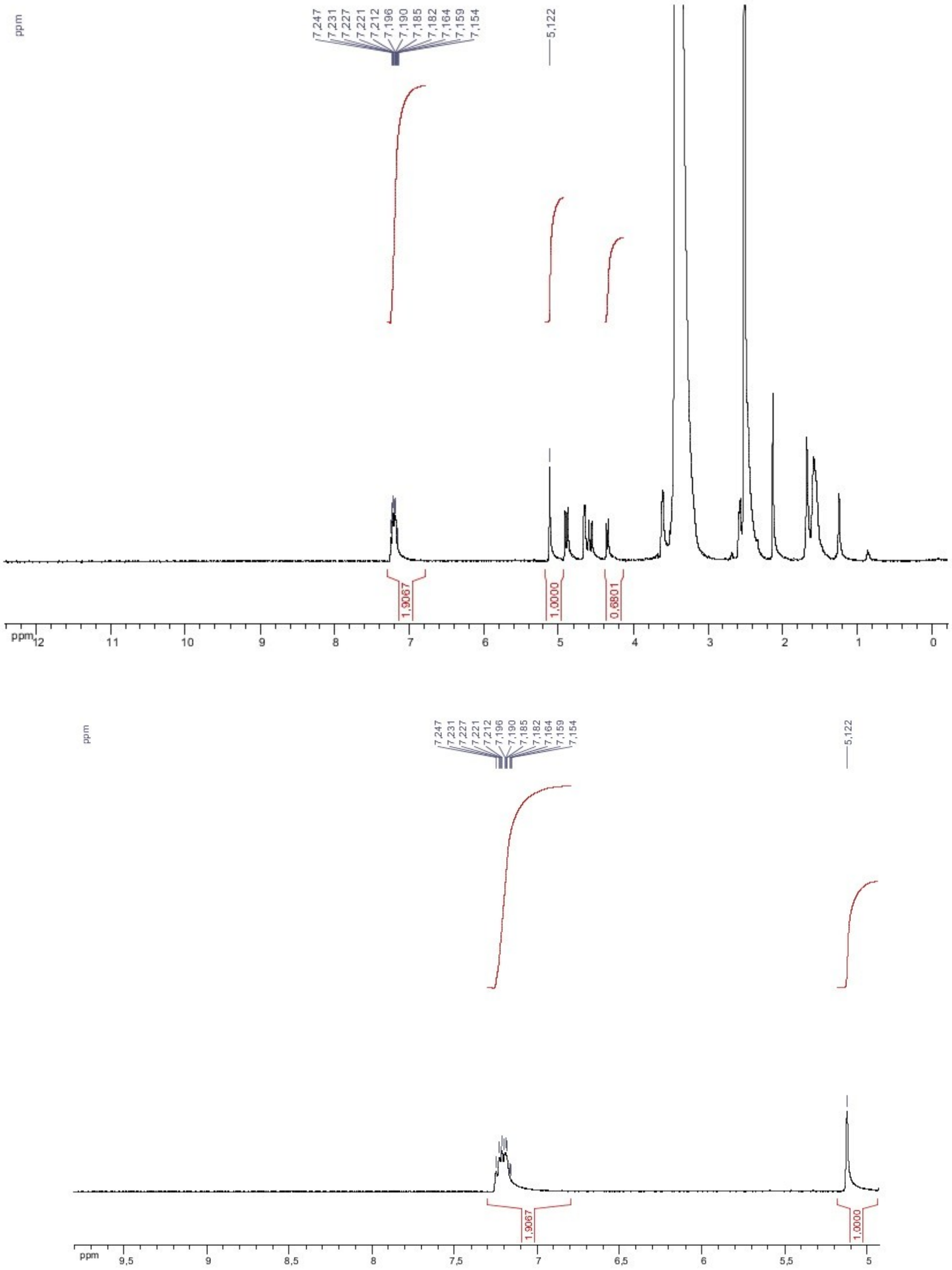

Figure S5: ^{1}H-NMR spectra of the crude sample of MG-14 (400 MHz, $(CD_3)_2SO$, 25°C) used to calculate the overall conversion.

The final overall molar monomer conversion (X_m) was calculated from NMR spectra of the crude microgels samples (Figure S5) in Dimethyl sulfoxide (DMSO)-d_6 using 1,3,5-trioxane (triox.) as an internal standard (see Equation (1).

$$X_m = 1 - \frac{[I(1HVAc + 1HVCL)/I(1Htriox.)]t}{[I(1HVAc + 1HVCL)/I(1Htriox.)]0}, \qquad \text{Equation (1)}$$

$I(_{1H,triox.})$ is the integral of one proton of 1,3,5-Trioxane (5.1 ppm, 6 H, **Figure S5**) used as an internal standard and $I(_{1H,VAc+1H,VCL})$ is the integral of the vinylic CH protons of VCL, VAc monomers and DVA crosslinker (7.1-7.4 ppm, 6 H, **Figure S5** and **Figure S6**).

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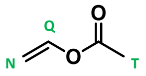
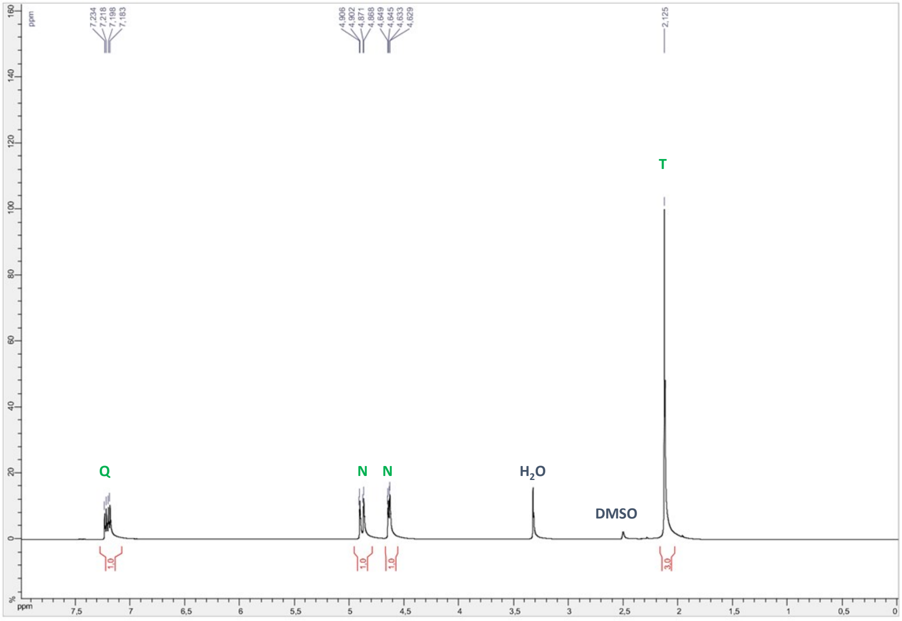
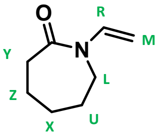
$$X_{m,corrected} = 1 - \frac{[I(1HVAc + 1HVCL + 1HDVA) - I(1HAcAl)]/I(1Htriox.)t}{[I(1HVAc + 1HVCL)/I(1Htriox.)]0} . \qquad \text{Equation (2)}$$



Vinyl Acetate

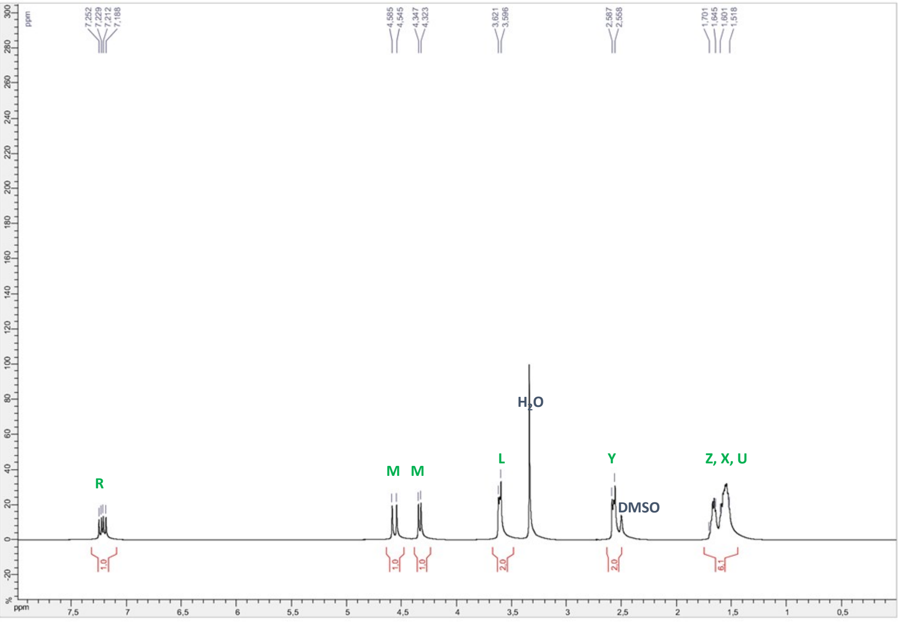
Vinyl Acetate (DMSO d$_6$, 25°C, 400 MHz)





N-Vinylcaprolactam

N-Vinylcaprolactam (DMSO d$_6$, 25°C, 400 MHz)



Divinyl Adipate (DMSO d_6, 25°C, 400 MHz)

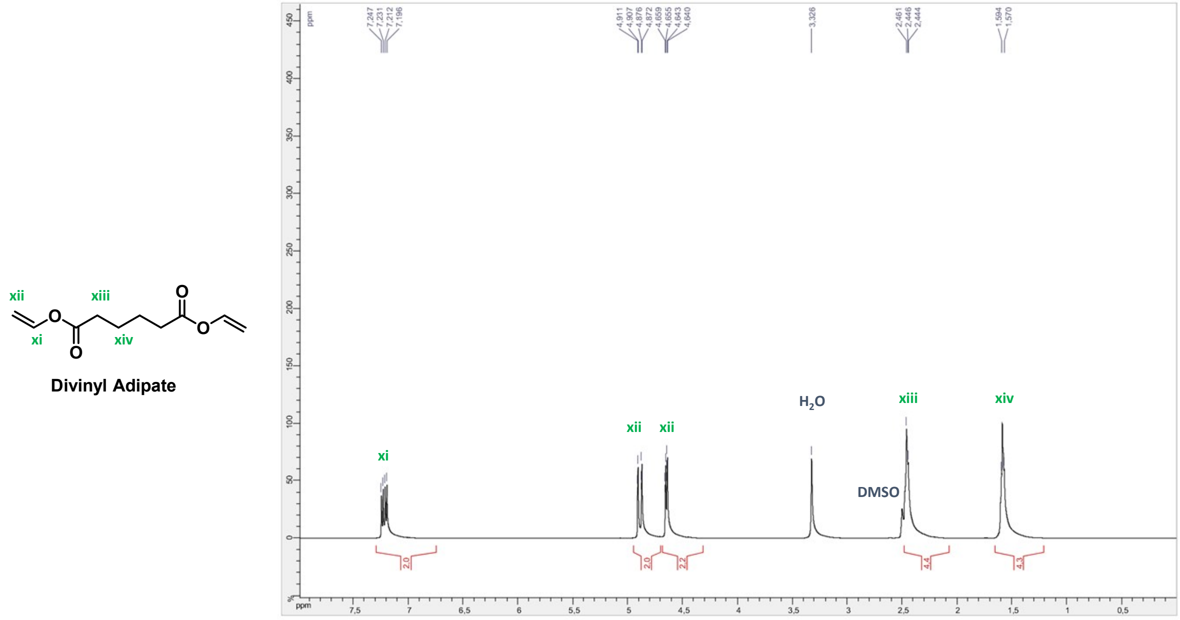


Figure S6: ^{1}H-NMR spectra of VAc, VCL and DVA (400 MHz, $(CD_3)_2SO$, 25°C).

Characterization of the P(VAc-co-VCL)-based microgels and polymer

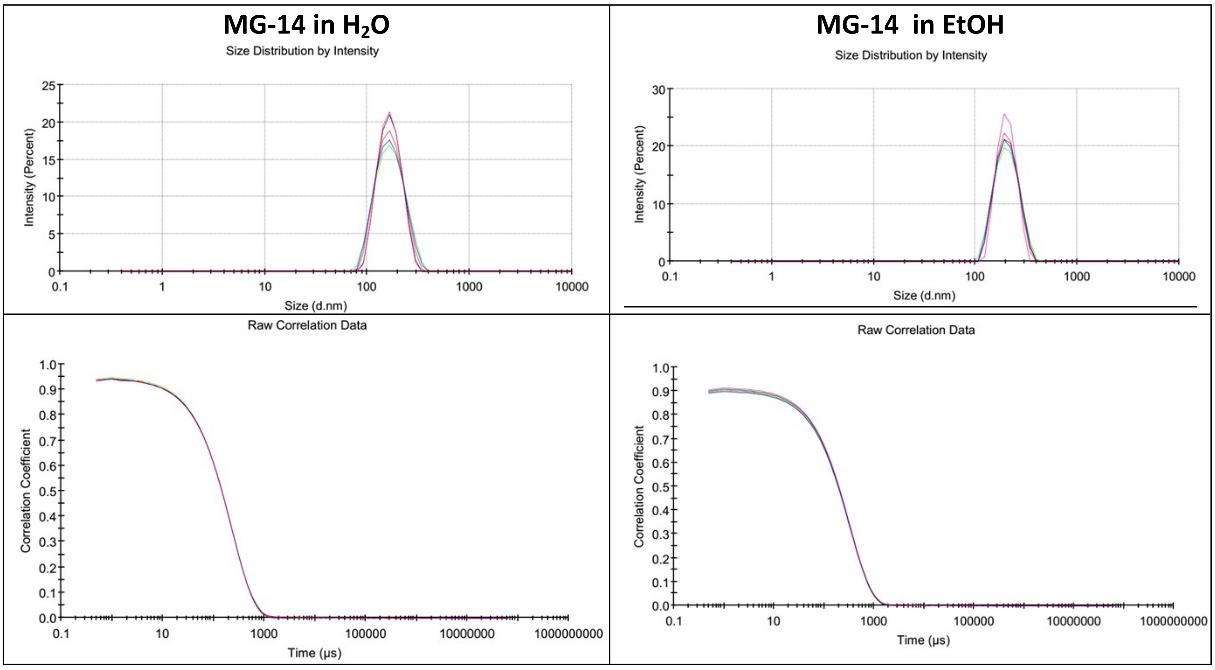


Figure S 7: (Left) Hydrodynamic diameter and auto-correlation function of MG-14 in water at [0.05 g L^{-1}] (Right) Hydrodynamic diameter and auto-correlation function of MG-14 in ethanol at [0.05 g L^{-1}] .

Table S2: Measured area of each signal (light scattering (LS) and UV-visible traces at 559 nm) under the A4F fractograms of MG-8 and VBRB@MG-8 dispersed in water from Figure 3 in the main text .

Sample	LS peak area (1/cm min)	UV -visible peak area at 559 nm (AU min)	Peak area LS/Peak area UV-Vis	**VBRB@MG-8/MG-8**
VBRB@MG-8	3.77E+00	1.39E-02	3.69E-03	5
MG-8	3.10E+00	2.09E-03	6.74E-04	

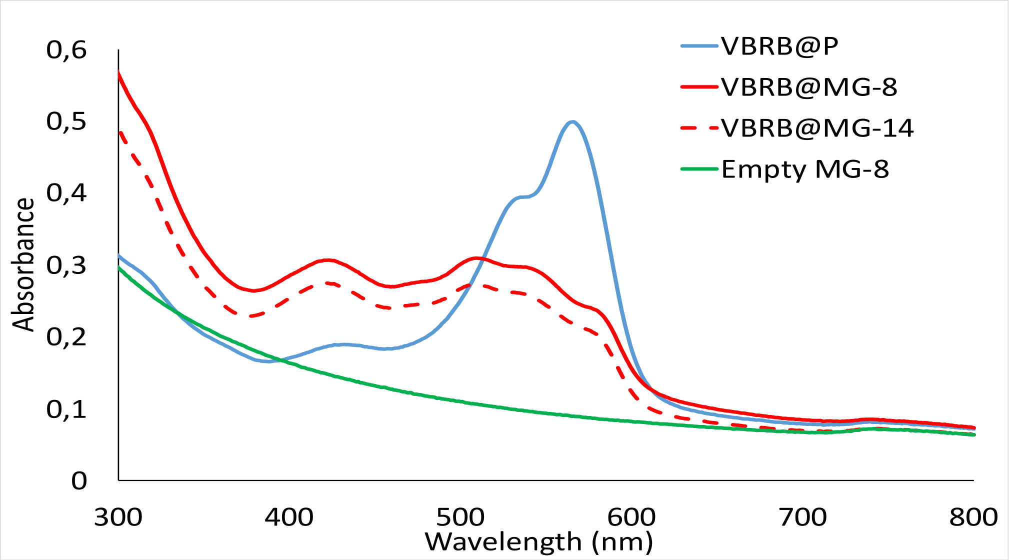


Figure S8: Raw absorption spectra in H_2O of the VBRB@P polymer (blue lines), VBRB@MG-8 (continuous red lines), VBRB@MG-14 (dotted red lines) and MG-8 (green line) microgels at concentration of [13.0 mg L^{-1}].

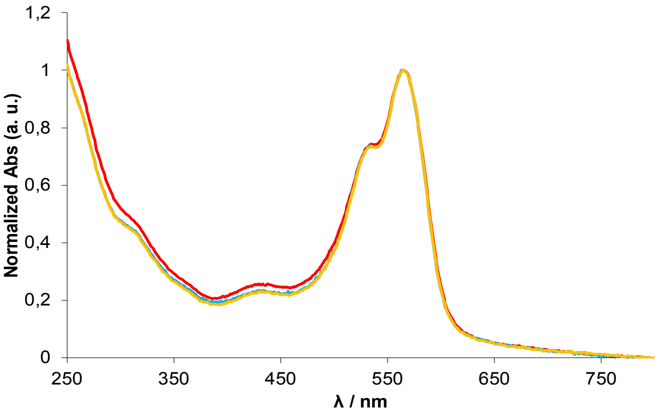


Figure S9: Absorption spectra in H_2O of VBRB@P recorded at: (red line) 25 °C, (yellow) 10 °C and (blue) 5 °C. [VBRB@P] = 8.0 mg L^{-1}.

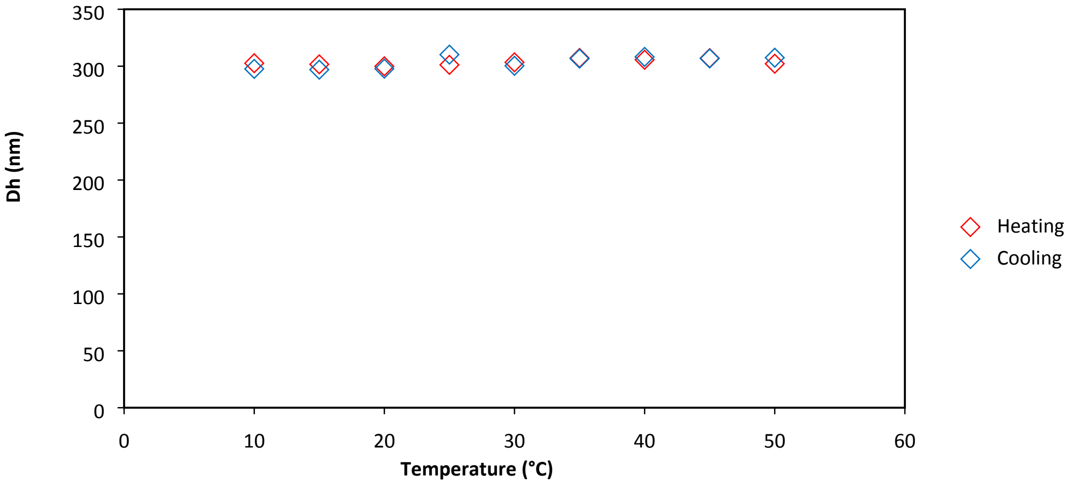


Figure S10: Hydrodynamic diameter of MG-8 versus temperature at 0.05g/L (Settings : trend from 10 to 50°C, step 5°C, stabilization time 15 min).

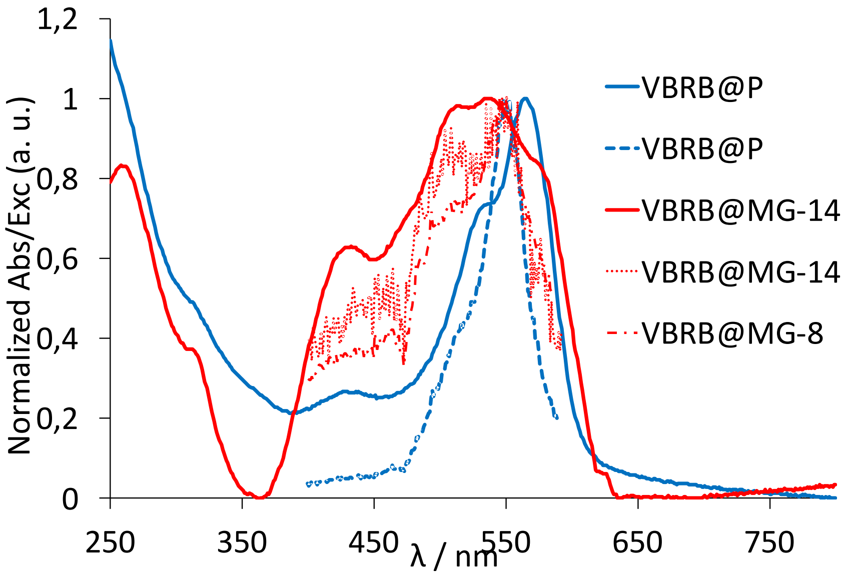


Figure S11: Absorption (full lines) and excitation with λ_{exc}=596 nm (dotted lines) spectra in water of VBRB@P (blue lines) and VBRB@MG-8 and VBRB@MG-14 (red lines). [13.0 mg L^{-1}]

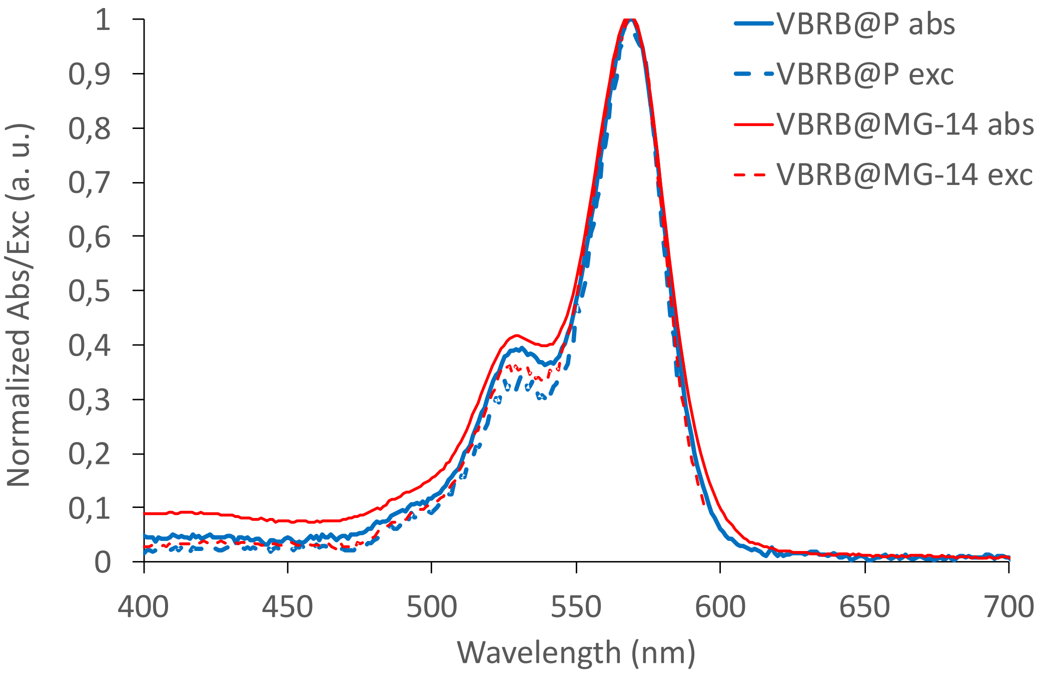


Figure S12: Absorption (continuous lines) and excitation (dotted lines, λ_{ex} 596 nm) spectra in ethanol of the VBRB@P linear polymer and of the VBRB@MG-14 microgel.

1. Production of singlet oxygen

Irradiation conditions

For all the experiments, the emission spectrum of the light source was measured with the spectroradiometer detector exactly at the same position as the sample cuvette (magnetically stirred quartz fluorescence cuvette Hellma). A calibrated Avaspec 2048L spectroradiometer (Avantes) with a 1 m x 600 µm UV optic fiber and a 3900 µm 180° cosin corrector was used for irradiance measurements.

Singlet oxygen measurement in water with furfuryl alcohol (FFA) FFA

A solution was prepared with a mixture of 1.1 10^{-4} M freshly distilled FFA, and either 1.8 10^{-2} (VB-RB@P) or 2.8 10^{-2} (VB-RB@MG-8 or-14) g L^{-1} copolymers dispersed in double deionized water. Several 3 mL samples of this solution were introduced one by one in the irradiation cuvette installed in the optical bench (**Figure SI-13**) in order to achieve the plot [FFA] *vs* time at room temperature. For each sample the light source was lighted on during variable time intervals (typically 0, 1, 2, 5, 10, 15 and 30 min). The light source was a high-power single chip led (Roithner LaserTechnik) with a maximum at 515 nm (irradiance around 3 mW cm^{-2}). After each irradiation period the samples were kept in the dark before monitoring their UV spectra and HPLC chromatrogram (**Figure SI-14**). For each irradiated sample, 1 mL was used for HPLC measurements, while the absorbance spectra of the remaining 2 mL samples were recorded to check the PS stability. Under these conditions no detectable bleaching of RB units was observed from the UV spectra of the solutions after irradiation. HPLC analysis was carried out with Agilent 1290 equipped with a Supelco Lichrosphere RP18-5 (25 mm x 4.6 mm, 5µm) column, 20 µL injection, eluting with 80% water with 0,1% H_3PO_4, 20% Acetonitrile, rate 2 mL min^{-1}, UV detection at 205 nm and 218 nm. The retention times of FFA were 2,9 min and 1,8 min for of 6-hydroxy-(2H)-pyran-3-one (**Figure SI-14**). An external calibration was done with five dilutions of FFA (from 1 10^{-4} to 1 10^{-6} M in double deionized water) to convert pic area to concentrations.

For the quantum yields calculation with VB-RB@MG-8 or-14, the UV spectra of the suspensions were affected by diffusion (Figure 3, main text). Accordingly, the PS concentration was determined after subtraction of the spectra of the corresponding photosensitizing-free microgels MG8 and MG14.

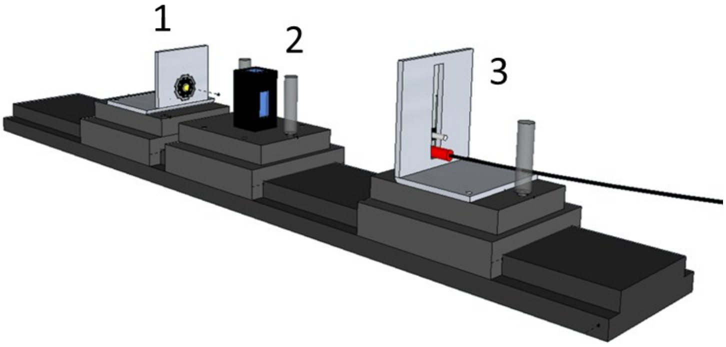


Figure S13: Scheme of the optical bench used for irradiation experiments (1 : light source (LED); 2 : Cuvette holder; 3 : Holder the for spectroradiometric cell

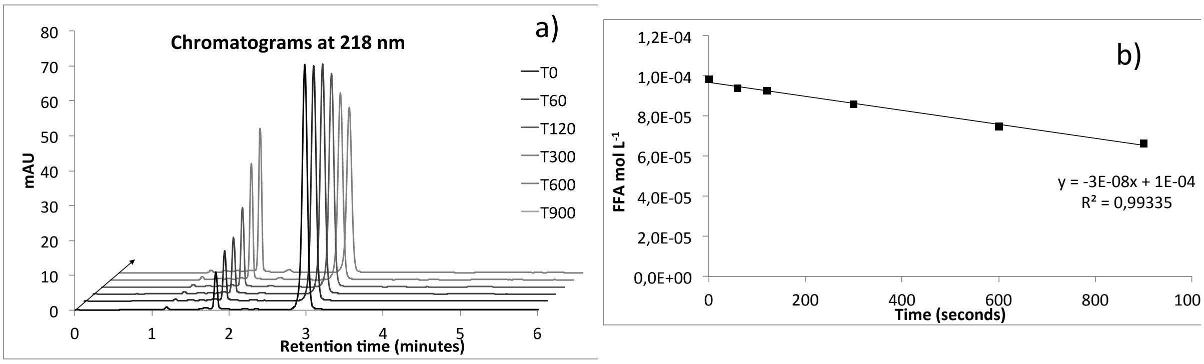


Figure S14: Irradiation of FFA with RB-Sty@MG4 at 515 nm a) HPLC-UC chromatogram (RT of FFA 2.9 min and of 6-hydroxy-(2H)-pyran-3-one 1.8 min; b) monitoring of FFA concentration with time calculated from the decrease of the intensity of the chromatographic peak of FFA.

Singlet oxygen measurement in ethanol with 1,3-diphenylisobenzofuran (DPBF)

An optical bench was equipped to irradiate and simultaneously analyze by UV-visible spectroscopy samples hosted in the holder of the Perkin-Elmer Lambda 850 spectrophotometer. The light source was an Apex Monochromator Illuminator with an off-axis F/4 corrected mirror used as host for a 200W Xe-Hg Lamp. A Cornerston 260 motorized 1/4m monochromator with a USB communication interface was used to set the irradiation wavelength at 547 nm. A CGA-375 nm filter and an IR filter were located between the light source and the monochromator (irradiance around 0.2 mW cm^{-2}). An LH1 M4 lens was placed between the monochromator and the analytical apparatus to collect and concentrate the light beam. A Perkin Elmer double beam, double monochromator Lambda 850 UV-Vis spectrophotometer was used for the analysis. All optical elements were purchased from Newport.

Irradiation of 3 mL samples containing a mixture of 3.8 10^{-5} M DPBF and either 8.8 x 10^{-2} (VB-RB@P) or 4.1 x 10^{-2} (VB-RB@MG-8 or-14) g L^{-1} copolymers dispersed in ethanol was carried out at 25°C in a thermo-regulated quartz fluorescence cuvette magnetically stirred. For each sample the light source was lighted on during 15 min. The kinetics of consumption of DPBF during irradiation was followed by the decrease of its absorbance at 410 nm ($\varepsilon_{410\ nm}$ DPBF = 24 320 L.mol^{-1}.cm^{-1} ^{1}) during irradiation (Figure S15). For each irradiated sample, absorbance spectra also allowed checking the PS stability.

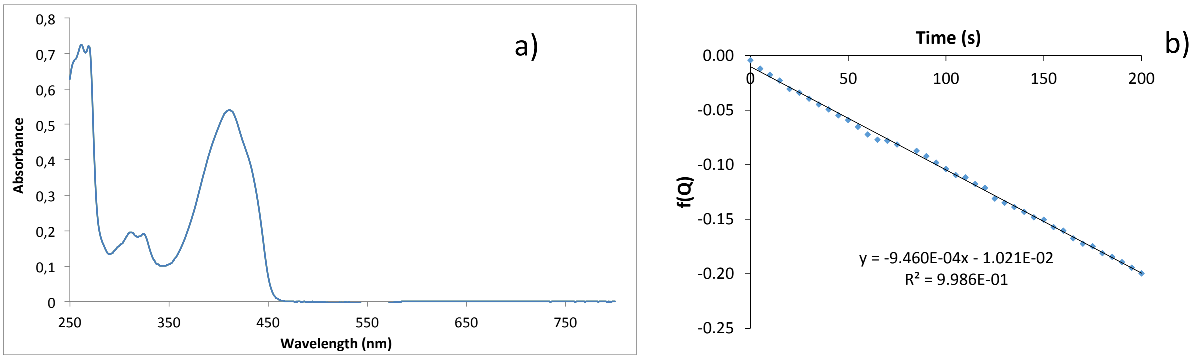


Figure S15. a) UV spectrum of DPBF in ethanol; b) Monitoring f([Q]) *vs* time for DPBF ethanol in the presence of VBRB@MG14 (see Eq (5), Absorbance DPBF at λ = 410 nm).

Quantum yields calculation

The rate of disappearance of a quencher Q by reaction with singlet oxygen (r_{ox}) is given by Eq. 1:

$$r_{ox} = -\frac{d[Q]}{dt} = k_r^Q[^1O_2][Q] \qquad \text{Eq. 1}$$

with

k_r^Q, rate constant of chemical quenching of FFA by $^1O_2$ or rate constant of chemical quenching of DPBF by $^1O_2$.

If singlet oxygen is produced by photosensitization under continuous irradiation, the steady-state concentration of $^1O_2$ is given by Eq. 2

$$[^1O_2] = P_a^{PS}\emptyset_\Delta^{PS}\frac{1}{k_d + k_t^Q[Q]} = P_a^{PS}\emptyset_\Delta^{PS}\tau_\Delta^Q \qquad \text{Eq. 2}$$

with

- P_a^{PS} (einstein L^{-1} s^{-1}) the photon flux absorbed by the sensitizer at the wavelength of excitation. According to the Beer-Lambert law, P_a^{PS} depends on the absorbance of the PS at the excitation wavelength (***A***$_{PS}$):

  $$P_a^{PS} = P_0(1 - 10^{-A_{PS}})$$

  And P_0 (einstein L^{-1} s^{-1}) incident photon flux
- k_d rate constant of the non-radiative decay of singlet oxygen in water
- And k_d rate constant of the non-radiative decay of singlet oxygen in ethanol or water
- k_t^Q rate constant of singlet oxygen $^1O_2$ total quenching by the probe Q and

$k_t^Q = k_r^Q + k_q^Q$, where k_q^Q is the rate constant of physical quenching of the probe Q by $^1O_2$

- $\emptyset_\Delta^{PS}$ quantum yield of singlet oxygen production by the PS under investigation
- τ_Δ^Q lifetime of singlet oxygen in the considered solvent in the presence of the probe Q

The values used for our experiments were the following.

	k_d (s^{-1})	k_r^Q (L mol^{-1} s^{-1})	k_t^Q (L mol^{-1} s^{-1})	$k_t^Q \times [Q]$ (s^{-1})	$\frac{k_t^Q \times [Q]_Q}{k_d}$
EtOH/DPBF	5.3×10^4	5.9×10^8	1.6×10^8	5.0×10^4	**0.9**
Water/FFA	3.03×10^5	7.0×10^7	1.2×10^8	1.2×10^4	**0.04 %**

In the previous calculation, it is assumed that $k_t^{PS}[PS] \ll k_d$, which means that the PS itself does not quench singlet oxygen, which is the case for Rose Bengal.

If there is no interference with the reaction products *i.e*. at the very beginning of the reaction (less than 10 % variation of $[Q]$), it is possible to combine Eq. 1 & Eq. 2 giving Eq. 3:

$$r_{ox} = -\frac{d[Q]}{dt} = P_a^{PS} \emptyset_\Delta^{PS} \frac{k_r^Q[Q]}{k_d + k_t^Q[Q]} \qquad \text{Eq. 3}$$

In water, under our conditions the term $k_t^Q[Q]$ is less than 10% of k_d. Accordingly, the first-order analysis of the reaction rate holds true (Eq. 4).

$$\ln\left(\frac{[Q]}{[Q_0]}\right) = -P_a^{PS} \emptyset_\Delta^{PS} \frac{k_r^Q}{k_d} t \qquad \text{Eq. 4}$$

In ethanol, integration of Eq.3 gives Eq.5

$$f([Q]) = \ln\left(\frac{[Q]}{[Q_0]}\right) - \left[\frac{k_t^Q}{k_d}([Q_0] - [Q])\right] = -P_a^{PS} \emptyset_\Delta^{PS} \frac{k_r^Q}{k_d} t \qquad \text{Eq. 5}$$

Under these conditions and with the previous values, we found for the reference Rose Bengale, $\emptyset_\Delta^{RB} =$ 0.76 in water and 0.75 in ethanol.

$\emptyset_\Delta^{PS}$ was then determined in comparative experiments according to Eq. 6 where the reference PS (PS_{Ref}) was Rose Bengale, using either the first order kinetic in water or $f([Q])$ function in ethanol:

$$\emptyset_\Delta^{PS} = \emptyset_\Delta^{RB} \frac{P_a^{RB} r_{ox}^{PS}}{P_a^{PS} r_{ox}^{RB}} \qquad \text{Eq. 6}$$

Photobleaching tests

Photobleaching experiments were carried out by irradiating a quartz spectroscopic cuvette containing a well-mixed ethanol solution of RB or VBRB@MG (initial absorbance of the solution 0.9 in both cases) with a 300 W Xenon lamp (irradiance 55 mW cm^{-2} between 400 and 800 nm).

The photobleaching was calculated from the ratio of the normalized intensity of the UV band at 559 nm for RB or 548 nm for VBRB at time 0 (I_0) and at *x* hours (I_x) under irradiation (Eq 7).

$$\%\ \text{photobleaching} = 100 \times \left(1 - \frac{I_x}{I_0}\right) \qquad \text{Eq (7)}$$

DPBF photooxygenation cycles

The photooxygenation of DPBF was repeated 5 times in ethanol by using VBRB@MG-14 microgels. The irradiation conditions are similar that the ones described above for singlet oxygen measurements.

Table S3. Photostability over 12 months of VBRB-MG14 colloids:

ϕ_Δ (EtOH) (t0)	ϕ_Δ (EtOH) (t0 + 12 months)
0.35	0.32

References:

1 X.-F. Zhang and X. Li, *J. Lumin.*, 2011, **131**, 2263–2266.