

## Photo-Induced Synthesis of Polymeric Nanoparticles and Chemiluminescent Degradable Materials via Flow Chemistry

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## 1. Instrumentation

**Nuclear Magnetic Resonance (NMR) Spectroscopy:**  $^1\text{H}$  and  $^{13}\text{C}$ -NMR spectra were recorded on a Bruker System 600 Ascend LH, equipped with a BBO-Probe (5 mm) with z-gradient ( $^1\text{H}$ : 600.13 MHz,  $^{13}\text{C}$  150.90 MHz) or on a Bruker AM 400 equipped with a PABBO-Probe (5 mm) ( $^1\text{H}$ : 400 MHz,  $^{13}\text{C}$ : 101 MHz). The  $\delta$ -scale was normalised relative to the solvent resonance of  $\text{CHCl}_3$ ,  $\text{DMSO-}d_6$ , acetonitrile- $d_3$  or tetrahydrofuran- $d_8$  for  $^1\text{H}$  spectra and for  $^{13}\text{C}$  spectra on the middle signal of  $\text{CDCl}_3$  triplet, the  $\text{DMSO-}d_6$  quintet, the acetonitrile- $d_3$  quintet or the tetrahydrofuran- $d_8$  singlet. The multiplicities were reported using the following abbreviations: s for singlet, d for doublet, t for triplet, q for quartet, quin for quintet, m for multiplet and br for broad signal.

**Interchim XS420:** Flash chromatography was performed on a Interchim XS420+ flash chromatography system consisting of a SP-in-line filter 20- $\mu\text{m}$ , an UV-VIS detector (200-800 nm). The separations were performed using an Interchim dry load column and a Interchim Puriflash Silica HP 30  $\mu\text{m}$  column after deposition on Celite<sup>®</sup> 565 (Sigma-Aldrich).

**Liquid Chromatography coupled Mass Spectroscopy (LC-MS):** LC-MS measurements were performed on an UltiMate 3000 UHPLC System (Dionex, Sunnyvale, CA, USA) consisting of a pump (LPG 3400SZ), autosampler (WPS 3000TSL) and a temperature-controlled column compartment (TCC 3000). Separation was performed on a C18 HPLC column (Phenomenex Luna 5  $\mu\text{m}$ , 100  $\text{\AA}$ , 250  $\times$  2.0 mm) operating at 40  $^\circ\text{C}$ . Water (containing 5 mmol  $\text{L}^{-1}$  ammonium acetate) and acetonitrile were used as eluents. A gradient of acetonitrile:  $\text{H}_2\text{O}$ , 5:95 to 100:0 (v/v) in 7 min at a flow rate of 0.40  $\text{mL}\cdot\text{min}^{-1}$  was applied. The flow was split in a 9:1 ratio, where 90% of the eluent was directed through a DAD UV-detector (VWD 3400, Dionex) and 10% was infused into the electrospray source. Spectra were recorded on an LTQ Orbitrap Elite mass spectrometer (Thermo Fisher Scientific, San Jose, CA, USA) equipped with a HESI II probe. The instrument was calibrated in the  $m/z$  range 74-1822 using premixed calibration solutions (Thermo Scientific). A constant spray voltage of 3.5 kV, a dimensionless sheath gas, and a dimensionless auxiliary gas flow rate of 5 and 2 were applied, respectively. The capillary temperature was set to 300  $^\circ\text{C}$ , the S-lens RF level was set to 68, and the aux gas heater temperature was set to 100  $^\circ\text{C}$ .

**Centrifuge:** The particles were isolated by centrifugation using a Sigma 3-16 L centrifuge, as specified in the experimental details in the supporting information.

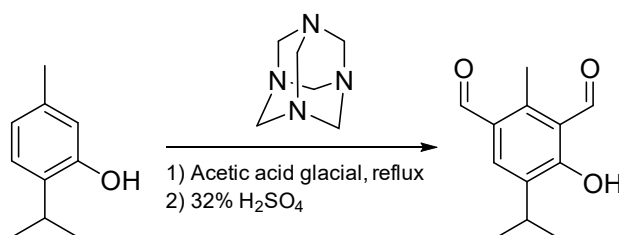
**Size Exclusion Chromatography (SEC):** The SEC measurements were conducted on a PSS SECurity system consisting of a PSS SECurity Degasser, PSS SECurity TCC6000 Column Oven (35  $^\circ\text{C}$ ), PSS SDV Column Set (8x150 mm 5  $\mu\text{m}$  Precolumn, 8x300 mm 5  $\mu\text{m}$  Analytical Columns, 100000  $\text{\AA}$ , 1000  $\text{\AA}$  and 100  $\text{\AA}$ ) and an Agilent 1260 Infinity Isocratic Pump, Agilent 1260 Infinity Standard Autosampler, Agilent 1260 Infinity Diode Array and Multiple Wavelength Detector (A: 254 nm, B: 360 nm), Agilent 1260 Infinity Refractive Index Detector (35  $^\circ\text{C}$ ). HPLC grade THF, stabilized with BHT, is used as eluent at a flow rate of 1  $\text{mL}\cdot\text{min}^{-1}$ . Narrow disperse linear poly(styrene) ( $M_n$ : 266  $\text{g}\cdot\text{mol}^{-1}$  to 2.52x10<sup>6</sup>  $\text{g}\cdot\text{mol}^{-1}$ ) and poly(methyl methacrylate) ( $M_n$ : 202  $\text{g}\cdot\text{mol}^{-1}$  to 2.2x10<sup>6</sup>  $\text{g}\cdot\text{mol}^{-1}$ ) standards (PSS ReadyCal) were used as calibrants. All samples were passed over 0.22  $\mu\text{m}$  PTFE membrane filters. Molecular weight and dispersity analysis was performed in PSS WinGPC UniChrom software (version 8.2).

**Brunauer-Emmett-Teller Method (BET):** Approximately 0.5 g of particles were weighed in a glass tube and degassed for 24 h at 100  $^\circ\text{C}$  under vacuum with a Micromeritics Smart VacPrep (temperature ramp rate: 10.0  $^\circ\text{C}/\text{min}$ ). The sample was subsequently weighed (0.5098 g) and the tube was transferred to a Micromeritics 3-Flex Surface and Porosity Analyzer. The sample was degassed in situ at 100  $^\circ\text{C}$  for 3h. The adsorption/desorption tests were then performed with nitrogen gas at 77K (the glass tube/sample was immersed in liquid nitrogen bath).

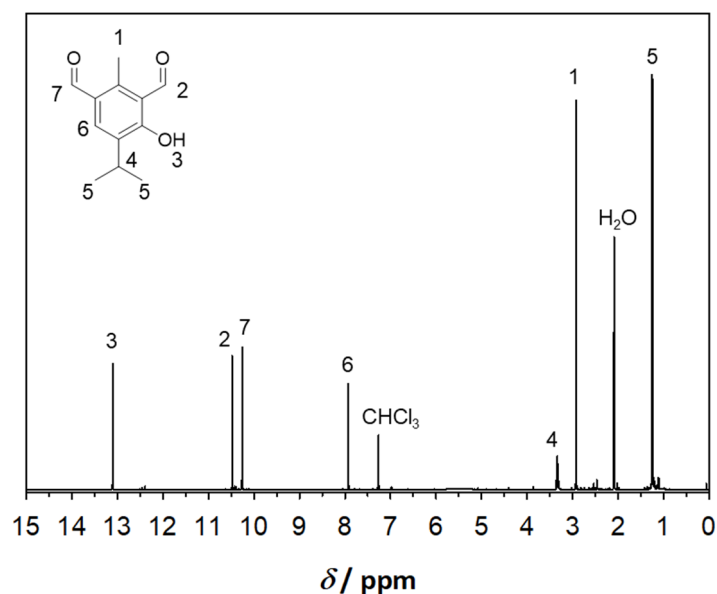
*Chemiluminescence measurements:* Chemiluminescence photon counts were recorded on a Spark microplate reader (Tecan). 200  $\mu\text{L}$  of the reaction mixture (in THF) was introduced in a PerkinElmer Flat 96-well optiplate and 20  $\mu\text{L}$  of  $\text{H}_2\text{O}_2$  was added prior to the measurement (luminescence mode, automatic attenuation OD0, integration time 1000 ms, kinetic mode for 15 min). Each measurement was duplicated; the average of the highest photon count and standard error were subsequently plotted. The scan was performed using the luminescence scan mode with an integration time of 100 ms, a central wavelength start at 398 nm and a central wavelength end at 653 nm.

## 2. Monomer Synthesis

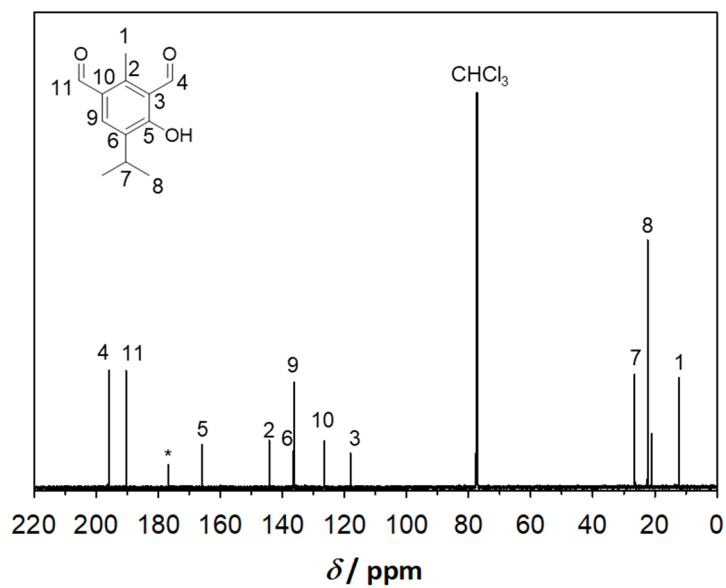
### 2.1. Synthesis of 4-hydroxy-5-isopropyl-2-methylisophthalaldehyde



**Scheme S1.** Synthesis of 4-hydroxy-5-isopropyl-2-methylisophthalaldehyde



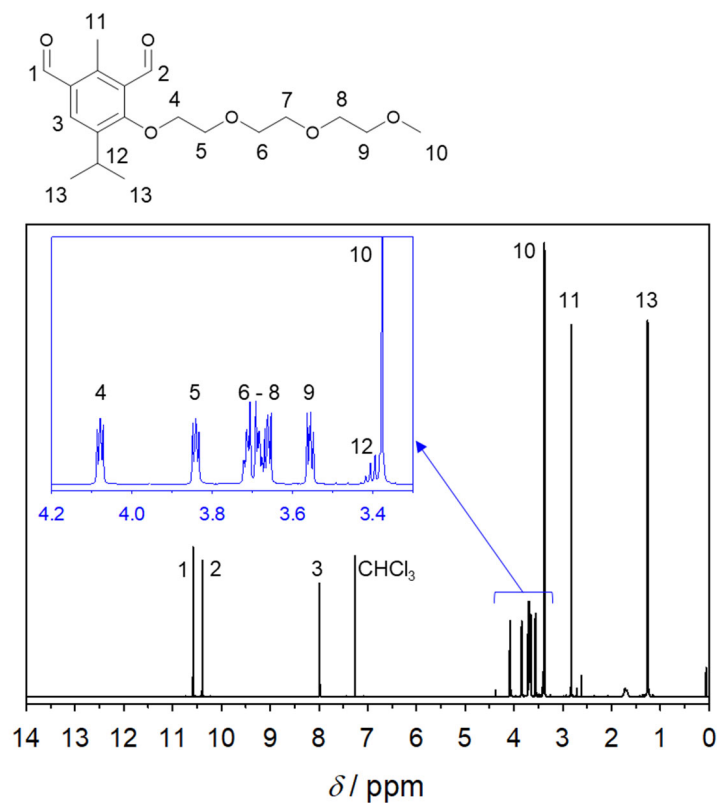
**Figure S1.**  $^1\text{H}$  NMR spectrum of 4-hydroxy-5-isopropyl-2-methylisophthalaldehyde (600 MHz,  $\text{CDCl}_3$ ).



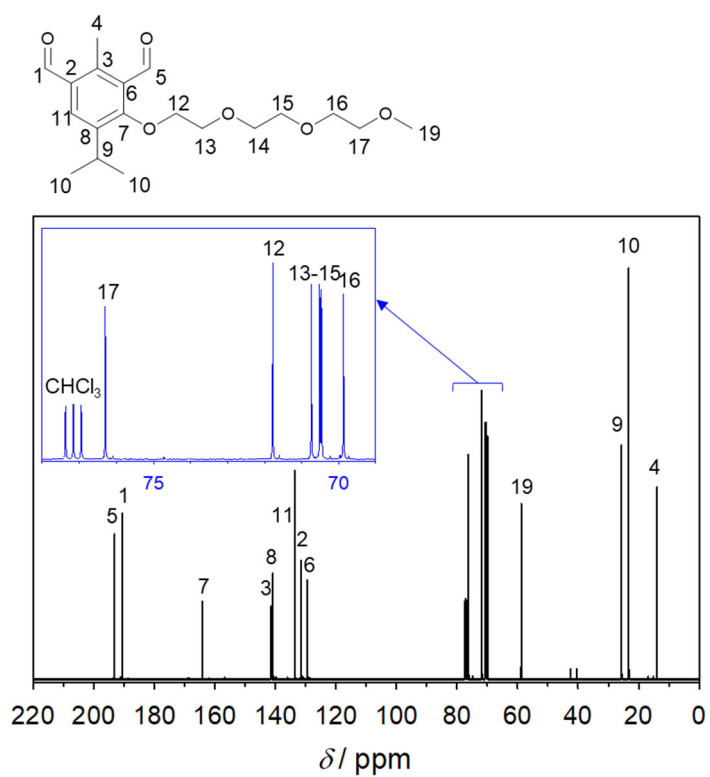
**Figure S2.** <sup>13</sup>C NMR spectrum of 4-hydroxy-5-isopropyl-2-methylisophthalaldehyde (150 MHz, CDCl<sub>3</sub>).

## 2.2. Synthesis of AA Monomer - 5-isopropyl-4-(2-(2-(2-methoxyethoxy)ethoxy) ethoxy)-2-methylisophthalaldehyde

**Scheme S2.** Synthesis of 5-isopropyl-4-(2-(2-(2-methoxyethoxy)ethoxy) ethoxy)-2-methylisophthalaldehyde



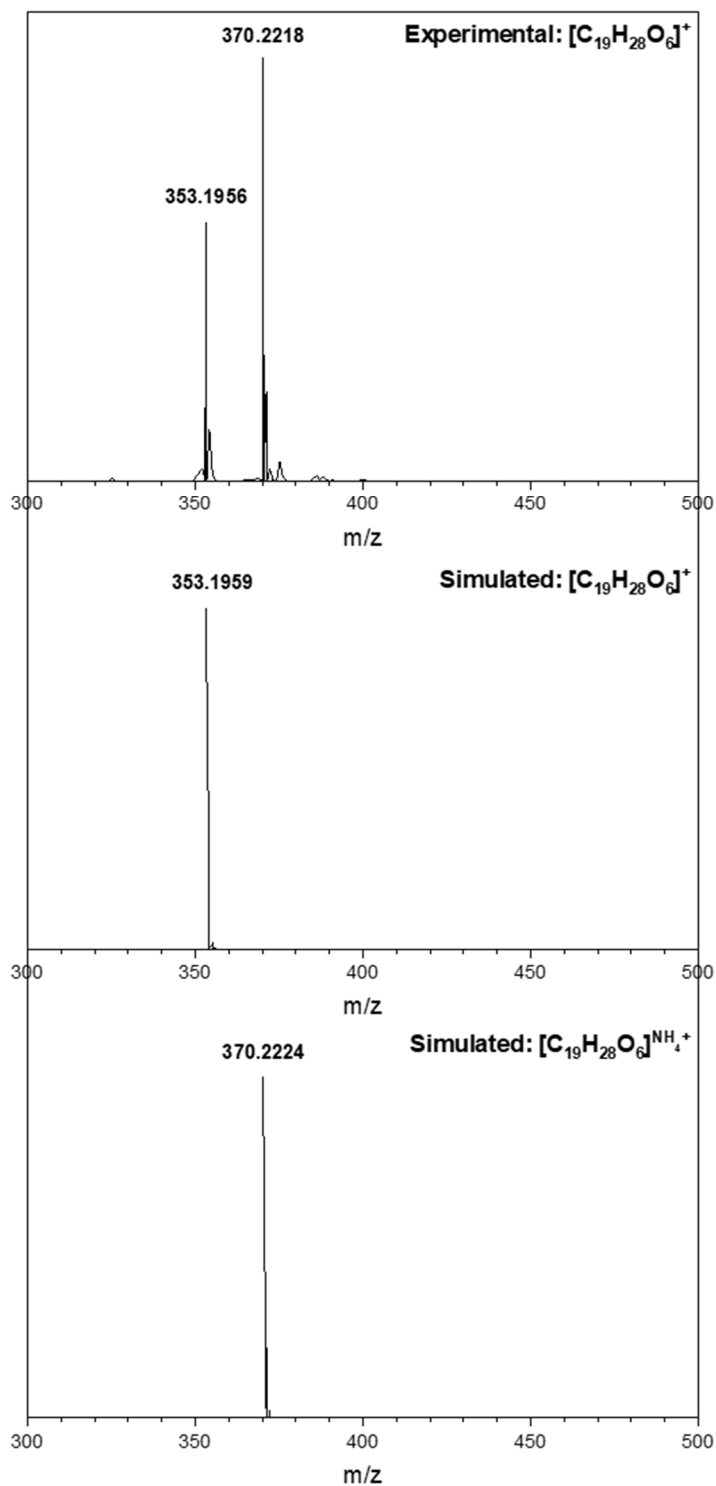
**Figure S3.**  $^1\text{H}$  NMR spectrum of 5-isopropyl-4-(2-(2-(2-methoxyethoxy)ethoxy) ethoxy)-2-methylisophthalaldehyde (600 MHz,  $\text{CDCl}_3$ ).



**Figure S4.**  $^{13}\text{C}$  NMR spectrum of 5-isopropyl-4-(2-(2-(2-methoxyethoxy)ethoxy) ethoxy)-2-methylisophthalaldehyde (150 MHz,  $\text{CDCl}_3$ ).

**Table S1.** Experimental and simulated  $m/z$  values for 5-isopropyl-4-(2-(2-methoxyethoxy)ethoxy)ethoxy)2-methylisophthalaldehyde in positive ion mode.

Symbol	$m/z_{\text{exp}}$	$m/z_{\text{theo}}$	Composition
$[M + H]^+$	353.1956	353.1959	$[C_{19}H_{28}O_6 - H]^+$
$[M + NH_4]^+$	370.2218	370.2224	$[C_{19}H_{28}O_6 - NH_4]^+$



**Figure S5.** LC-MS spectra (experimental and simulated) of 5-isopropyl-4-(2-(2-methoxyethoxy)ethoxy)ethoxy)2-methylisophthalaldehyde

### 2.3. NMR of BB1 monomer 2,4-toluene bismaleimide

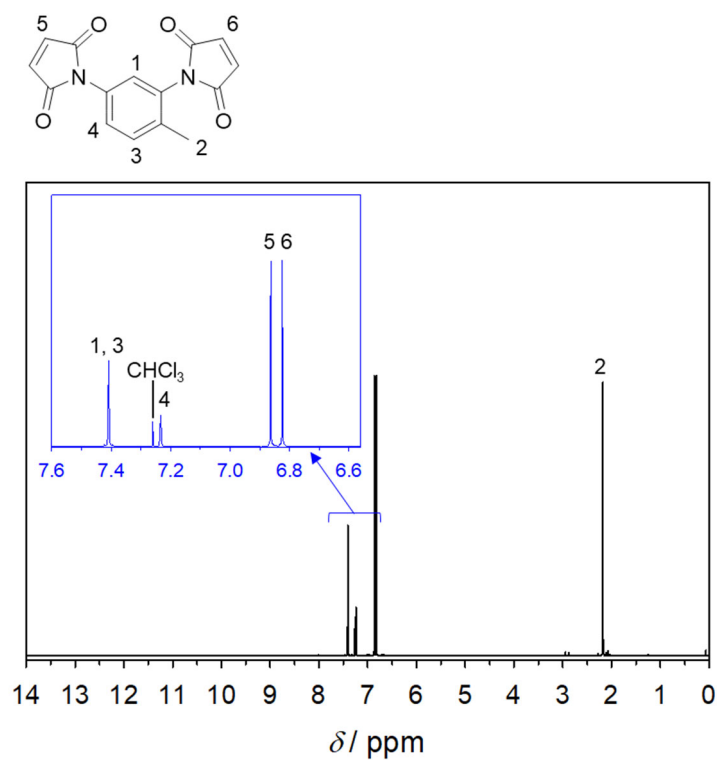


Figure S6. <sup>1</sup>H NMR spectrum of 2,4-toluene bismaleimide (BB1) (600 MHz, CDCl<sub>3</sub>).

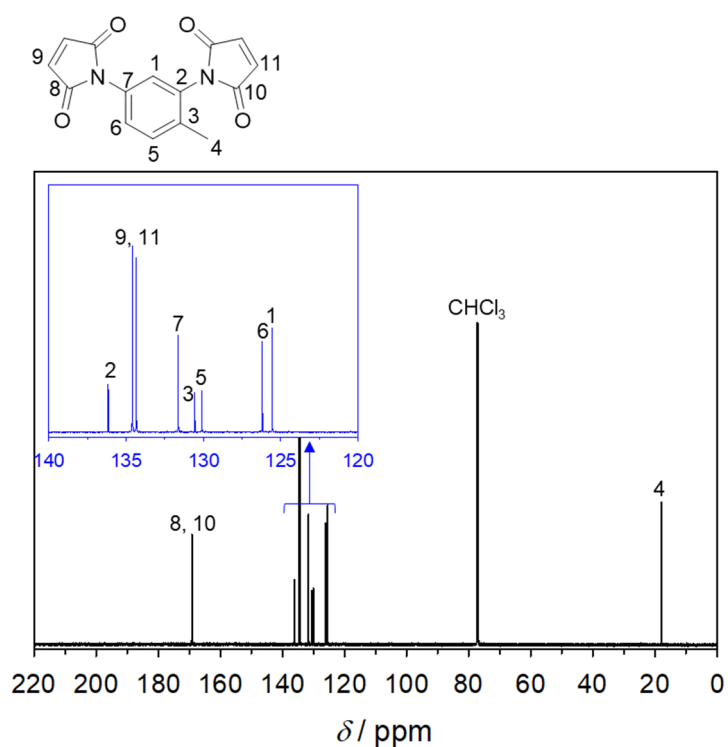
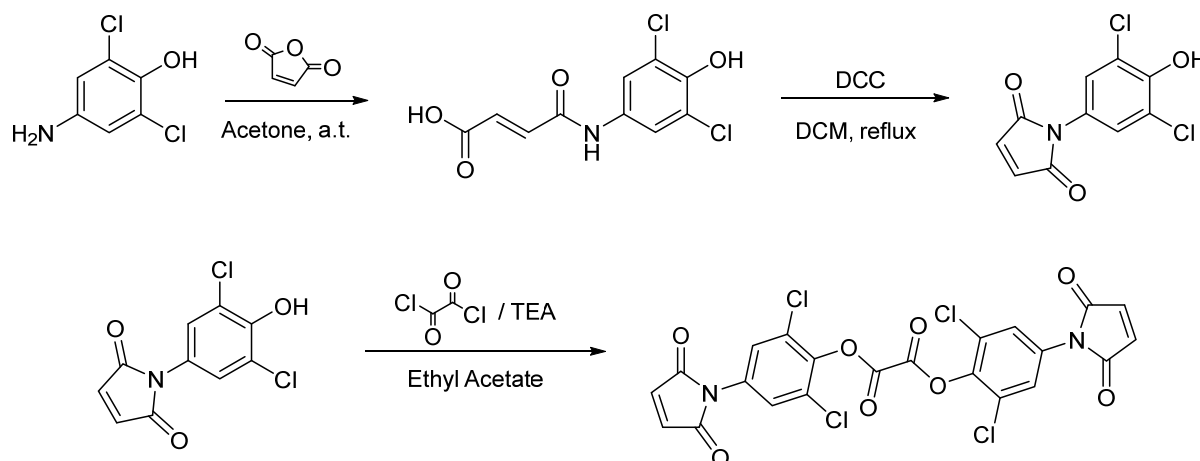


Figure S7. <sup>13</sup>C NMR spectrum of 2,4-toluene bismaleimide (BB1) (150 MHz, CDCl<sub>3</sub>).

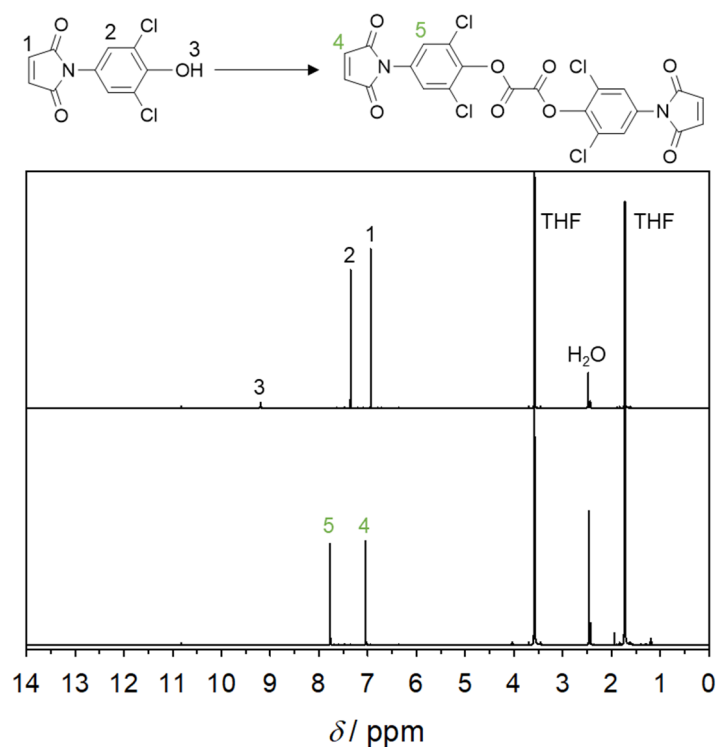
**Table S2.** Experimental and simulated  $m/z$  values for 2,4-toluene bismaleimide in positive ion mode.

Symbol	$m/z_{\text{exp}}$	$m/z_{\text{theo}}$	Composition
$[M + H]^+$	283.0712	283.0713	$[C_{19}H_{28}O_6 - H]^+$
$[M + NH_4]^+$	305.0528	305.0533	$[C_{19}H_{28}O_6 - NH_4]^+$

#### 2.4. Synthesis of BB2 monomer Bis(2,6-dichloro-4-N-maleimido)phenyl) oxalate

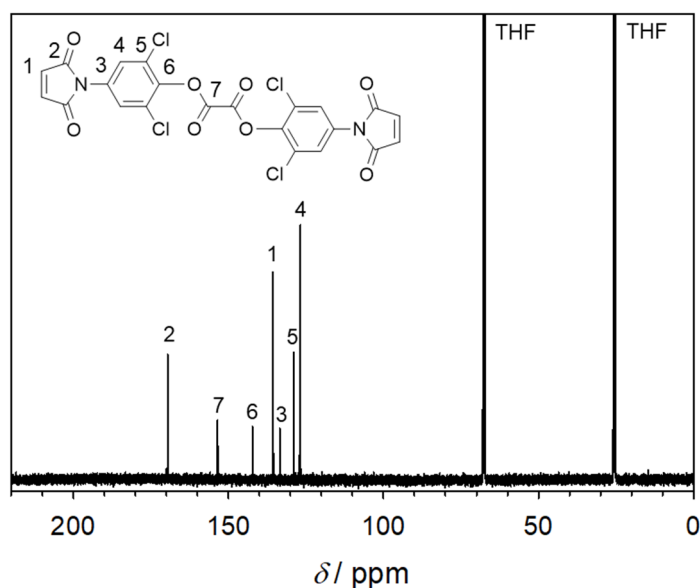


**Scheme S3.** Synthesis of bis(2,6-dichloro-4-N-maleimido)phenyl) oxalate (BB2)



**Figure S8.**  $^1\text{H}$  NMR spectra of 1-(3,5-dichloro-4-hydroxyphenyl)-1H-pyrrole-2,5-dione (precursor, top) and bis(2,6-dichloro-4-N-maleimido)phenyl) oxalate (BB2, bottom) (600 MHz,  $\text{THF}-d_8$ ).



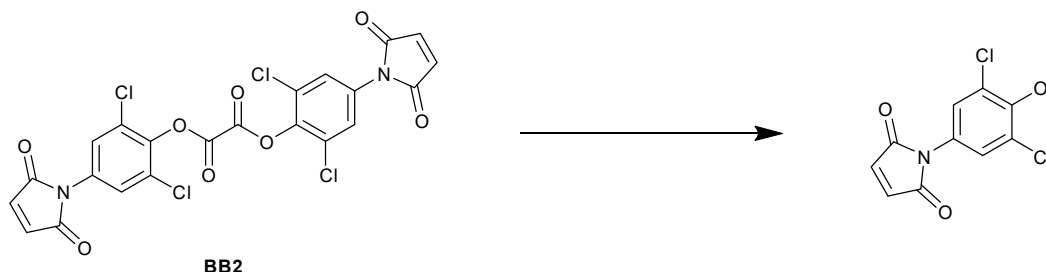


**Figure S9.**  $^{13}\text{C}$  NMR spectrum of bis(2,6-dichloro-4-N-maleimido)phenyl oxalate (BB2) (600 MHz, THF- $d_8$ ).

**Table S3.** Experimental and simulated  $m/z$  values for a) 1-(3,5-dichloro-4-hydroxyphenyl)-1H-pyrrole-2,5-dione precursor and b) bis(2,6-dichloro-4-N-maleimido)phenyl oxalate in negative ion mode.

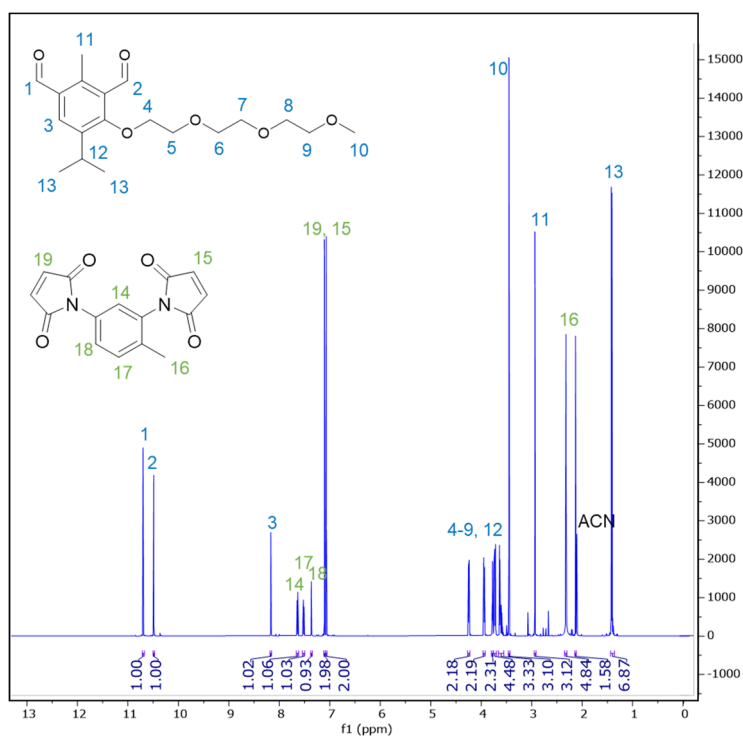
	Symbol	$m/z_{\text{exp}}$	$m/z_{\text{theo}}$	Composition
a)	[M] <sup>-</sup>	255.9574	255.9563	[C <sub>10</sub> H <sub>4</sub> Cl <sub>2</sub> NO <sub>3</sub> ] <sup>-</sup>
		257.9544	257.9533	[C <sub>10</sub> H <sub>4</sub> Cl <sub>2</sub> NO <sub>3</sub> ] <sup>-</sup>
b) *	[M] <sup>-</sup>	255.9573	255.9563	[C <sub>10</sub> H <sub>4</sub> Cl <sub>2</sub> NO <sub>3</sub> ] <sup>-</sup>
	[M] <sup>-</sup>	257.9542	257.9533	[C <sub>10</sub> H <sub>4</sub> Cl <sub>2</sub> NO <sub>3</sub> ] <sup>-</sup>

\*Cleavage appears to occur during LCMS measurement of bis(2,6-dichloro-4-N-maleimido)phenyl oxalate (see Scheme S4) because whilst the same mass is obtained as the 1-(3,5-dichloro-4-hydroxyphenyl)-1H-pyrrole-2,5-dione precursor, a clear shift in LC retention time from 6.4 to 5.9 minutes is observed, indicating a different product. Moreover, the NMR spectra shown in Figure S8, shows a clear shift in the aromatic protons, 2 (to 5), indicating successful formation of the oxalate moiety. Furthermore, the fact that particles were formed in this experiment (Figure 7), further proves that a bifunctional molecule must have been obtained.

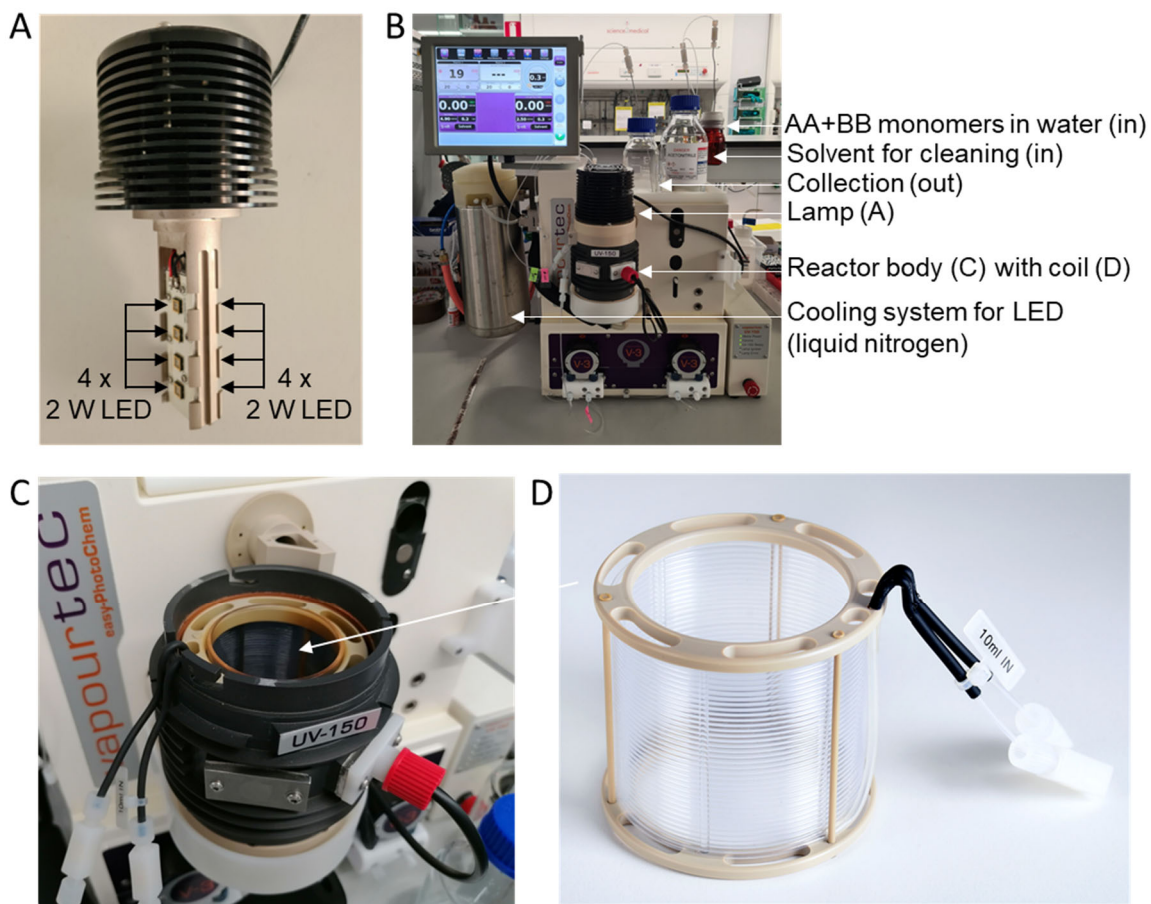


**Scheme S4.** Proposed cleavage of BB2 to its precursor material during LCMS analysis.

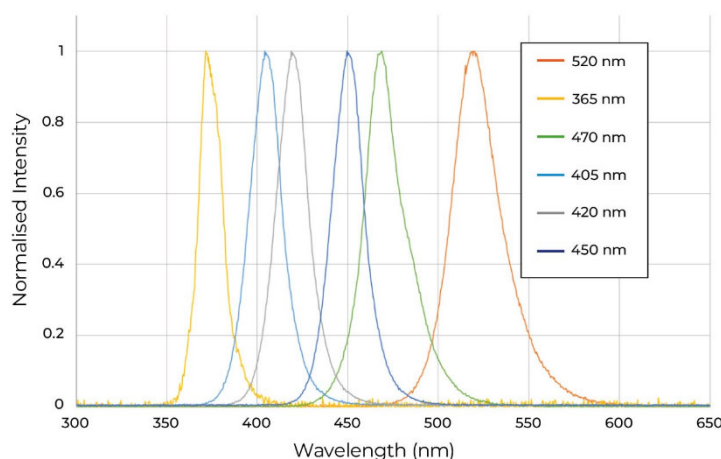
### 3. Particle Synthesis



**Figure S10.**  $^1\text{H}$  NMR of the stock solution after filtration (600 MHz,  $\text{ACN-}d_3$ ).



**Figure S11.** Pictures of the photoflow reactor set-up. (A) 365 nm LED lamp (8 x 2W), (B) Vapourtec UV-150 E-series photoflow set-up, (C) Reactor body, and (D) 10 mL coil cartridge.



**Figure S12.** Normalised intensity of the LED lamps from Vapourtec. The yellow spectrum represents the 365 nm LED lamp employed in this work (yellow curve).

From <https://www.vapourtec.com/products/flow-reactors/photochemistry-uv-150-photochemical-reactor-features/>

### Particle Synthesis (Experiment 1)

A 10 mmol L<sup>-1</sup> equimolar stock solution of the AA and BB1 monomer were prepared in acetonitrile, in amber glassware. The solution was subsequently passed through a 2.5 μm PTFE syringe filter and then bubbled with argon for at least 10 minutes. The flow reactor was flushed with water. Afterwards, the stock solution was sent through the flow coil at a constant temperature of 20 °C at a flow rate of 0.50 mL min<sup>-1</sup>, irradiated at 365 nm (16 W) and collected in a pre-weighed centrifuge tube. The volume collected ( $V_{\text{collected}}$ ) was noted and used for the yield calculations. As we observed that particles could be stuck within the tube, the flow reactor was subsequently purged (without irradiation) with 5 mL of water at 5.0 mL min<sup>-1</sup>, which was also collected in the pre-weighed centrifuge tube. The turbid solution was centrifuged (1000 rpm for 30"), then the supernatant was decanted into a pre-weighed round bottom flask, and the solid pellet was washed with 25 mL of THF (solution was sonicated until well dispersed and then centrifuged (1500 rpm for 1'30") and the THF solution decanted off into the flask with the rest of the supernatant). The resulting particles were redispersed in methanol (10 mL) and 10 μL was drop cast for SEM analysis (Figure S13). The methanol was subsequently evaporated, and the particles dried in an oven at 50 °C so that the mass could be determined. Similarly, the solvent was removed *in vacuo* from the supernatant, and the residue was subsequently dried in the oven at 50 °C so that the mass could be determined.

### Particle Synthesis (Experiment 2)

A 10 mmol L<sup>-1</sup> equimolar stock solution of the AA and BB1 monomer were prepared in acetone, in amber glassware. The solution was subsequently passed through a 2.5 μm PTFE syringe filter and then bubbled with argon for at least 10 minutes. The flow reactor was flushed with water. Afterwards, the stock solution was sent through the flow coil at a constant temperature of 20 °C at a flow rate of 0.50 mL min<sup>-1</sup>, irradiated at 365 nm (16 W) and collected in a pre-weighed centrifuge tube. The volume collected ( $V_{\text{collected}}$ ) was noted and used for the yield calculations. As we observed that particles could be stuck within the tube, the flow reactor was subsequently purged (without irradiation) with 5 mL of water at 5.0 mL min<sup>-1</sup>, which was also collected in the pre-weighed centrifuge tube. No turbid solution resulted. 10 μL of the crude reaction mixture was drop cast for SEM analysis. No particles were discovered.

### Particle synthesis (Experiment 3)

A 10 mmol L<sup>-1</sup> equimolar stock solution of the AA and BB1 monomer were prepared in a 1:1 mixture of water and acetone, in amber glassware. The solution was subsequently passed through a 2.5 μm

PTFE syringe filter and then bubbled with argon for at least 10 minutes. The flow reactor was flushed with water. Afterwards, the stock solution was sent through the flow coil at a constant temperature of 20 °C at a flow rate of 0.50 mL min<sup>-1</sup>, irradiated at 365 nm (16 W) and collected in a pre-weighed centrifuge tube. The volume collected ( $V_{\text{collected}}$ ) was noted and used for the yield calculations. As we observed that particles could be stuck within the tube, the flow reactor was subsequently purged (without irradiation) with 5 mL of water at 5.0 mL min<sup>-1</sup>, which was also collected in the pre-weighed centrifuge tube. The turbid solution was centrifuged (1500 rpm for 1'30"), then the supernatant was decanted into a pre-weighed round bottom flask, and the solid pellet was washed with 25 mL of THF (solution was sonicated until well dispersed and then centrifuged (1500 rpm for 1'30") and the THF solution decanted off into the flask with the rest of the supernatant). The resulting particles were redispersed in methanol (10 mL) and 10  $\mu$ L was drop cast for SEM analysis (Figure S14). The methanol was subsequently evaporated, and the particles dried in an oven at 40 °C so that the mass could be determined. Similarly, the solvent was removed *in vacuo* from the supernatant, and the residue was subsequently dried in the oven at 40 °C so that the mass could be determined.

#### Particle synthesis (Experiment 4)

A 10 mmol L<sup>-1</sup> equimolar stock solution of the AA and BB1 monomer were prepared in a 1:1 mixture of water and acetonitrile, in amber glassware. The solution was subsequently passed through a 2.5  $\mu$ m PTFE syringe filter and then bubbled with argon for at least 10 minutes. The flow reactor was flushed with water. Afterwards, the stock solution was sent through the flow coil at a constant temperature of 20 °C at a flow rate of 0.50 mL min<sup>-1</sup>, irradiated at 365 nm (16 W) and collected in a pre-weighed centrifuge tube. The volume collected ( $V_{\text{collected}}$ ) was noted and used for the yield calculations. As we observed that particles could be stuck within the tube, the flow reactor was subsequently purged (without irradiation) with 5 mL of water at 5.0 mL min<sup>-1</sup>, which was also collected in the pre-weighed centrifuge tube. The turbid solution was centrifuged (9000 rpm for 5'30"), then the supernatant was decanted into a pre-weighed round bottom flask, and the solid pellet was washed with 25 mL of THF (solution was sonicated until well dispersed and then centrifuged (1500 rpm for 1'30") and the THF solution decanted off into the flask with the rest of the supernatant). The resulting particles were redispersed in methanol (10 mL) and 10  $\mu$ L was drop cast for SEM analysis (Figure S15). The methanol was subsequently evaporated, and the particles dried in an oven at 40 °C so that the mass could be determined. Similarly, the solvent was removed *in vacuo* from the supernatant, and the residue was subsequently dried in the oven at 40 °C so that the mass could be determined.

#### Particle synthesis (Experiments 5-16)

10 mmol L<sup>-1</sup> equimolar stock solutions of the AA and BB1 monomer were prepared in a 1:1 mixture of water and acetone, in amber glassware. The solution was subsequently passed through a 2.5  $\mu$ m PTFE syringe filter and then bubbled with argon for at least 10 minutes. The flow reactor was flushed with water. Afterwards, the stock solution was sent through the flow coil at a constant temperature of 20 °C at a flow rate ranging from 0.10 to 5.0 mL min<sup>-1</sup>, (see **Table 1**) irradiated at 365 nm (16 W) and collected in a pre-weighed centrifuge tube. The volume collected ( $V_{\text{collected}}$ ) was noted and used for the yield calculations. As we observed that particles could be stuck within the tube, the flow reactor was subsequently purged (without irradiation) with 5 mL of water at 5.0 mL min<sup>-1</sup>, which was also collected in the pre-weighed centrifuge tube. The turbid solution was centrifuged (9000 rpm for 5'30"), then the supernatant was decanted into a pre-weighed round bottom flask, and the solid pellet was washed with 25 mL of THF (solution was sonicated until well dispersed and then centrifuged (9000 rpm for 1'30") and the THF solution decanted off into the flask with the rest of the supernatant). The resulting particles were redispersed in methanol (10 mL) and 10  $\mu$ L was drop cast for SEM analysis. The methanol was subsequently evaporated, and the particles dried in an oven at 40 °C so that the mass could be determined. Similarly, the solvent was removed *in vacuo* from the supernatant, and the residue was subsequently dried in the oven at 40 °C so that the mass could be determined.

NB: At some point during the experiment runs, the tubing became blocked due to particle deposition on the tubing walls and so the tubing was replaced. Whilst the same specification tubing was ordered from the manufacturer, there was a slight difference in length, which accounts for the slight inconsistency in retention times observed in Table 1.

#### **Particle synthesis (Experiments 17-20)**

Equimolar stock solutions of the AA and BB1 monomer were prepared in a 1:1 mixture of water and acetone, in amber glassware, at a range of concentrations from 2.5 to 15 mmol L<sup>-1</sup> (see **Table 2**). The solution was subsequently passed through a 2.5 µm PTFE syringe filter and then bubbled with argon for at least 10 minutes. The flow reactor was flushed with water. Afterwards, the stock solution was sent through the flow coil at a constant temperature of 20 °C at a flow rate of 0.80 min<sup>-1</sup>, irradiated at 365 nm (16 W) and collected in a pre-weighed centrifuge tube. The volume collected ( $V_{\text{collected}}$ ) was noted and used for the yield calculations. As we observed that particles could be stuck within the tube, the flow reactor was subsequently purged (without irradiation) with 5 mL of water at 5.0 mL min<sup>-1</sup>, which was also collected in the pre-weighed centrifuge tube. The turbid solution was centrifuged (9000 rpm for 5'30"), then the supernatant was decanted into a pre-weighed round bottom flask, and the solid pellet was washed with 25 mL of THF (solution was sonicated until well dispersed and then centrifuged (9000 rpm for 1'30") and the THF solution decanted off into the flask with the rest of the supernatant). The resulting particles were redispersed in methanol (10 mL) and 10 µL was drop cast for SEM analysis. The methanol was subsequently evaporated, and the particles dried in an oven at 40 °C so that the mass could be determined. Similarly, the solvent was removed *in vacuo* from the supernatant, and the residue was subsequently dried in the oven at 40 °C so that the mass could be determined.

#### **Control experiment (Experiment 21)**

A 10 mmol L<sup>-1</sup> stock solution of AA monomer was prepared in a 1:1 mixture of water and acetone, in amber glassware. The solution was subsequently passed through a 2.5 µm PTFE syringe filter and then bubbled with argon for at least 10 minutes. The flow reactor was flushed with water. Afterwards, the stock solution was sent through the flow coil at a constant temperature of 20 °C at a flow rate of 0.80 min<sup>-1</sup>, irradiated at 365 nm (16 W) and collected in a pre-weighed centrifuge tube. The volume collected ( $V_{\text{collected}}$ ) was noted and used for the yield calculations. As we observed that particles could be stuck within the tube, the flow reactor was subsequently purged (without irradiation) with 5 mL of water at 5.0 mL min<sup>-1</sup>, which was also collected in the pre-weighed centrifuge tube. The resulting solution was not turbid, but a clear yellow. An SEM sample (10 µL drop cast) was prepared from the crude. From this it was clear that no nanoparticles were present. Nonetheless, the solution was centrifuged (9000 rpm for 5'30") to see if any solid precipitated out, however, this was not the case.

#### **Particles synthesis for chemiluminescent output (Experiment 22)**

A 2.5 mmol L<sup>-1</sup> stock solution of AA and BB2 monomer was prepared in acetonitrile, in amber glassware. The solution was subsequently passed through a 2.5 µm PTFE syringe filter and then bubbled with nitrogen for at least 10 minutes. The flow reactor was flushed with acetonitrile. Afterwards, the stock solution was sent through the flow coil at a constant temperature of 20 °C at a flow rate of 0.50 min<sup>-1</sup>, irradiated at 365 nm (16 W) and collected in a pre-weighed centrifuge tube. The turbid solution was centrifuged (9000 rpm for 5'), then the supernatant was decanted into a pre-weighed round bottom flask, and the solid pellet was washed with 25 mL of THF (solution was sonicated until well dispersed) and then centrifuged (9000 rpm for 5'). The THF solution was decanted off into the flask with the rest of the supernatant. The resulting particles were redispersed in methanol (10 mL) and 10 µL was drop cast for SEM analysis. The methanol was subsequently evaporated, and the particles dried in an oven at 40 °C so that the mass could be determined. Similarly, the solvent was

removed *in vacuo* from the supernatant, and the residue was subsequently dried in the oven at 40 °C so that the mass could be determined.

#### 4. Additional Scanning Electron Microscopy (SEM) Images

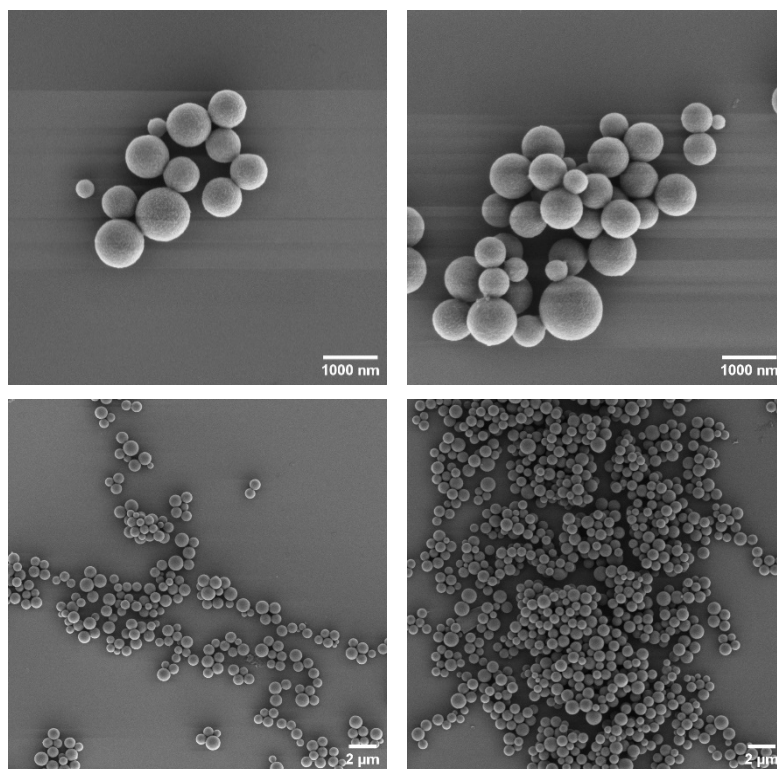


Figure S13. SEM images of the nanoparticles synthesised in Experiment 1.

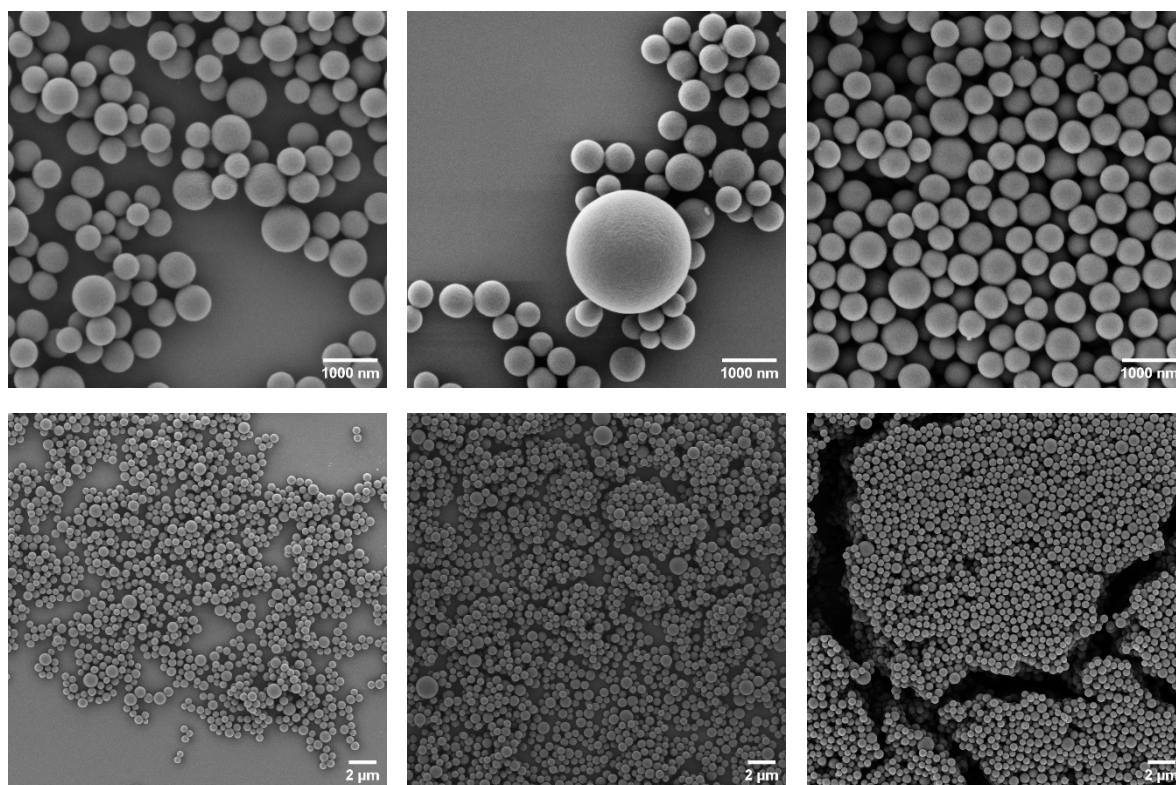
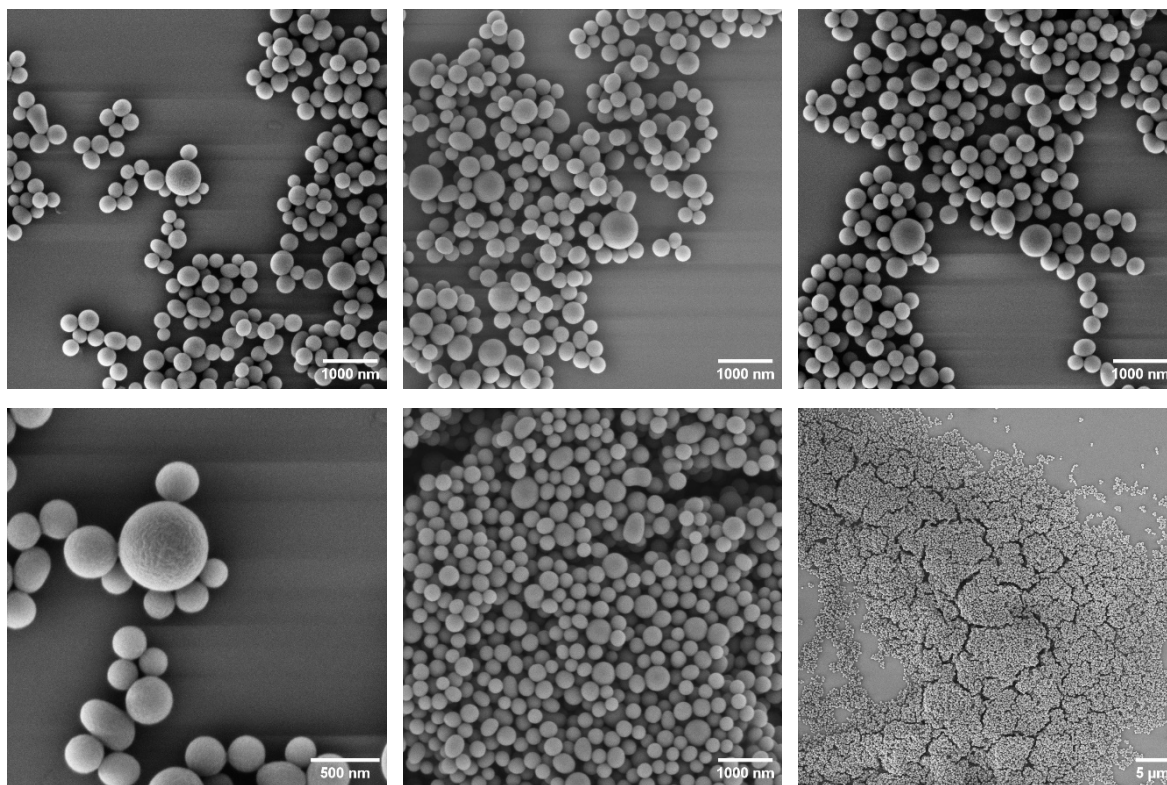
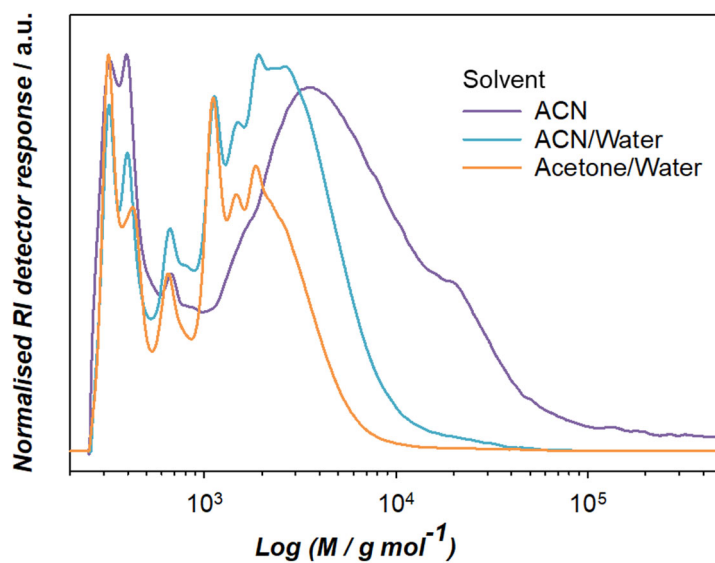


Figure S14. SEM images of the nanoparticles synthesised in Experiment 3.

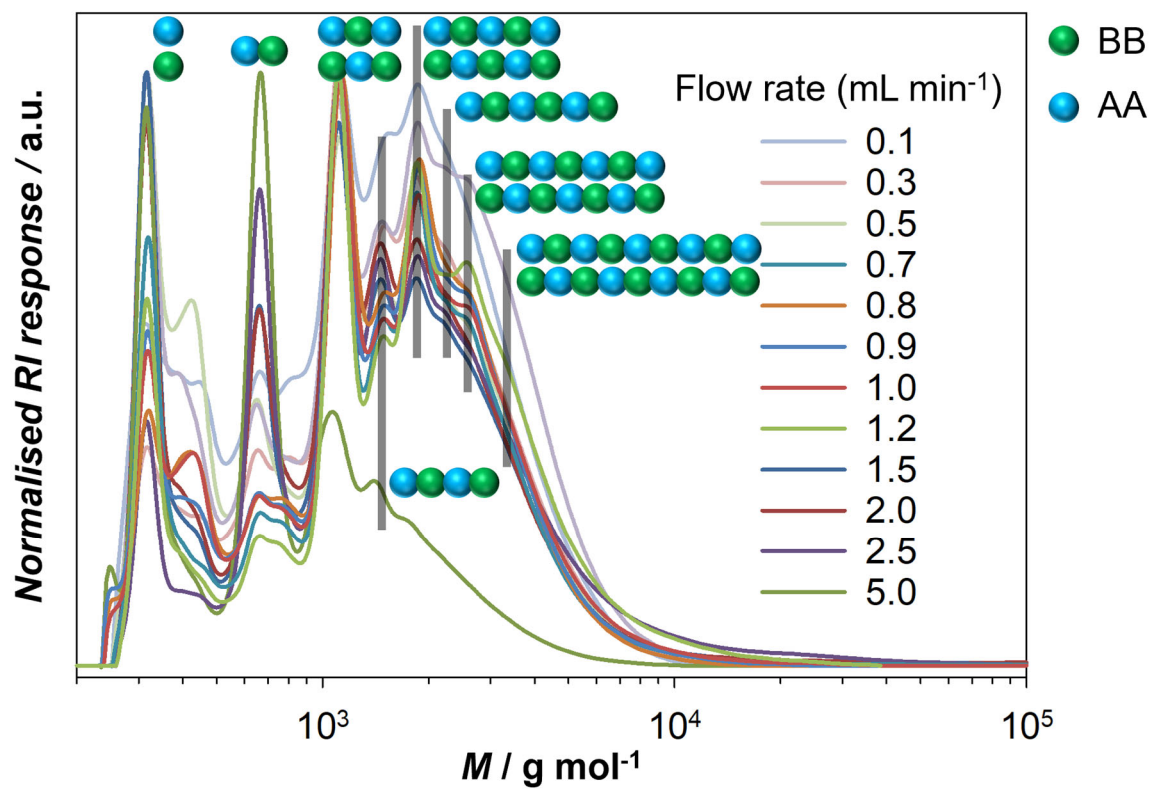


**Figure S15.** SEM images of the nanoparticles synthesised in Experiment 4.



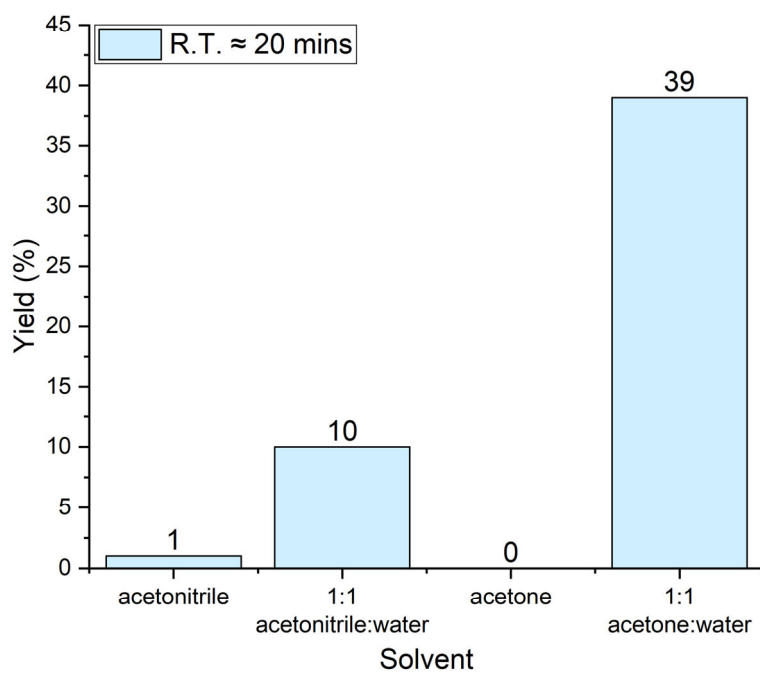
**Figure S16.** SEC analyses of supernatant at with varying solvent. Flow rate =  $0.5 \text{ ml min}^{-1}$ , concentration =  $10 \text{ mmol L}^{-1}$ . (THF, calibration PS).

## 5. Particle Analysis

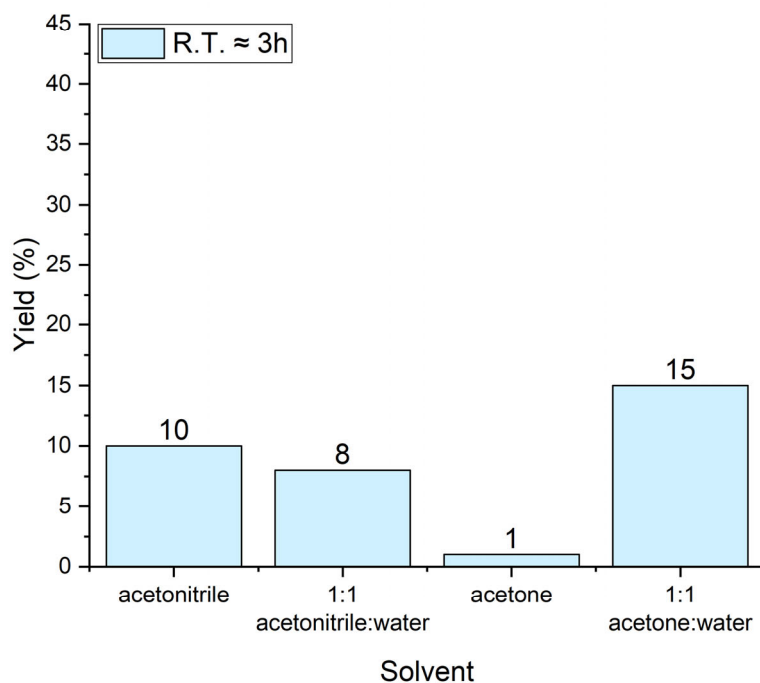


**Figure S17.** SEC analyses of supernatant for various flow rates ( $[AA] = [BB] = 10 \text{ mmol L}^{-1}$ , acetone/water 1:1 mixture) (THF, calibration PS).





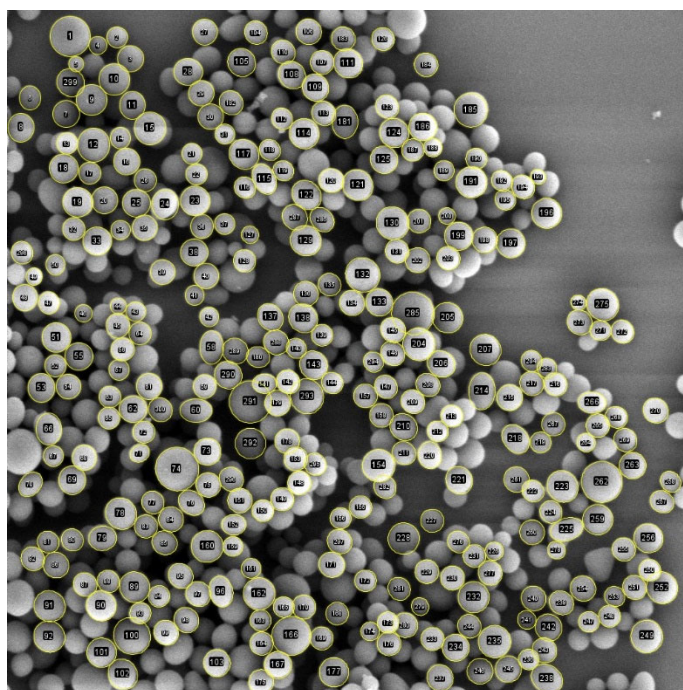
**Figure S18.** Particle mass recovery depending on the solvent used when the reaction solution was passed just once through the photo-flow reactor (retention time (R.T.) ≈ 20 minutes).



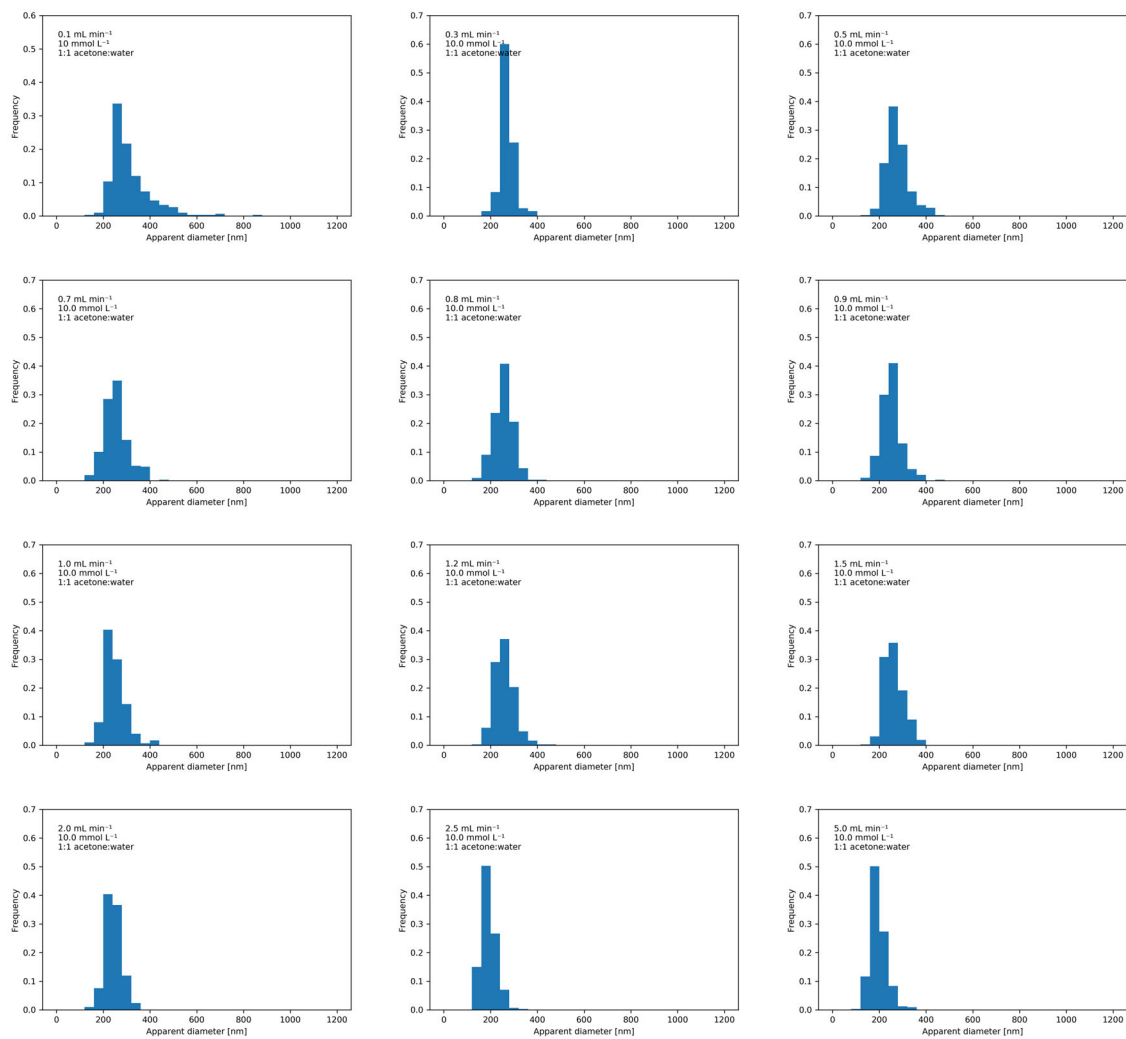
**Figure S19.** Particle mass recovery depending on the solvent used when the reaction solution was passed multiple times through the photo-flow reactor (retention time (R.T.) ≈ 3 hours).

**Table S4.** Volume collected, supernatant and particle masses obtained from experiments 5-16, which were subsequently used to calculate the reaction yield. Supplementary to Table 1.

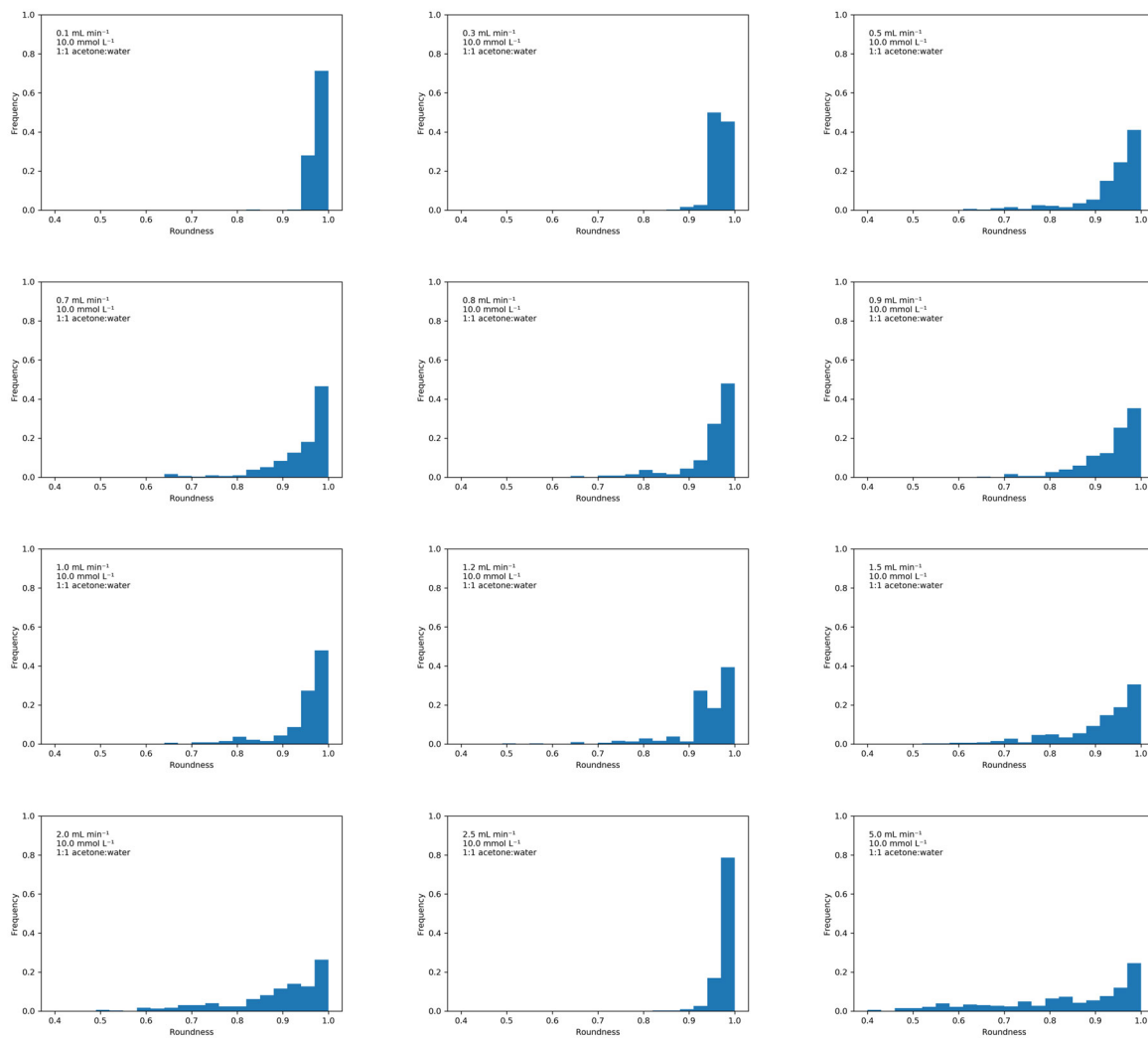
Experiment Number	Volume collected / mL	Supernatant mass / mg	Particle mass / mg	Particle yield %
5	8	20	31	61
6	12	26	48	63
7	12	80	36	47
8	7	13	22	50
9	10	42	34	54
10	10	56	32	50
11	11	47	32	46
12	10	38	24	38
13	15	91	29	30
14	13	81	21	25
15	13	69	8	10
16	14	79	15	17



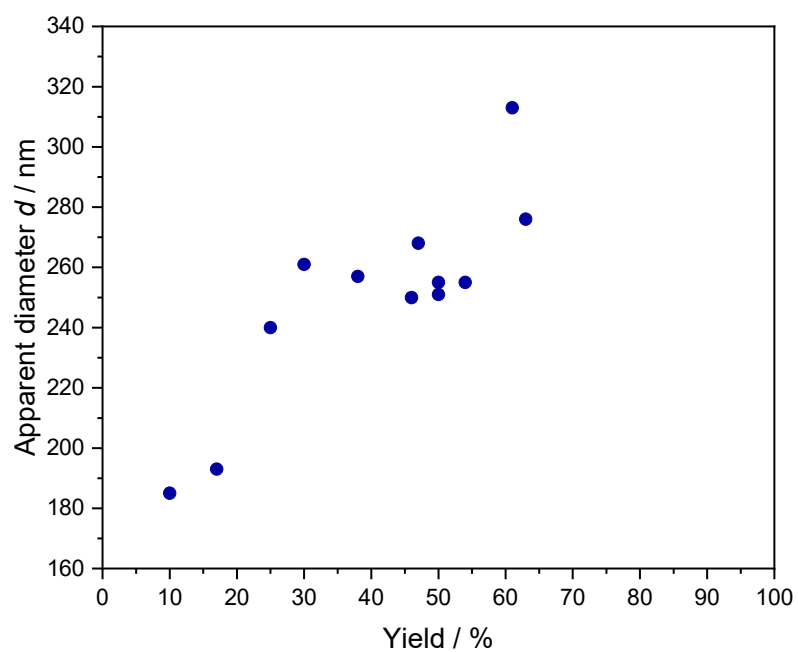
**Figure S20.** Example of particle counting (Experiment 12), carried out in ImageJ. From these fitted ellipses, the apparent diameter, dispersity and roundness were obtained and statistically analysed.



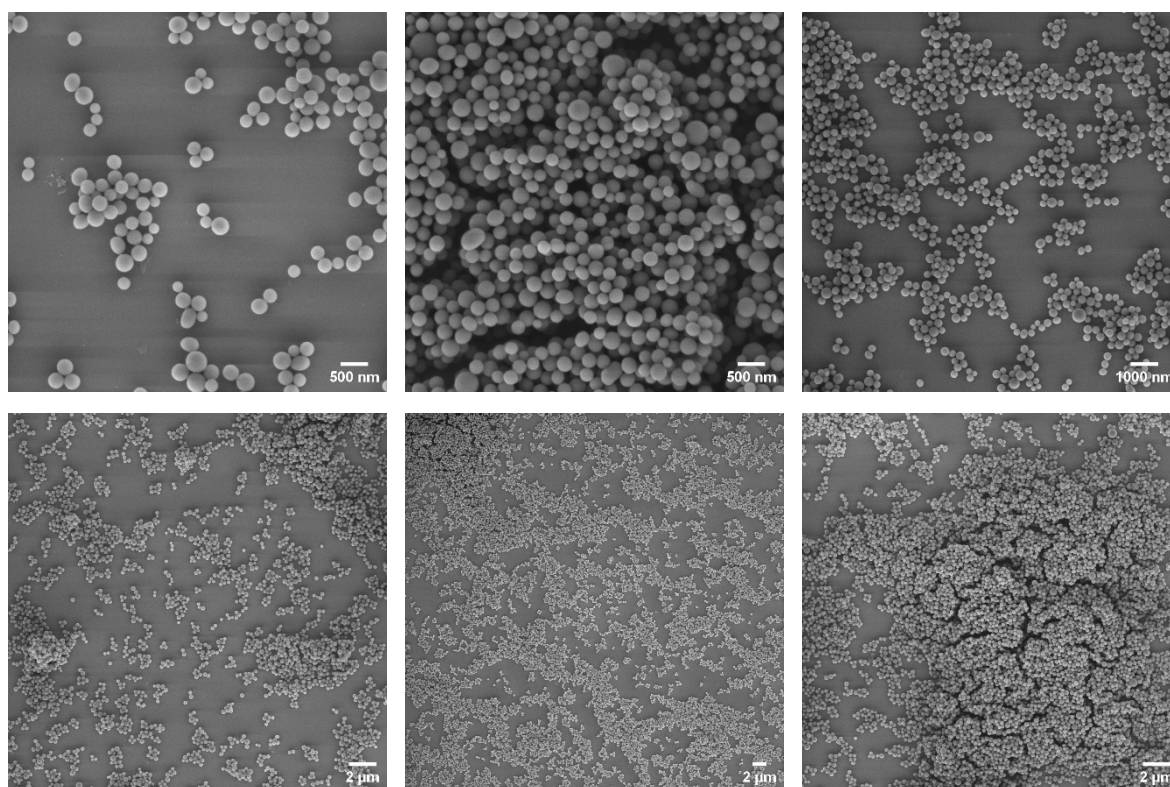
**Figure S21.** Histograms of the apparent diameter as measure of the particle size for different flow rates at fixed concentrations of 10 mmol L<sup>-1</sup> in 1:1 acetone:water.



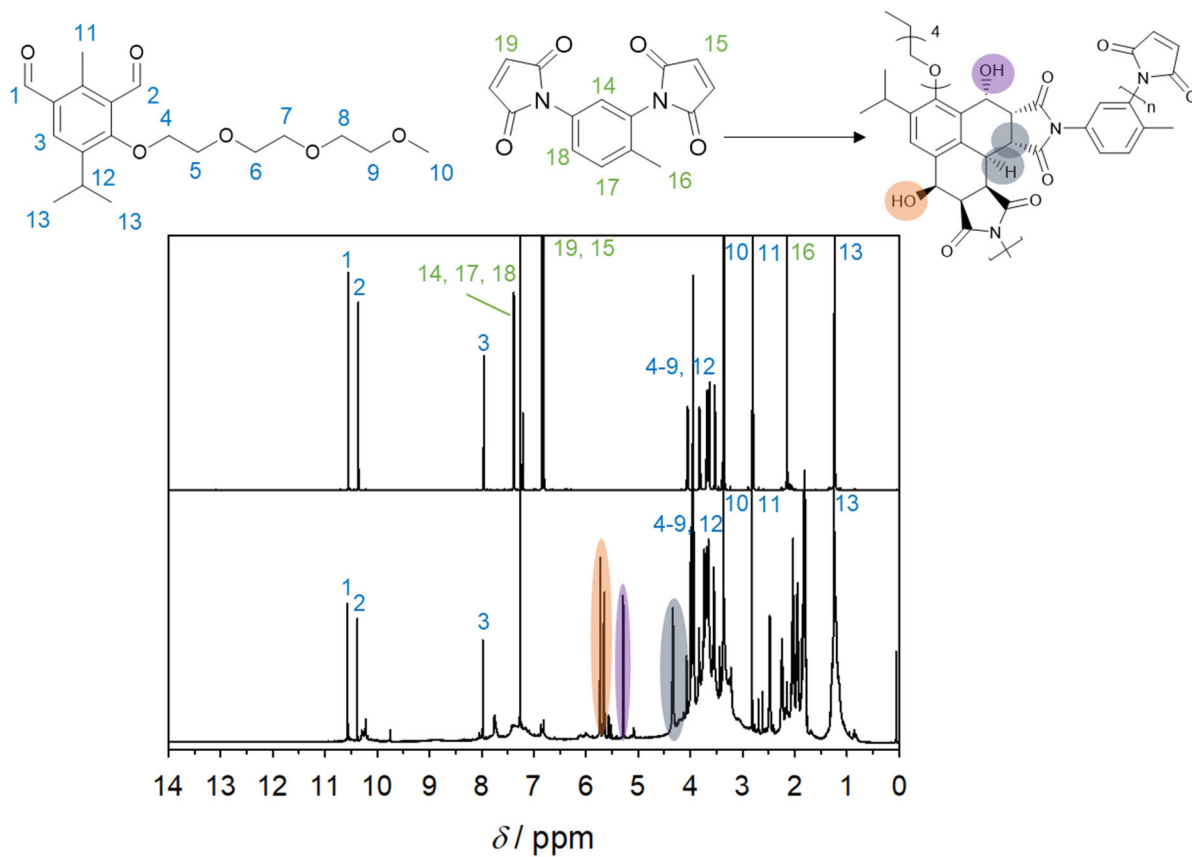
**Figure S22.** Histograms of the roundness for different flow rates at fixed concentrations of 10 mmol L<sup>-1</sup> in 1:1 acetone:water.



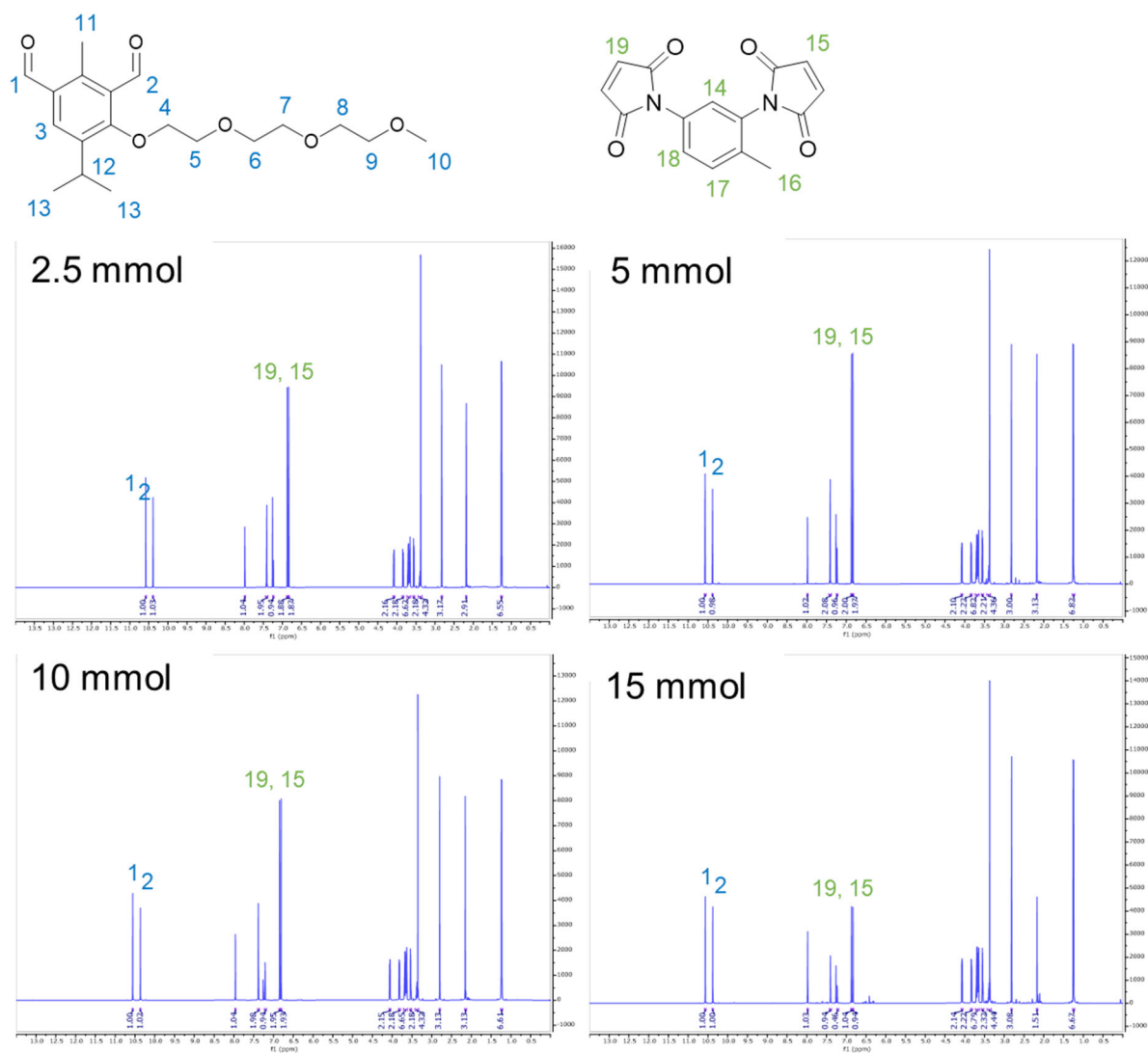
**Figure S23.** Graphical representation of the correlation between yield and apparent particle diameter.



**Figure S24.** SEM images of nanoparticles synthesised in  $10 \text{ mmol L}^{-1}$  1:1 mixture of acetone and water, at a flow rate of  $0.8 \text{ mL min}^{-1}$  (Experiment 10).



**Figure S25.**  $^1\text{H}$  NMR spectra of the  $10 \text{ mmol L}^{-1}$  stock solution after filtration (top) and supernatant (bottom). Experiment 14. Flow rate  $2.0 \text{ mL min}^{-1}$ . (600 MHz,  $\text{CDCl}_3$ ).



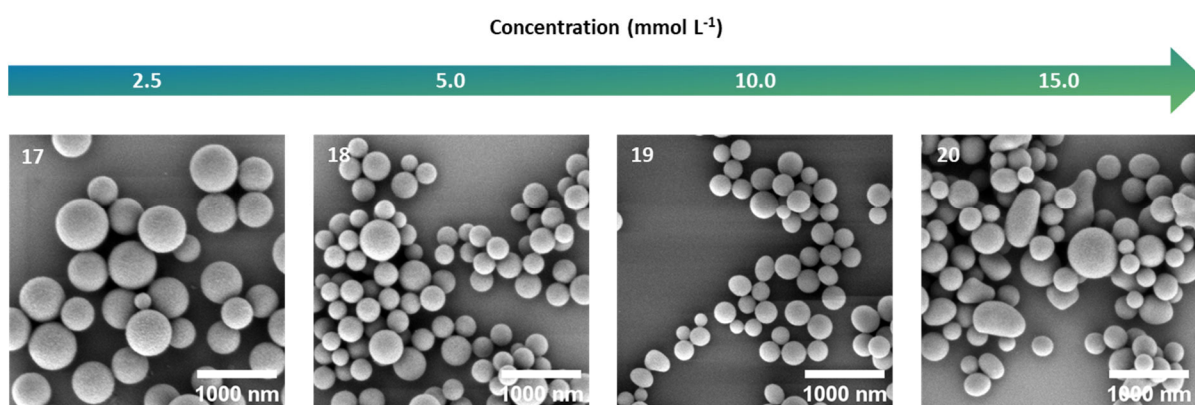
**Figure S26.**  $^1\text{H}$  NMR of the stock solution after filtration for various concentrations of 1:1 acetone/water (600 MHz,  $\text{CDCl}_3$ ).

**Table S5.** Integrals of protons H1, H2 (AA monomer) and H15, H19 (BB monomer) employed to calculate molar ratio.

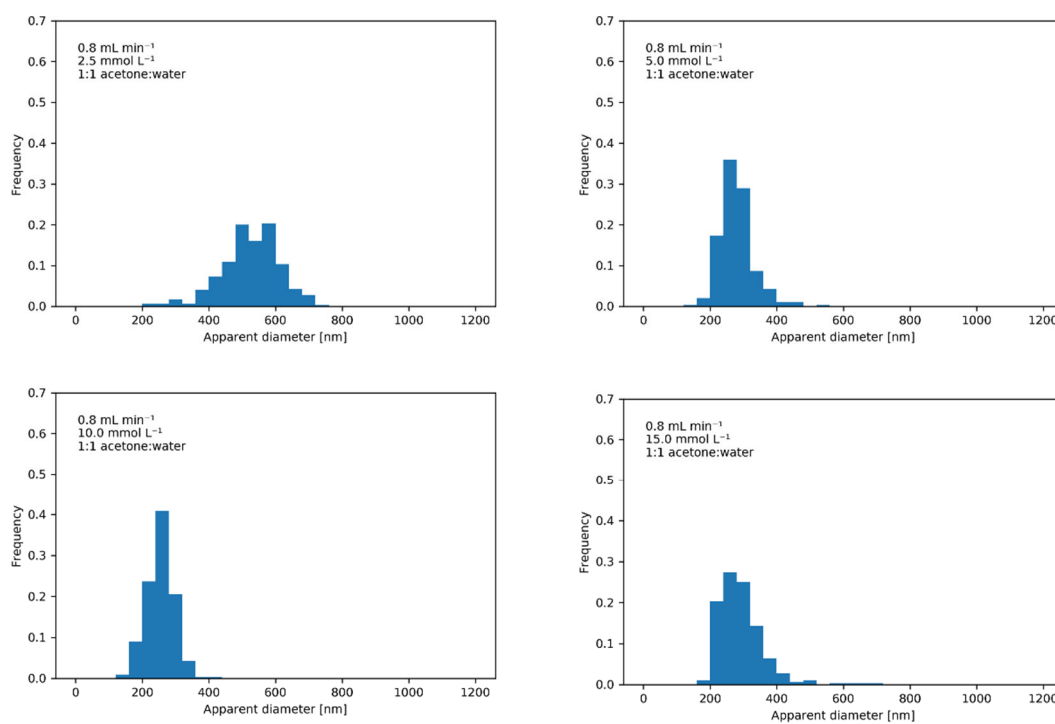
$\int$ Proton	2.5 mmol	5 mmol	10 mmol	15 mmol
H1	1.0	1.0	1.0	1.0
H2	0.99	0.98	1.02	1.0
H15	1.87	1.97	1.93	0.94
H19	1.88	2.0	1.95	1.04
Ratio AA/BB	1 / 0.8	1 / 1	1 / 1	1 / 0.5

**Table S6.** Volume collected, supernatant and particle masses obtained from experiments 17-20, which were subsequently used to calculate the reaction yield. Supplementary to Table 2.

Experiment Number	Volume collected / mL	Supernatant mass / mg	Particle mass / mg	Particle yield %
17	12	15	7	37
18	11	20	17	48
19	10	42	34	54
20	14	81	63	47

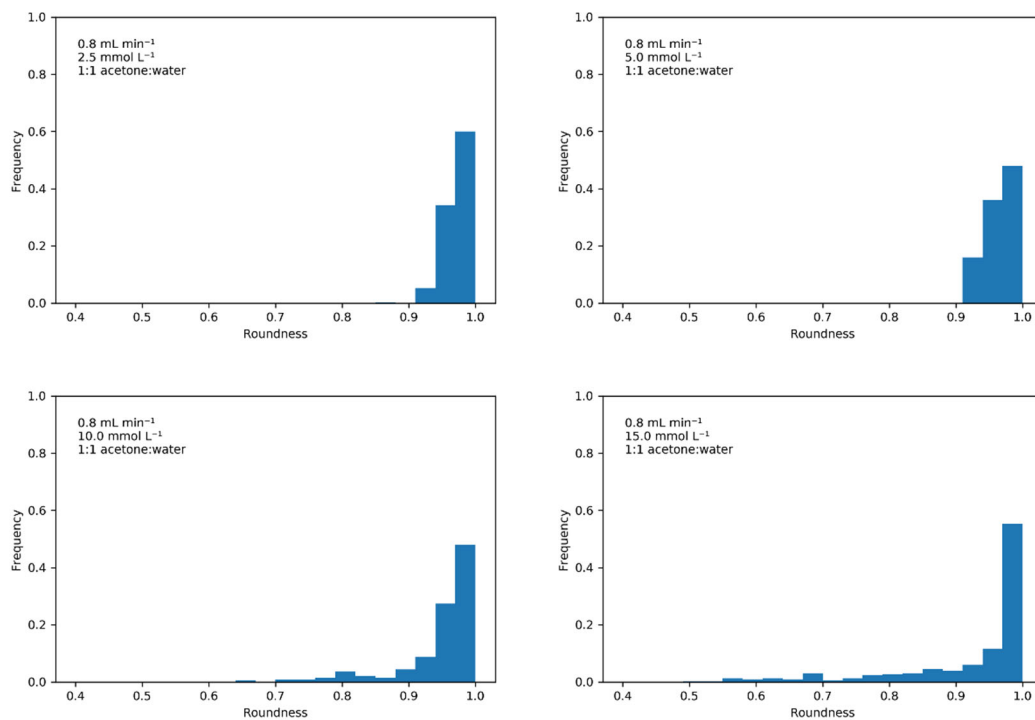


**Figure S27.** SEM of the isolated particles produced with increasing concentration. [AA] = [BB]. 0.8 mL min<sup>-1</sup>, 1:1 acetone/water.

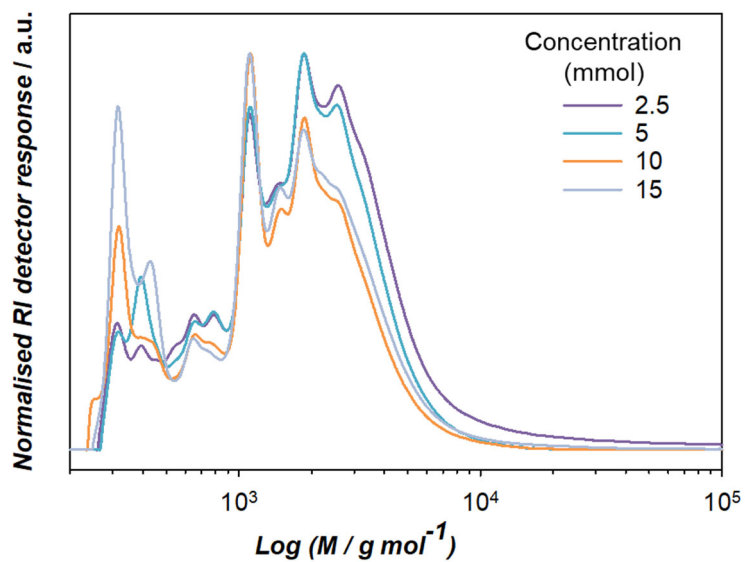


**Figure S28.** Histograms of the apparent diameter as measure of the particle size for different concentrations at a fixed flow rate of 0.8 mL min<sup>-1</sup> in 1:1 acetone:water.





**Figure S29.** Histograms of the roundness for different concentrations at a fixed flow rate of 0.8 mL min<sup>-1</sup> in 1:1 acetone:water.



**Figure S30.** SEC analyses of supernatant at various concentrations. Flow rate = 0.8 ml min<sup>-1</sup>. Acetone/water 1:1 mixture. (THF, calibration PS).

## 6. BET analyses

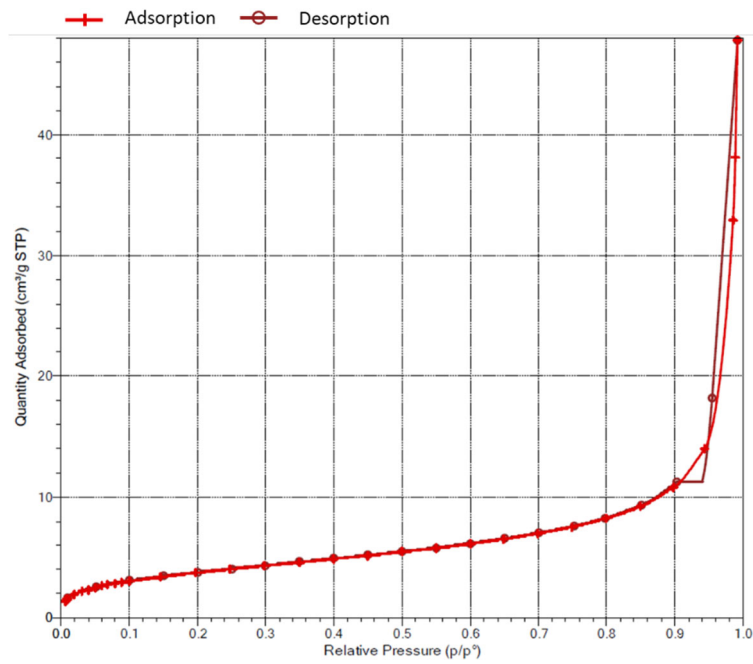


Figure S31. BET Isotherm Linear Plot.

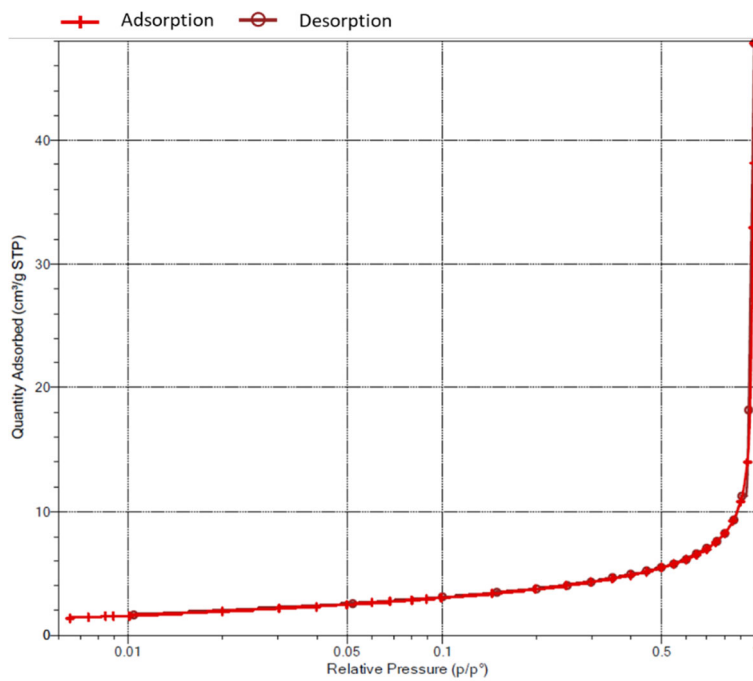


Figure S32. BET Isotherm Log Plot.

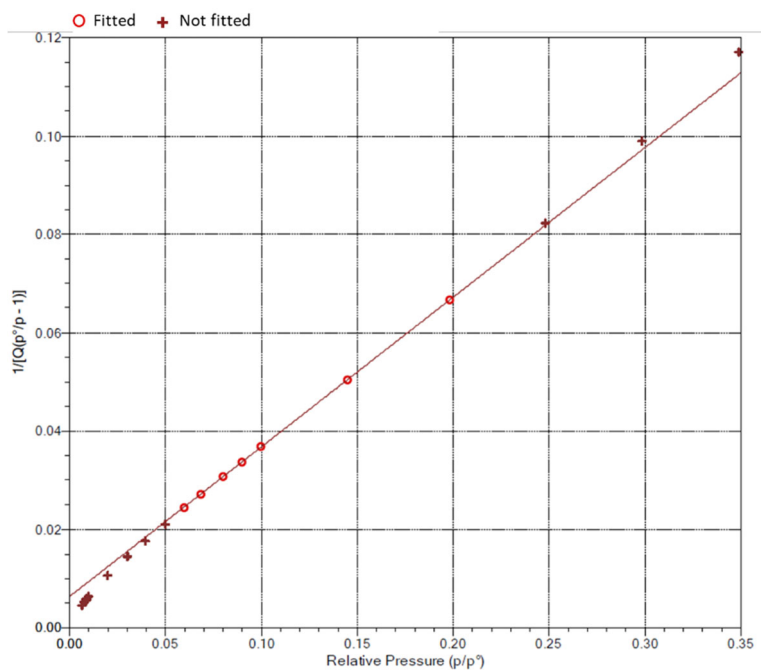


Figure S33. BET Surface Area Plot

## 7. Chemiluminescence Analyses

Experiment 22 was used for chemiluminescence analyses. 1 mg of particles was isolated and 1 mL of THF was added to have a  $1 \text{ mg mL}^{-1}$  stock solution. 200  $\mu\text{L}$  was added into the well and 20  $\mu\text{L}$  was poured and the photon count was subsequently recorded for 15 min (Figure S36). The stock solution was divided into aliquots and THF was added for subsequent dilutions.

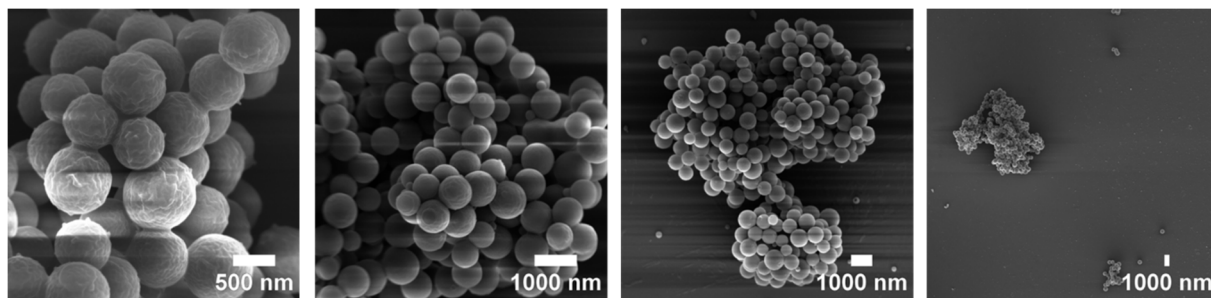
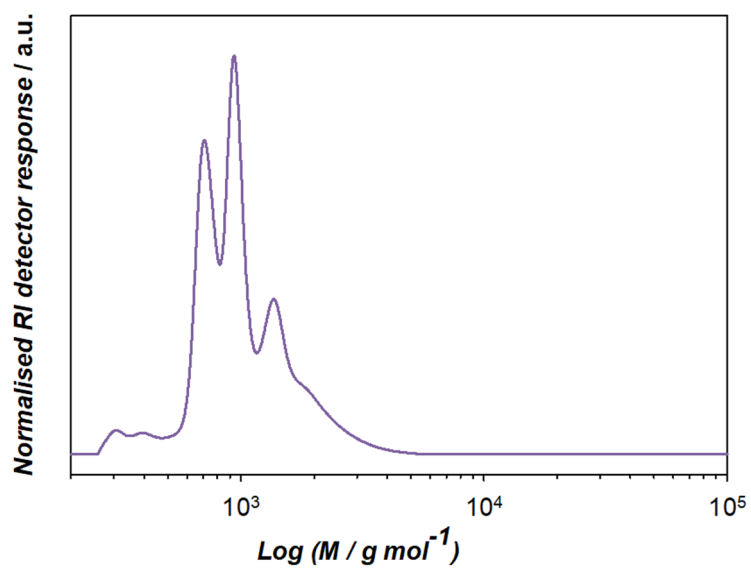
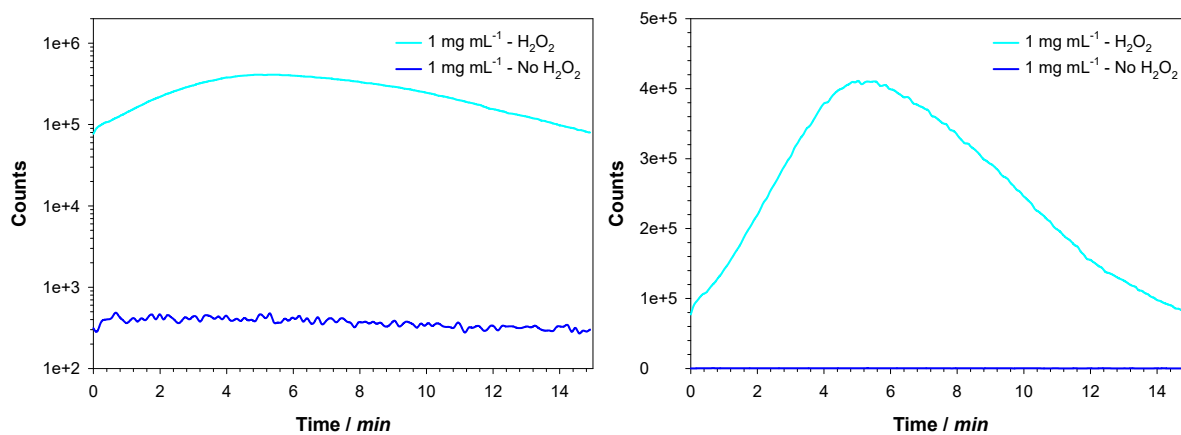


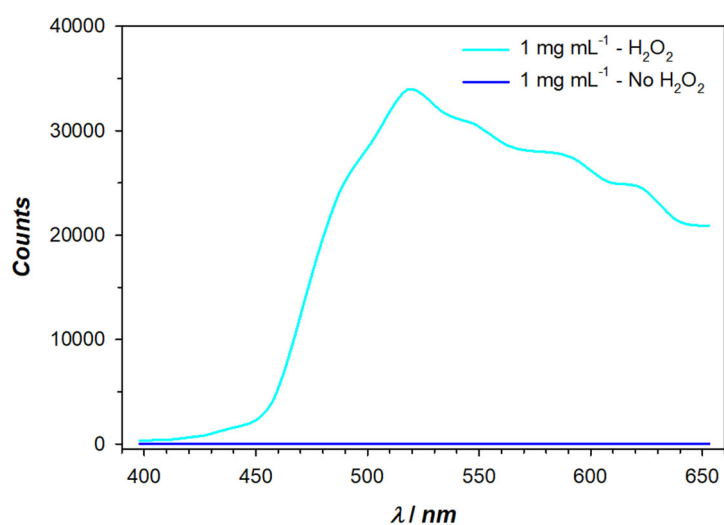
Figure S34. SEM images of AA+BB2 nanoparticles synthesised in  $2.5 \text{ mmol L}^{-1}$  in acetonitrile, at a flow rate of  $0.5 \text{ mL min}^{-1}$  (Experiment 22).



**Figure S35.** SEC analyse of supernatant of the AA+BB2 (experiment 22). ACN, Flow rate = 0.5 ml min<sup>-1</sup> (THF, calibration PS).



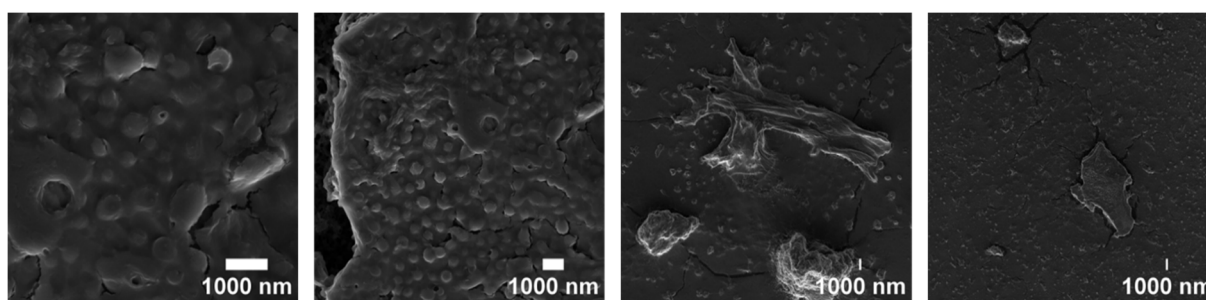
**Figure S36.** Kinetics of photons count over time for the AA+BB2 experiment at 1 mg mL<sup>-1</sup> in THF with (cyan) and without (navy) H<sub>2</sub>O<sub>2</sub>. Left: Logarithmic scale. Right: Linear scale.



**Figure S37.** Light emission of the AA+BB2 experiment at  $1 \text{ mg mL}^{-1}$  in THF with (cyan) and without (navy)  $\text{H}_2\text{O}_2$ .

**Table S7.** Photocount of the AA+BB2 particle at various concentration in THF when the PO\_CL reaction is triggered by  $\text{H}_2\text{O}_2$  ( $20 \mu\text{L}$ ).

Concentration / $\text{mg mL}^{-1}$	Max Photo Count / Run 1	Max Photo Count / Run 2	Average
1 (No $\text{H}_2\text{O}_2$ )	487		$4.87\text{E}+02$
1	372126	411085	$3.92\text{E}+05 \pm 1.95\text{E}+04$
0.5	315245	290027	$3.03\text{E}+05 \pm 1.26 \text{E}+04$
0.25	194207	193755	$1.94\text{E}+05 \pm 2.26\text{E}+02$
0.125	74880	50104	$6.25\text{E}+04 \pm 1.24\text{E}+04$
0.0625	24049	25575	$2.48\text{E}+04 \pm 7.63\text{E}+02$
0.03125	6089	10776	$8.43\text{E}+03 \pm 2.34\text{E}+03$



**Figure S38.** SEM images of AA+BB2 nanoparticles synthesised in  $2.5 \text{ mmol L}^{-1}$  in acetonitrile, at a flow rate of  $0.5 \text{ mL min}^{-1}$  after exposure to  $\text{H}_2\text{O}_2$  for 5 min (Experiment 23).