

Supporting Information

Comparison of metal free polymer-dye conjugation strategies in protic solvents

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Table of Contents

1. Structure of Cy-3.....	2
2. Polymers – Synthesis and Characterisation.....	2
2.1. PMPC-PDPA Polymers.....	2
2.2. POEGMA-PDPA Polymer.....	4
2.3. PEG-PDPA Polymer.....	5
3. Coupling with NHS Ester on Polymer.....	5
4. Disulphide-Maleimide coupling.....	6
5. Synthesis of TAD modified Sudan Red II.....	7
5.1. Synthesis of the butyrate ester (3) from Sudan II (1).....	7
5.2. Synthesis of the cleaved ester (4) from the butyrate ester (3).....	7
5.3. Synthesis of Sudan-semicarbazide (5).	8
6. Polymer for TAD modification.....	8
6.1. GPC Analysis.....	8
7. References.....	9

1. Structure of Cy-3

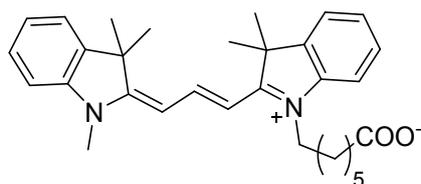


Figure S1: Structure of the plain Cy-3 Dye without any modifications as published on <http://www.lumiprobe.com/tech/cyanine-dyes>

All modifications are attached to the carboxylic acid and the exact structure can be found on the website mentioned.

2. Polymers – Synthesis and Characterisation

2.1. PMPC-PDPA Polymers

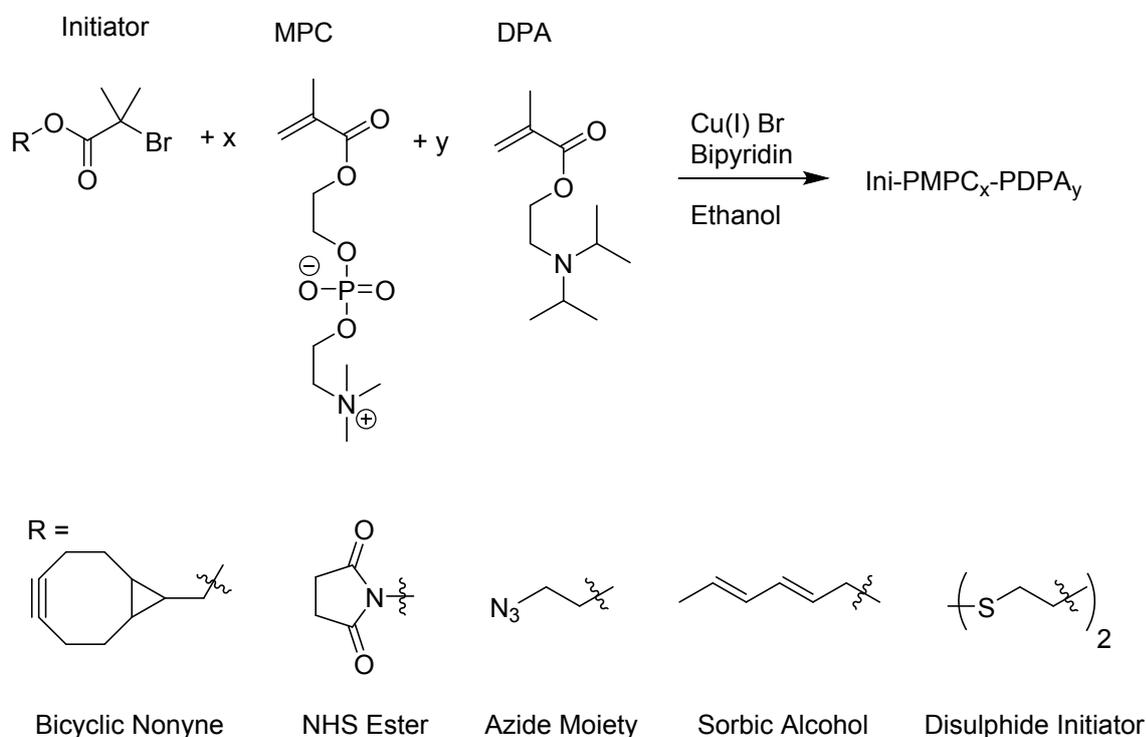


Figure S2: Synthetic scheme for the synthesis of the various PMPC-PDPA block-copolymers

Polymerisation procedure based on previously published procedures:^{1, 2} Briefly, 0.05 mmol of the corresponding initiators were dissolved in 1mL EtOH in a round-bottom flask and 25 eq. of MPC were added (370 mg, 1.25 mmol, 25 eq.). The mixture was stirred to be homogeneous and purged with nitrogen for 30 minutes at 30 °C. Then, a mixture of bpy (16 mg, 0.10 mmol, 2 eq.) and Cu(I)Br (7.5 mg, 0.05 mmol, 1 eq.) was added under a constant nitrogen flow. The mixture was stirred for 60 minutes to yield a highly viscous brown substance and sampled for NMR to estimate conversion (gave full conversion, no monomer peaks present). Meanwhile, a solution of DPA (695 mg, 3.25 mmol, 85 eq.) in 2 mL ethanol

was prepared and purged with nitrogen for 60 minutes in a separate flask. Then, the DPA solution was added to the polymerisation mixture, the reaction mixture was purged for another 10 minutes and then left overnight at 30°C. After 18 h, ¹H NMR analysis confirmed that the conversion was > 99 % and the reaction was opened to the atmosphere and diluted with ethanol. The solution gradually turned green, indicating oxidation of the copper-based catalyst system. The green solution was passed through silica using ethanol and evaporated partially to give an opaque solution. The solution was then dialyzed (MWCO 1 kDa) against Chloroform/Methanol (vol/vol, 3:1) (2 times), methanol (2 times) and water (2 times) for 8-14 hours each dialysis cycle. The polymer was first freeze-dried under vacuum.

The properties of the PMPC-PDPA polymers were as follows. Due to the full conversion of the 1st step, the degree of polymerisation is set to 25 for PMPC. The length of the PDPA is calculated from the signal intensity ratio in the corresponding NMR spectra of the final polymer:

PMPC-PDPA with bicyclic nonyne: NMR (ppm, ¹H, 600 MHz, all broad): 0.89 (CH₃, PMPC+PDPA), 1.00 (CH₃, PDPA), 1.81 (CH₂, PMPC+PDPA), 2.63 (CH, PDPA), 2.98 (CH₂, PDPA), 3.26 (CH₃, PMPC), 3.71 (CH₂, PMPC), 3.84 (CH₂, PDPA), 4.03 (CH₂, PMPC), 4.18 (CH₂, PMPC), 4.26 (CH₂, PMPC). Degrees of Polymerisation: PMPC₂₅-PDPA₆₂, M_n = 20.5 Kg/mol, Dispersity: 1.25

PMPC-PDPA with N₃ moiety: NMR (ppm, ¹H, 600 MHz, all broad): 0.89 (CH₃, PMPC+PDPA), 1.00 (CH₃, PDPA), 1.80 (CH₂, PMPC+PDPA), 2.64 (CH, PDPA), 2.99 (CH₂, PDPA), 3.25 (CH₃, PMPC), 3.70 (CH₂, PMPC), 3.84 (CH₂, PDPA), 4.00 (CH₂, PMPC), 4.16 (CH₂, PMPC), 4.25 (CH₂, PMPC). Degrees of Polymerisation: N₃-PMPC₂₅-PDPA₆₀, M_n = 20.1 Kg/mol, Dispersity: 1.28

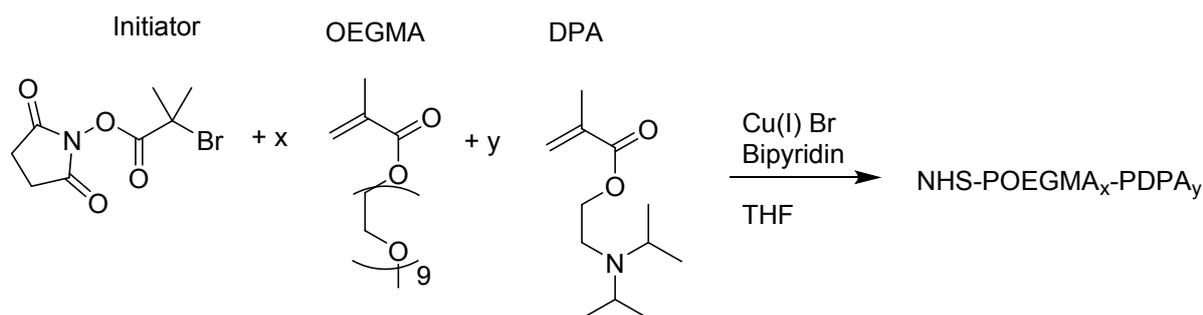
PMPC-PDPA with N-hydroxysuccinimide (NHS ester): NMR (ppm, ¹H, 600 MHz): 0.86 (CH₃, b, PMPC+PDPA), 0.99 (CH₃, b, PDPA), 1.79 (CH₂, b, PMPC+PDPA), 2.61 (CH, b, PDPA), 2.97 (CH₂, b, PDPA), 3.24 (CH₃, b, PMPC), 3.69 (CH₂, b, PMPC), 3.82 (CH₂, b, PDPA), 3.99 (CH₂, b, PMPC), 4.15 (CH₂, b, PMPC), 4.24 (CH₂, b, PMPC). Degrees of Polymerisation: NHS-PMPC₂₅-PDPA₅₈, M_n = 19.7 Kg/mol, Dispersity: 1.26

PMPC-PDPA with sorbic alcohol unit: NMR (ppm, ¹H, 600 MHz): 0.88 (CH₃, b, PMPC+PDPA), 1.00 (CH₃, b, PDPA), 1.80 (CH₂, b, PMPC+PDPA), 2.62 (CH, b, PDPA), 2.98 (CH₂, b, PDPA), 3.25 (CH₃, b, PMPC), 3.69 (CH₂, b, PMPC), 3.83 (CH₂, b, PDPA), 4.00 (CH₂, b, PMPC), 4.16 (CH₂, b, PMPC), 4.24 (CH₂, b, PMPC). 5.55 (CH, m), 5.75 (CH, m), 6.02 (CH, t, *j* = 12 Hz), 6.21 (CH, dd, *j*₁ = 14.9 Hz, *j*₂ = 10.7 Hz) Degrees of Polymerisation: Sorb-PMPC₂₅-PDPA₇₁, M_n = 22.5 Kg/mol Dispersity: 1.22

Synthesis of PDPA-PMPC-SS-PMPC-PDPA: Similar procedure to the one above but since the initiator has two sites, we used a double amount of solvent and all chemicals, e.g. 50 eq. of MPC, 170 eq. of DPA, 4 eq. of bipyridin and 2 eq. of CuBr.

PMPC-PDPA with disulphide bond: NMR (ppm, ^1H , 600 MHz, all broad): 0.85 (CH_3 , PMPC+PDPA), 0.98 (CH_3 , PDPA), 1.78 (CH_2 , PMPC+PDPA), 2.61 (CH , PDPA), 2.95 (CH_2 , PDPA), 3.24 (CH_3 , PMPC), 3.68 (CH_2 , PMPC), 3.81 (CH_2 , PDPA), 3.99 (CH_2 , PMPC), 4.13 (CH_2 , PMPC), 4.23 (CH_2 , PMPC). Degrees of Polymerisation: $\text{PDPA}_{72}\text{-PMPC}_{25}\text{-SS-PMPC}_{25}\text{-PDPA}_{72}$, $M_n = 44.9 \text{ Kg/mol}$, Dispersity: 1.24

2.2. POEGMA-PDPA Polymer

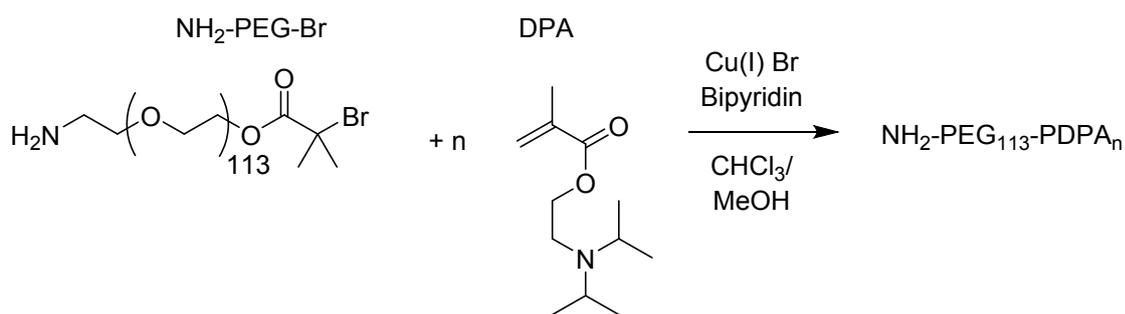


Polymerisation procedure based on previously published procedures:^{1, 2} Briefly, 0.05 mmol of the initiator was dissolved in 1 mL dry THF in a round-bottom flask and 20 eq. of OEGMA were added (495 mg, 1.00 mmol, 20 eq.). The mixture was stirred to be homogeneous and purged with nitrogen for 30 minutes at 30 °C. Then, a mixture of bpy (16 mg, 0.10 mmol, 2 eq.) and Cu(I)Br (7.5 mg, 0.05 mmol, 1 eq.) was added under a constant nitrogen flow. The mixture was stirred for 120 minutes to yield a highly viscous brown substance and sampled for NMR to estimate conversion (gave full conversion, no monomer peaks present). Meanwhile, a solution of DPA (1.06 g, 5.00 mmol, 100 eq.) in 2 mL THF were prepared and purged with nitrogen for 60 minutes in a separate flask. Then, the DPA solution was added to the polymerisation mixture, the reaction mixture was purged for another 10 minutes and then left overnight at 30°C. After 40 h, ^1H NMR analysis confirmed that the conversion was > 99 % and the reaction was opened to the atmosphere and diluted with ethanol. The solution gradually turned green, indicating oxidation of the copper-based catalyst system. The green solution was passed through silica using ethanol and evaporated partially to give an opaque solution. The solution was then dialyzed (MWCO 1 kDa) against Chloroform/Methanol (vol/vol, 3:1) (2 times), methanol (2 times) and water (2 times) for 8-14 hours each dialysis cycle. The polymer was first freeze-dried under vacuum.

POEGMA-PDPA with NHS ester: NMR (ppm, ^1H , 600 MHz): 0.89 (CH_3 , b, POEGMA+PDPA), 0.99 (CH_3 , b, PDPA), 1.80 (CH_2 , b, POEGMA+PDPA), 2.62 (CH , b, PDPA), 2.98 (CH_2 , b, PDPA), 3.38 (CH_3 , b, POEGMA), 3.64 (CH_2 , b, POEGMA), 3.82 (CH_2 , b, PDPA), 4.07 (CH_2 , b, POEGMA).

Degrees of Polymerisation: NHS-POEGMA₂₀-PDPA₁₀₀, $M_n = 31.2 \text{ Kg/mol}$, Dispersity: 1.36

2.3. PEG-PDPA Polymer



Polymerisation procedure based on previously published procedures:^{2, 3} Briefly, $\text{NH}_2\text{-PEG}(113)\text{-Br}$ (110 mg, 0.022 mmol) was dissolved in 1 ml Chloroform/Ethanol (1:1 vol/vol ratio) in a round-bottom flask and 115 eq. of DPA (515 mg, 2.42 mol) were added. The mixture was stirred to be homogeneous and purged with nitrogen for 30 minutes at 30 °C. Then, a mixture of bpy (6.9 mg, 0.046 mmol, 2 eq.) and Cu(I)Br (3.2 mg, 0.023 mmol, 2 eq.) was added under a constant nitrogen flow. The mixture was purged for another 10 minutes and then left overnight at 30°C. After 18 h, ^1H NMR analysis confirmed that the conversion was > 99 % and the reaction was opened to the atmosphere and diluted with ethanol. The solution gradually turned green, indicating oxidation of the copper-based catalyst system. The green solution was passed through silica using ethanol and evaporated partially to give an opaque solution. The solution was then dialyzed (MWCO 1 kDa) against Chloroform/Methanol (vol/vol, 3:1) (2 times), methanol (2 times) and water (2 times) for 8-14 hours each dialysis cycle. The polymer was first freeze-dried under vacuum.

$\text{NH}_2\text{-PEG-PDPA}$ (ppm, ^1H , 600 MHz): 0.85 (CH_3 , PDPA), 0.97 (CH_3 , PDPA)), 1.78 (CH_2 , PDPA)), 2.60 (CH , PDPA)), 2.96 (CH_2 , PDPA), 3.61 (CH_2 , PEG), 3.81 (CH_2 , PDPA). Degrees of Polymerisation: $\text{NH}_2\text{-PEG}_{113}\text{-PDPA}_{165}$, $M_n = 40.1$ kg/mol, Dispersity: 1.33

3. Coupling with NHS Ester on Polymer

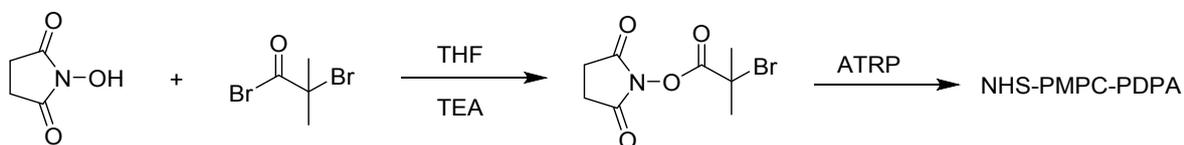


Figure S3: Reaction scheme for the production of NHS-PMPC-PDPA . For actual procedures see experimental part of the main manuscript

The reaction of the NHS-PMPC-PDPA with Cy-3 Amine is monitored with HPLC. Since no change in peak can be observed, we had to conclude that no reaction is occurring.

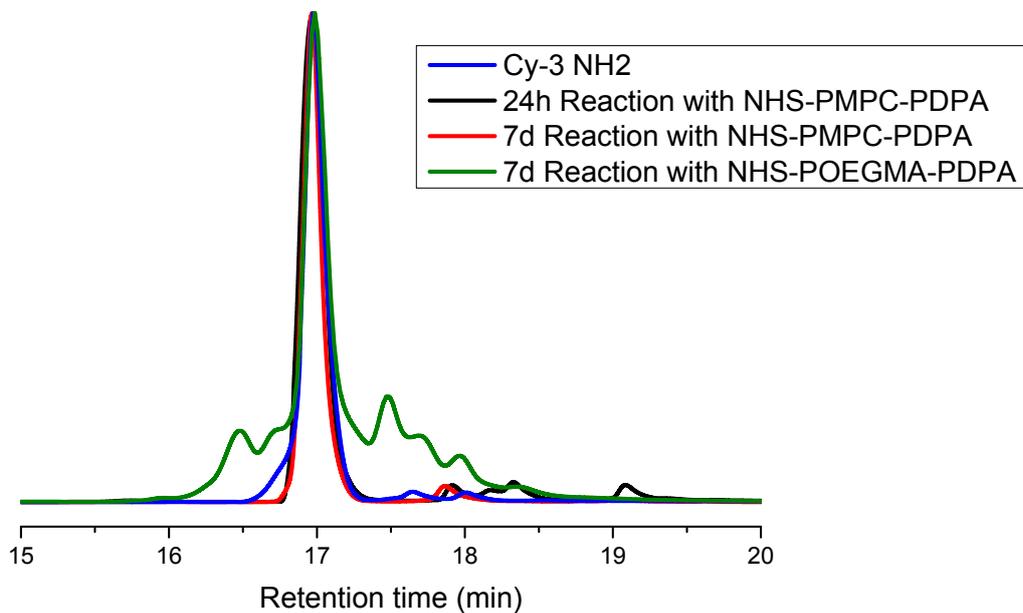


Figure S4: HPLC traces of the Amine-to-NHS Ester modification. The amine is on the dye, NHS Ester on the Polymer.

4. Disulphide-Maleimide coupling

In order to prove that the disulphide bond is cleaved during the reaction, compared the GPC traces before and after the conjugation. As expected, the polymer is shorter after the conjugation than it is beforehand.

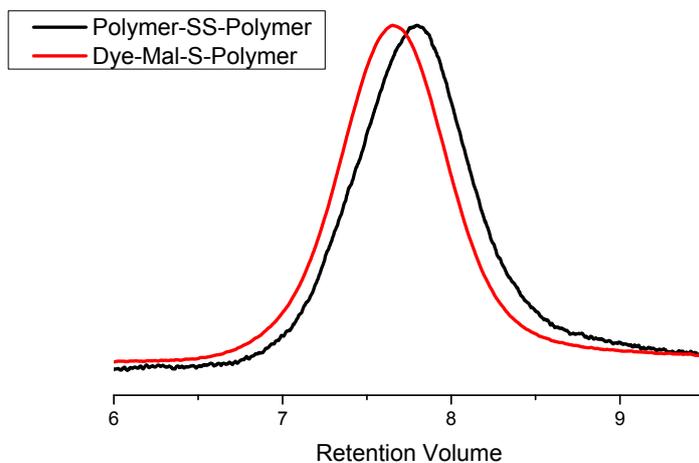


Figure S5: GPC traces before and after disulphide cleavage with consecutive dye conjugation.

5. Synthesis of TAD modified Sudan Red II

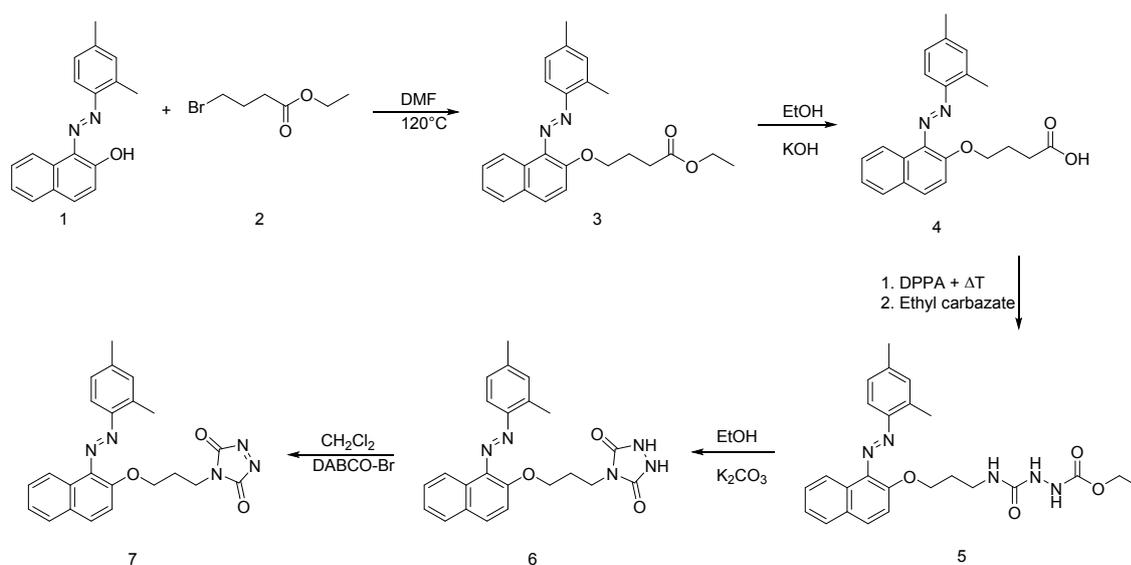


Figure S6: Scheme to synthesise the TAD modified Dye (Figure also in Main Paper, shown here for reasons of being able to follow the procedure).

5.1. Synthesis of the butyrate ester (3) from Sudan II (1)

A mixture of 2.50 g Sudan II (**1**, 9.05 mmol, 1 eq), 1.94 mL ethyl-4-bromobutyrate (**2**, 13.6 mmol, 1.5 eq), 6.75 g potassium carbonate (42.5 mmol, 4.7 eq) and dimethyl sulfoxide (25 mL) was placed under inert atmosphere. The mixture was vigorously stirred for 24 hours at 65 °C and monitored by thin layer chromatography (hexane:ethylacetate 1:1). Water (120 mL) was added, followed by extraction with ethyl acetate (3 × 50 mL). The combined organic layers were washed with brine (1 × 50 mL) and dried with magnesium sulphate. The organic layer was concentrated *in vacuo* to give **3** (3.50 g, 9 mmol, 99 %).

Brutoformula: C₂₄H₂₆N₂O₃. MW.: 390.48 g/mol. ¹H-NMR (300 MHz, DMSO

d₆): (ppm) = 1.13 (t, 3 H, O-CH₂-CH₃), 1.95 (quint, 2 H, CH₂-CH₂-CH₂), 2.37 (s, 3 H, Ar-CH₃), 2.44 (t, 2 H, CH₂-CO), 2.63 (s, 3 H, Ar-CH₃), 4.01 (q, 2 H, O-CH₂-CH₃), 4.21 (t, 2 H, Ar-O-CH₂), 7.17 (d, 1 H, Ar-H), 7.27 (s, 1 H, Ar-H), 7.45 (m, 1 H, Ar-H), 7.54 (d, 1 H, Ar-H), 7.59 (d, 2 H, Ar-H), 7.95 (d, 1 H, Ar-H), 8.00 (d, 1 H, Ar-H), 8.27 (d, 1 H, Ar-H).

5.2. Synthesis of the cleaved ester (4) from the butyrate ester (3)

Hydrolysis of (**3**) to its carboxylic acid (**4**), was done as described in literature (B. Vaisman, A. Shikanov, A. J. Domb *J. Am. Oil. Chem. Soc.* **2008**, *85*, 169–184).

The butyrate ester (**3**, 3.50 g, 9 mmol, 1 eq) was dissolved in ethanol (45 mL), together with potassium hydroxide (6 g, 108 mmol, 12 eq) and placed under an inert atmosphere. 3 mL (166.8 mmol) of water was added and the mixture was stirred at 100 °C for 1 hour. Water (120 mL) was added and the mixture was acidified with concentrated hydrogen chloride to a pH of 2, followed by extraction with ethyl acetate (4 × 150 mL). The combined organic layers were dried with MgSO₄ and concentrated *in vacuo* to give **4** (3.2 g, 8.8 mmol, 98 %).

Brutoformula: C₂₂H₂₂N₂O₃. MW.: 362.43 g/mol. ESI-MS (m/z): 363.0 [MH]⁺,

722.9 [M₂H]⁻. ¹H-NMR (300 MHz, DMSO d₆): (ppm) = 1.93 (quint, 2 H, CH₂-CH₂-CH₂), 2.37 (band, 5 H, Ar-CH₃ + CH₂-CO), 2.63 (s, 3 H, Ar-CH₃), 4.21 (t, 2 H, Ar-O-CH₂), 7.17 (d, 1 H, Ar-H), 7.27 (s, 1 H, Ar-H), 7.45 (m, 1 H, Ar-H), 7.54 (d, 1 H, Ar-H), 7.59 (d, 2 H, Ar-H), 7.95 (d, 1 H, Ar-H), 8.01 (d, 1 H, Ar-H), 8.29 (d, 1 H, Ar-H).

5.3. Synthesis of Sudan-semicarbazide (5).

A solution of 0.929 g **4** (2.56 mmol, 1 eq), 0.55 mL diphenylphosphoryl azide (2.56 mmol, 1 eq) and 0.72 mL triethylamine (5.12 mmol, 2 eq) in 25 mL dry toluene, was placed under an inert atmosphere. The solution was vigorously stirred at room temperature for 1 hour, followed by stirring at reflux for 3 hours. After the addition of ethyl carbazate (0.267 g, 2.56 mmol, 1 eq), the solution was stirred overnight. The solution was concentrated under reduced pressure, followed by chromatography over silica (ethyl acetate:hexane, 25-100% ethyl acetate), to give Sudan-semicarbazide (**5**, 0.859 g, 1.85 mmol, 72 %).

Brutoformula: C₂₅H₂₉N₅O₄. MW.: 463.54 g/mol. ESI-MS (m/z): 464.0 [MH]⁺.

¹H-NMR (300 MHz, DMSO d₆): (ppm) = 1.17 (t, 3 H, O-CH₂-CH₃), 1.85 (quint, 2 H, CH₂-CH₂-CH₂), 2.38 (s, 3 H, Ar-CH₃), 2.64 (s, 3 H, Ar-CH₃), 3.16 (q, 2 H, CH₂-NH), 4.01 (q, 2 H, O-CH₂-CH₃), 4.19 (t, 2 H, Ar-O-CH₂), 6.46 (br. s, 1 H, N-H), 7.17 (d, 1 H, Ar-H), 7.27 (s, 1 H, Ar-H), 7.45 (m, 1 H, Ar-H), 7.54 (d, 1 H, Ar-H), 7.59 (d, 2 H, Ar-H), 7.67 (br. s, 1 H, N-H), 7.95 (d, 1 H, Ar-H), 8.01 (d, 1 H, Ar-H), 8.29 (d, 1 H, Ar-H), 8.73 (br. s, 1 H, N-H).

6. Polymer for TAD modification

6.1. GPC Analysis

In order to prove that the sorbic alcohol unit did not interfere significantly with the polymerisation (as it could have because of the double bonds), the final polymer was also analysed by GPC. Because only a slight tailing towards lower molar masses and together with the NMR data shown in the main paper, we were certain that a pure polymer was formed.

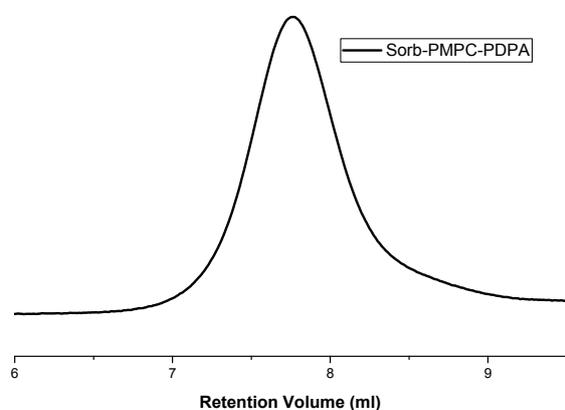


Figure S7: GPC trace of the PMPC-PDPA synthesised from the initiator made from sorbic alcohol.

7. References

1. Lomas, H.; Canton, I.; MacNeil, S.; Du, J.; Armes, S. P.; Ryan, A. J.; Lewis, A. L.; Battaglia, G. *Adv. Mater.* **2007**, *19*, (23), 4238-4246.
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3. Gaitzsch, J.; Appelhans, D.; Wang, L. G.; Battaglia, G.; Voit, B. *Angew. Chem., Int. Ed.* **2012**, *51*, (18), 4448-4451.