Supplementary Material (ESI) for Chemical Communications

Supporting Information

Bioconjugation onto Biological Surfaces with Fluorescently Labeled Polymers

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Experimental section

Materials. Copper(I) bromide (Cu(I)Br, Aldrich, 98 %) was purified according to the method of Keller and Wycoff.[1] *N*-(ethyl)-2-pyridylmethanimine was prepared as described earlier[2] and stored at 0 °C under inert atmosphere. Anhydrous dimethylsulfoxide (DMSO, Aldrich, 99.9 %), triethylamine (TEA, VWR, >99 %), poly(ethylene glycol) methyl ether methacrylate ($PEGMA_{475}$, M_n = 475 g.mol^{-1}, Aldrich), benzylamine (Aldrich), and dichloromethane (Fischer, >99 %) were used as received. Hostasol (Thioxantheno[2,1,9-dej]isochromene-1,3-dione) was supplied by Clariant and hostasol methacrylate monomer (HMA) was synthesised as described elsewhere.[3] *N*-hydroxysuccinimide-2-bromopropionate was synthesised as previously reported.[4] Untreated hairs were kindly supplied by Unilever.

Synthesis of NHS-poly($PEGMA_{475}$-*co*-HMA) copolymers. In a typical polymerisation (copolymer **P1**), a dry Schlenk tube was charged with Cu(I)Br (0.179 g, 1.25 mmol), *N*-succinimidyl 2-bromopropionate (0.31 g, 1.25 mmol), $PEGMA_{475}$ (4.75 g, 10.0 mmol) and HMA (0.08 g, 0.17 mmol, 1.9 mol.% with respect to $PEGMA_{475}$), toluene (15.0 g, 0.16 mol) and a magnetic follower. The mixture was then subjected to five freeze-pump-thaw degassing cycles. Degazed *N*-(ethyl)-2-pyridylmethanimine (0.39 ml, 2.76 mmol) was added and the resulting brown solution was stirred at 50 °C (time zero of the polymerisation) for 48 h. Samples were removed periodically using degassed syringes and quenched in liquid nitrogen for conversion and molecular weight analysis (samples for molecular weight analysis were prepared by passing over an acidic alumina column to remove the copper complexes). The final polymer was passed over acidic alumina column, precipitated in petroleum ether to removed unreacted HMA, dialyzed against slightly acidic water and lyophilized. For copolymers **P2** and **P3**, same experimental procedures were applied except that monomer over initiator

molar ratios were 8.2 and 31.8 respectively. Hostasol methacrylate over PEGMA initial molar ratios were kept constant.

Synthesis of Bn-poly(PEGMA$_{475}$-*co*-HMA) copolymers. In a typical experiment, the NHS-poly(PEGMA$_{475}$-*co*-HMA) copolymer **P2** (0.26 g,18.3 μmol) was reacted at room temperature during 24 hours with benzylamine (0.10 g, 0.93 mmol, 42 molar equivalents with respect to the copolymer) in dichloromethane (50.0 g, 0.59 mol). The reaction was followed by ^{1}H NMR in $CDCl_3$. The obtained copolymer (**P4**) was then recovered by evaporation under reduced pressure and used without further purification. Fluorescence spectroscopy indicated that no change occurred about its excitation and emission spectra.

Conjugation reaction with hair. A typical bioconjugation reaction (Expt. **1**) was the following. In a 100 mL round-bottom flask, the NHS-poly(PEGMA$_{475}$-*co*-HMA) copolymer **P1** were introduced (0.10 g, 8.44 μmol) followed by anhydrous DMSO (67.06 g, 0.86 mol) and TEA (3.48 g, 0.034 mol, ~5 wt.% with respect to the DMSO). After 10 min of stirring, 0.90 g of untreated hairs were introduced. The reaction medium was gently stirred during 70 hours at room temperature. After the reaction, hairs were collected and washed for several days in deionized water at room temperature under gentle stirring (water was changed twice a day). Before further analysis, hairs were dried and stored at room temperature.

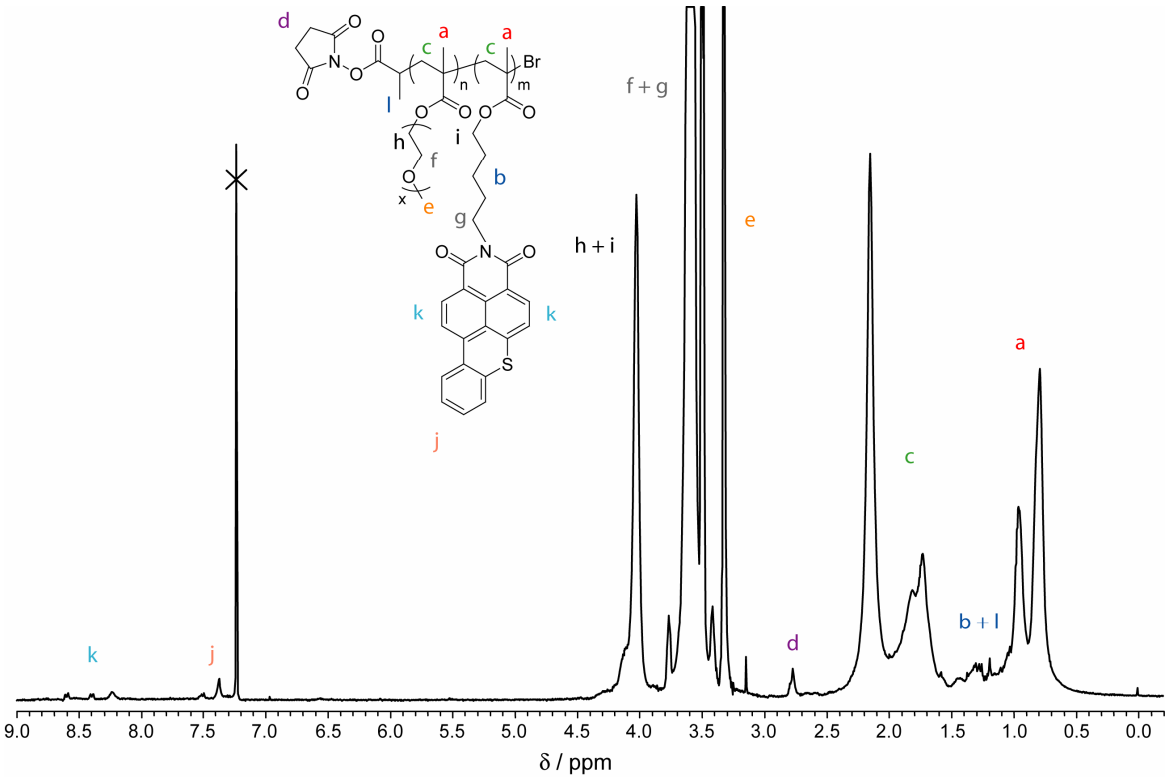
Bleaching treatment of hair. European hair fibers were bleached two times with l'Oréal platifiz precision powder and Oxydant cream (1:1.5) for 30 min, rinsed with water and naturally dried overnight before use.

Standard shampooing procedure. Hair was washed for 1 min in a simple shampoo formulation (Dove Shampoo, Beautifully Clean, 12 oz) and then rinsed under running water for 1 min. The hair switch was allowed to dry naturally.

Analytical techniques. Molar mass distributions were measured using size exclusion chromatography (SEC), on a system equipped with two PL gel 5 μm mixed D-columns (300 × 7.5 mm) and one PL gel 5 mm guard S2 column (50 × 7.5 mm) (Polymer Laboratories) with differential refractive index detection using chloroform/triethylamine 95:5 at 1.0 $mL.min^{-1}$ as the eluent. PMMA standards (1×10^6-200 g mol^{-1}) were used to calibrate the SEC. The analysed samples contained (0.2 vol.%) toluene as the flow marker.

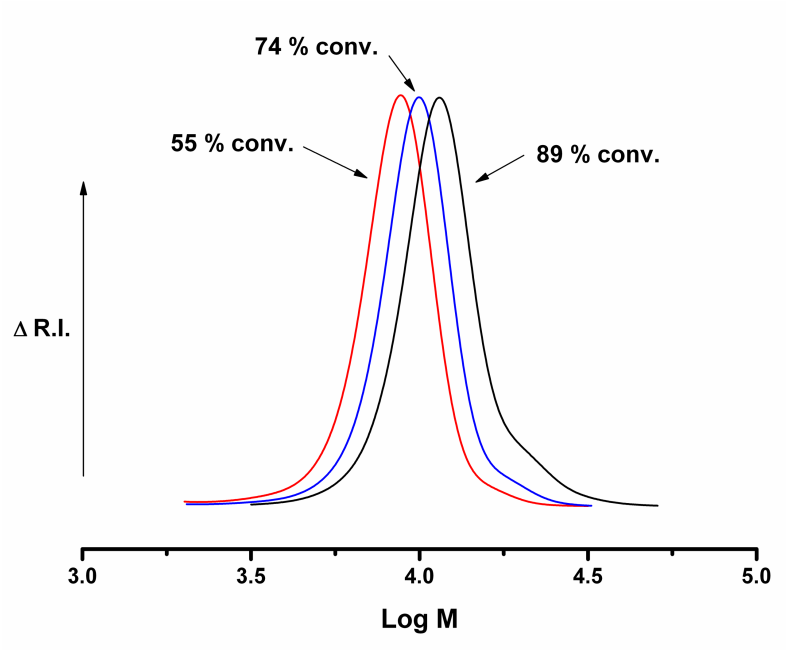
Hairs were observed with a confocal laser scanning microscope (LSM 510, Axioplan 2, Carl Zeiss, Jena, Germany) using an argon laser (λ = 488 nm) in conjunction with a long-pass filter (λ = 505 nm). All CLSM images (512 × 512 pixels, 12 bit pixel depth) were acquired using an objective lens (Zeiss, Epiplan-Neofluar 20×/0.50NA W) with a 10× tube lens. Images were processed using the LSM Image Browser software (Zeiss).

Differential Scanning Calorimetry (DSC) experiments were conducted on a DSC-7 from Perkin Elmer, using pressure resistant (25 bar), stainless steel, large-volume capsules (60 μL) in the temperature range of 120-190 °C (heating rate: 5 $°C.min^{-1}$, sample weight: 6 mg, 30 $mL.min^{-1}$ nitrogen atmosphere). The DSC device was calibrated using indium and palmitic acid, both of high purity. Samples of ~ 2 mm length were weighted and placed into the pan. 50 μl of water was then added and the pan was sealed. Samples were then mixed using a rotary mixer and left overnight to allow the water to equilibrate throughout the samples. For each sample, the helix transition temperature (T_d) was collected and analysed with one-way ANOVA. Each sample was analysed fivefold.

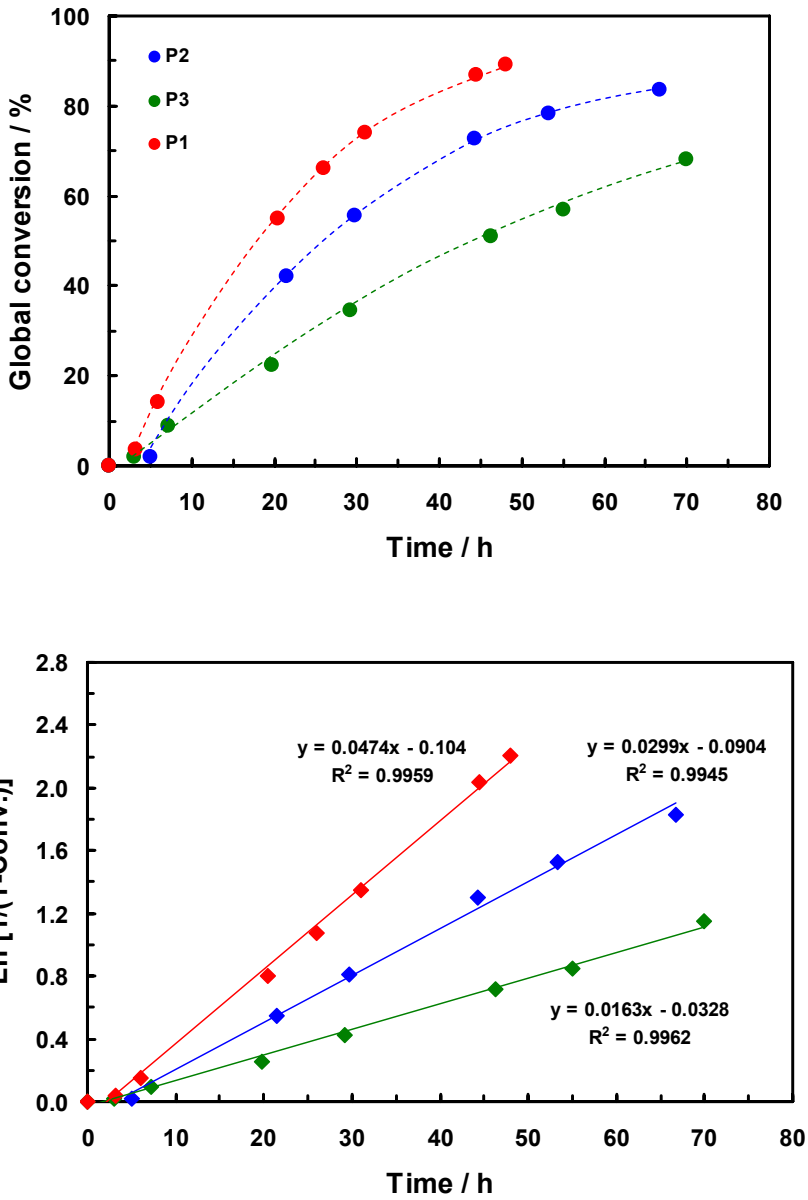


S1: 400 MHz ^{1}H NMR spectrum in $CDCl_3$ of the NHS-poly(PEGMA-*co*-HMA) copolymer **P1**.

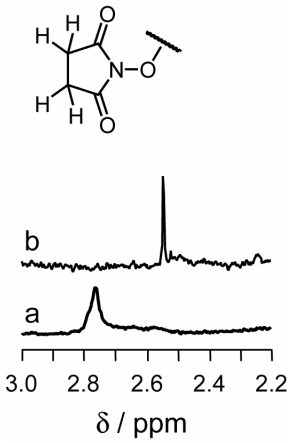
From the initial feed ratio, final copolymer should exhibit 1.9 mol.% of hostasol methacrylate units. Using integrals from the peak "*e*" and from the peaks "*j*+*k*" (Figure S1), ^{1}H NMR gave 1.5, 1.7 and 1.6 mol.% for copolymer P1, P2 and P3 respectively which demonstrated a rather good matching with the initial feed ratio.



S2: Size exclusion chromatograms recorded at various conversions for the synthesis of a NHS-poly(PEGMA$_{475}$-*co*-HMA) copolymer initiated by *N*-hydroxysuccinimide-2-bromopropionate (**P1**).



S3: Living radical copolymerisation of $PEGMA_{475}$ and HMA initiated by *N*-hydroxysuccinimide-2-bromopropionate (**P1–P3**). (Top) global monomer conversion versus time; (bottom) ln[1/(1-conv.)] versus time.



S4: Selected region (3.0–2.2 ppm) of 400 MHz ^{1}H NMR spectra in $CDCl_3$ of the NHS-poly(PEGMA-*co*-HMA) copolymer **P2** (a) and of the reaction medium after amidification with benzylamine in dichloromethane during 24 h (b).

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(3) Limer, A. J.; Rullay, A. K.; San Miguel, V.; Peinado, C.; Kelly, S.; Fitzpatrick, E.; Carrington, S. D.; Brayden, D.; Haddleton, D. M. *React. Funct. Polym*. **2006**, *66*, 51-64.

(4) Lecolley, F.; Tao, L.; Mantovani, G.; Durkin, I.; Lautru, S.; Haddleton, D. M. *Chem. Commun.* **2004**, 2026-2027.