Supplementary Information

Microwave-assisted hydrolysis for the physicochemical characterization of functional methacrylic polymers and their bioconjugates

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Experimental

Lysozyme functionalization with AROMA-1 ATRP initiator. Lysozyme from hen egg white (20 mg, 0.0014 mmol, 1 eq.) was inserted in a glass vial together with 2-methylpyridine borane complex (4.49 mg, 0.042 mmol, 30 eq.) and dissolved in 10 mL of PO^{3-} 0.05 M NaCl 0.1 M buffer. 4-Formylphenyl 2-bromo-2-methylpropanoate (AROMA-1, A1) ATRP initiator was synthesized according to the procedure previously reported.[34,35] 21 µL of AROMA-1 initiator solution (0.0069 mmol, 5 eq.) were added to the reaction mixture withdrawing an aliquot from a previously prepared stock solution in DMSO ([A1] = 90.33 mg/mL). The reaction mixture was stirred at 25°C for 4 h. The final product (LYS-A1) was purified via dialysis against deionized water for 48 h (3.5 kDa MWCO membranes) then freeze-dried.

Synthesis of LYS-PGMA$_m$. 20 mL of PBS 100 mM were inserted in a two-necks round bottom flask, 3 cycles of vacuum/N_2 of 5 min each were performed followed by 15 min of N_2 purge. GMA monomer (14.23 mg, 0.0889 mmol, m eq., where m = 125) was inserted in a two-necks round bottom flask together with LYS-A1 (10.4 mg, 0.000711 mmol, 1 eq.) and 3 vacuum/N_2 cycles of 5 min each were performed. 548 mL of degassed PBS 100 mM were added to the Schlenk vial and the reaction mixture was left under continuous flow of N_2. $CuCl_2$ (0.00717 mg, 0.053 mmol, 0.075 eq) and TPMA (0.1238 mg, 0.0443 mmol, 0.6 eq) were added to the reaction mixture from a stock solution in PBS 100 mM ([$CuCl_2$] = 25 mM, [TPMA] = 200 mM). A stock solution of AA was prepared in a two-necks round bottom flask by firstly introducing dry AA (degassed with 3 vacuum/N_2 cycles), then adding degassed PBS 100 mM ([AA] = 5.28 mg/mL)**.** An aliquot of AA stock solution (0.0976 mg, 0.0554 mmol, 0.779 eq.) was inserted in the reaction flask to start the polymerization. The reaction mixture was stirred at 30°C for 16 h. The resulting product was purified by ultrafiltration with centrifugal filters (Amicon® Ultra 15, 30 kDa MWCO, Merck Scientific) at 2500 RCF for 10 min, washing four times with 1 mL of PBS and lyophilized. Yield 70–80%.

Results and discussion

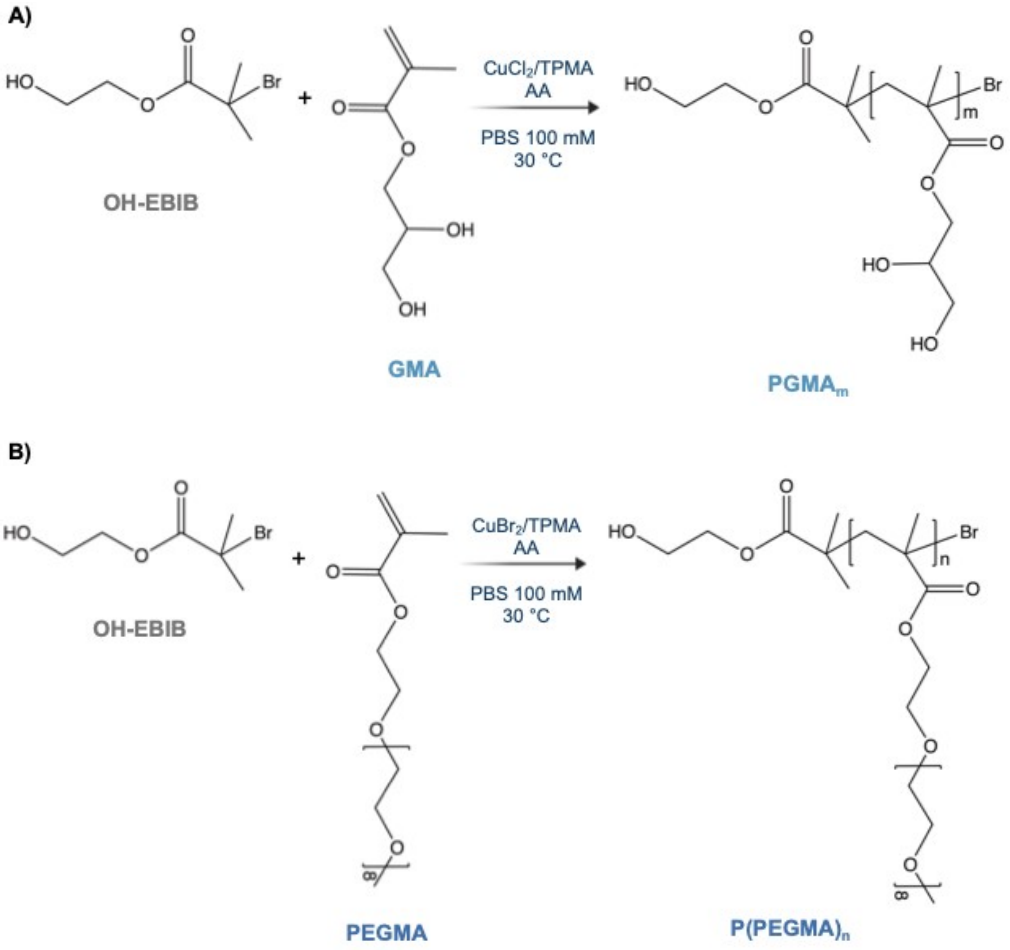

Figure S1. Synthetic pathway followed for the polymerization of: **A)** $PGMA_m$ **B)** $P(PEGMA)_n$ from commercial initiator via AGET ATRP.

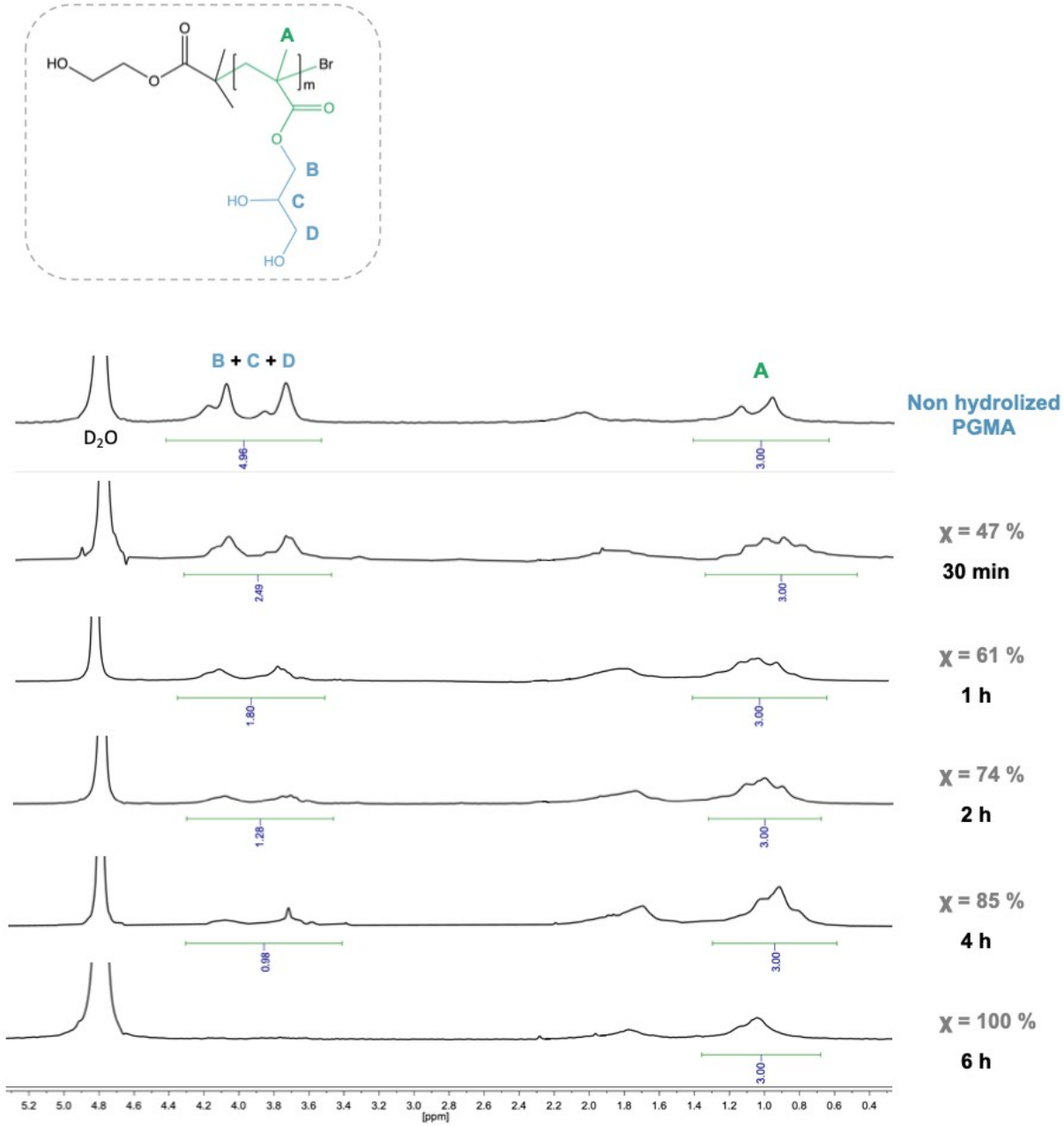

Figure S2. Sequence of ^{1}H-NMR spectra recorded before the hydrolysis of PGMA, using an oil bath-based heating system, and after various reaction time points.

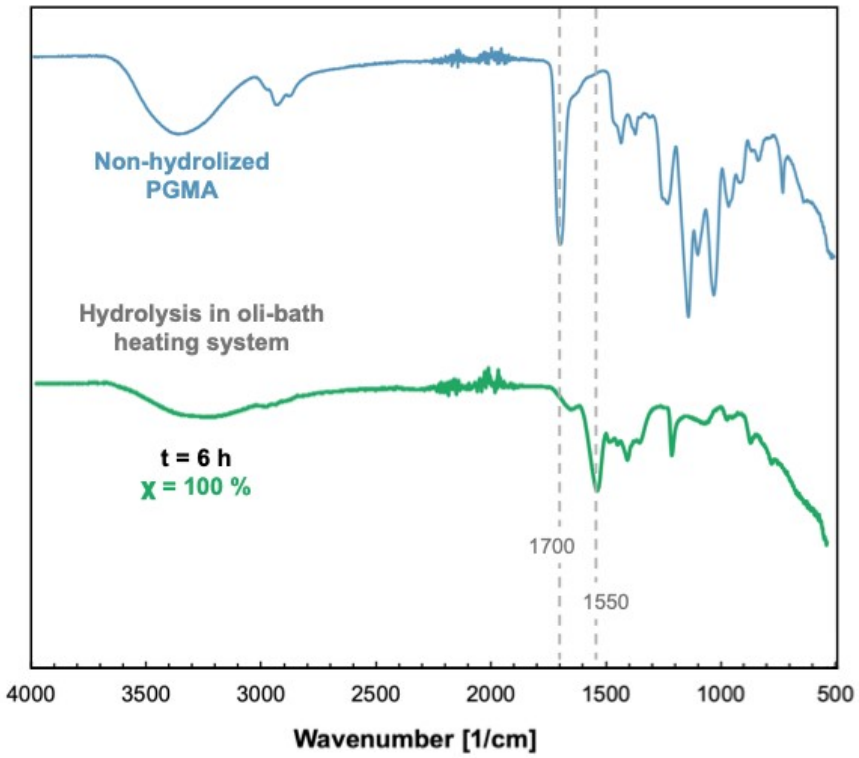

Figure S3. FTIR spectra recorded before PGMA hydrolysis (light blue) and after 6 h-hydrolysis leading 100% of conversion employing an oil-bath based heating system.

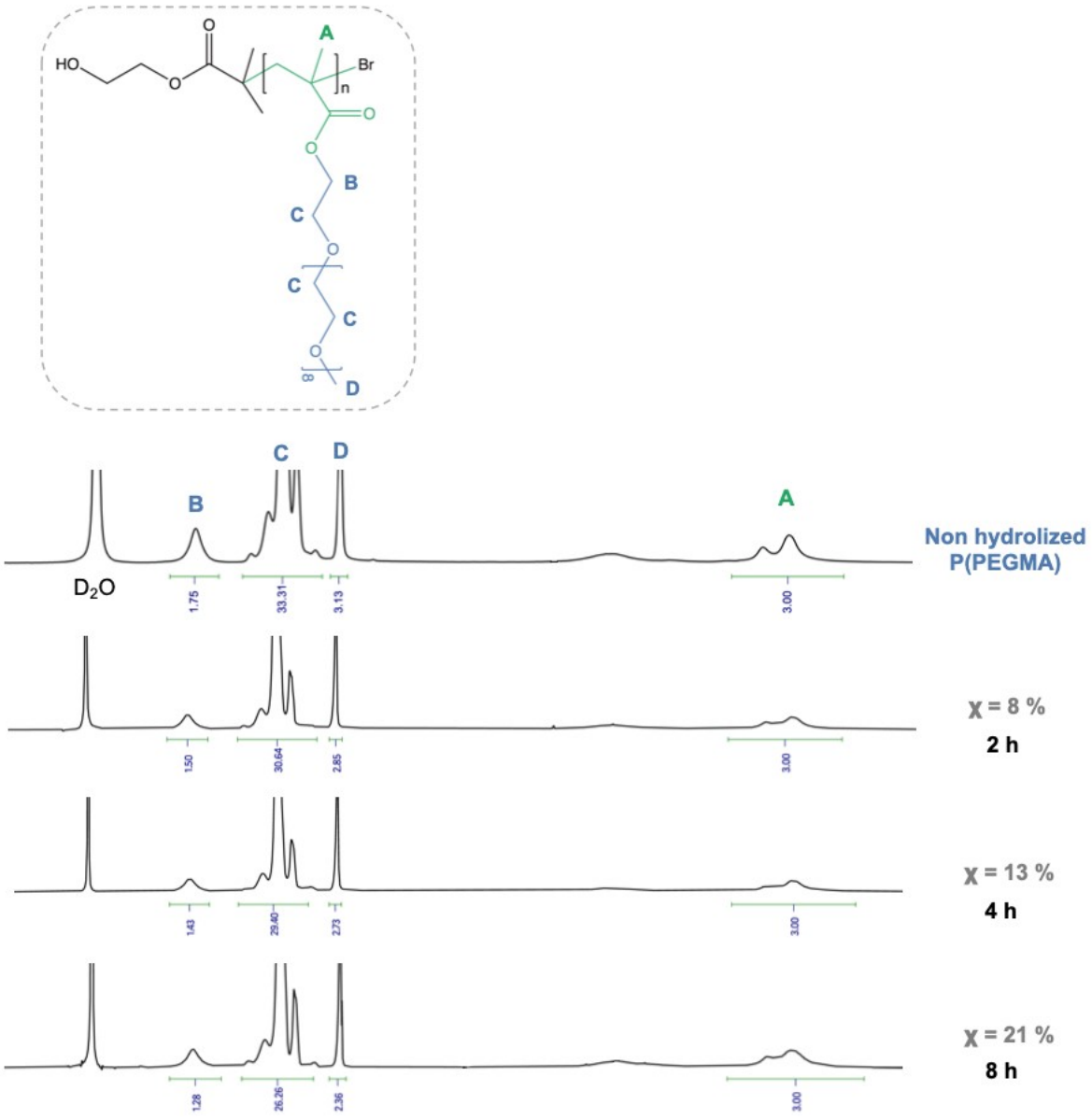

Figure S4. Sequence of ^{1}H-NMR spectra recorded before the hydrolysis of P(PEGMA), using an oil bath-based heating system, and after various reaction time points.