Electronic Supplementary Information

Graft modification of cold water-soluble starch via nitroxide-mediated polymerisation

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^{1}H NMR Preparation and Measurement

Proton nuclear magnetic resonance (^{1}H NMR) measurements were performed according to the methods of Tizzotti et al.[1] with slight modifications. A 0.5 wt% solution of dry LiBr in deuterated dimethyl sulfoxide (DMSO-d_6 solution) was made by quickly weighing LiBr and adding DMSO-d_6 to prevent water absorption, followed by ultrasonication for 2 min at 5 W power output. The solution vial was sonicated in a water bath to prevent excessive temperature increase. The DMSO-d_6 solution was stored sealed in a desiccator over Drierite until use. For ^{1}H NMR measurement, raw cold water-soluble starch (CWS) samples were weighed (~10 mg mL^{-1}), sealed, and allowed to dissolve in the DMSO-d_6 solution for 24 hours on an IKA KS 130 basic orbital shaker (orbital shaker). Modified CWS samples were weighed wet (15-25 mg mL^{-1}), sealed, and dissolved in the DMSO-d_6 solution for 24 hours on the orbital shaker.

Following the method of Tizzotti et al.,[1] the optimal amount of trifluoracetic acid (TFA) added to the native and 4-vinylbenzyl chloride (VBC) modified CWS was determined for ^{1}H NMR, as seen in Figure S1. It was desired to shift the TFA peak to >9.0 ppm to accommodate aromatic substituents and obtain a flat baseline. The optimal dosage was found to be 25 µL, as seen in Table S1. The TFA was added immediately before ^{1}H NMR measurement to prevent potential sample decomposition.

Once 25 µL had been identified as the optimal amount of TFA, a CWS sample was prepared in DMSO-d_6 solution, 25 µL TFA was added, and the sample was analysed by ^{1}H NMR at 25 °C and then from 30 °C to 90 °C at 10 °C increments. The temperature sensitivity of the starch was due to the high molecular weight of the chains and hydrogen bonding interactions. High temperature and LiBr in the DMSO-d_6, which acted as a chaotropic agent,[2] helped to disrupt the inter-chain hydrogen bonding. The α(1-4) and α(1-6) linkage protons and reducing end signals (integral 4.10 ppm to 5.70 ppm) were compared to the residual dimethyl sulfoxide (DMSO) signal (integral 2.30 ppm to 2.70 ppm) until the ratio became stable. At that temperature, the CWS was fully solubilised and further increasing the temperature did not increase the relative CWS signal intensity. The ^{1}H NMR spectra are given in Figure S2 and the corresponding integral ratios are shown in Figure S3. The integral ratio was found to stabilise at 60 °C which was determined to

be the optimal temperature for NMR analysis. Thus, all NMR experiments were run at 60 °C to give the mildest possible measurement conditions while ensuring quantitative results could be obtained.

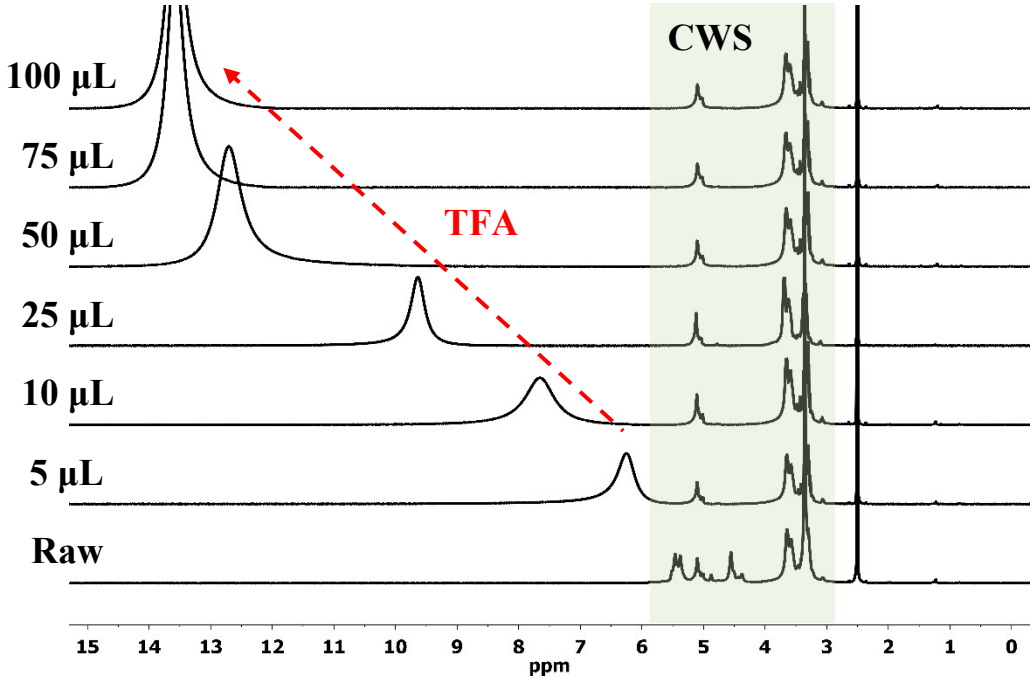

Figure S1: ^{1}H NMR spectra for native CWS with 5 µL, 10 µL, 25 µL, 50 µL, 75 µL, and 100 µL TFA in DMSO-d_6 at 25 °C.

Table S1: Shift of trifluoroacetic acid (TFA) signal in ^{1}H NMR with TFA volume, in DMSO-d_6 at 30 °C.

TFA Volume (µL)	5	10	25	50	75	100
TFA Shift (ppm)	6.25	7.65	9.68	12.70	13.58	14.18

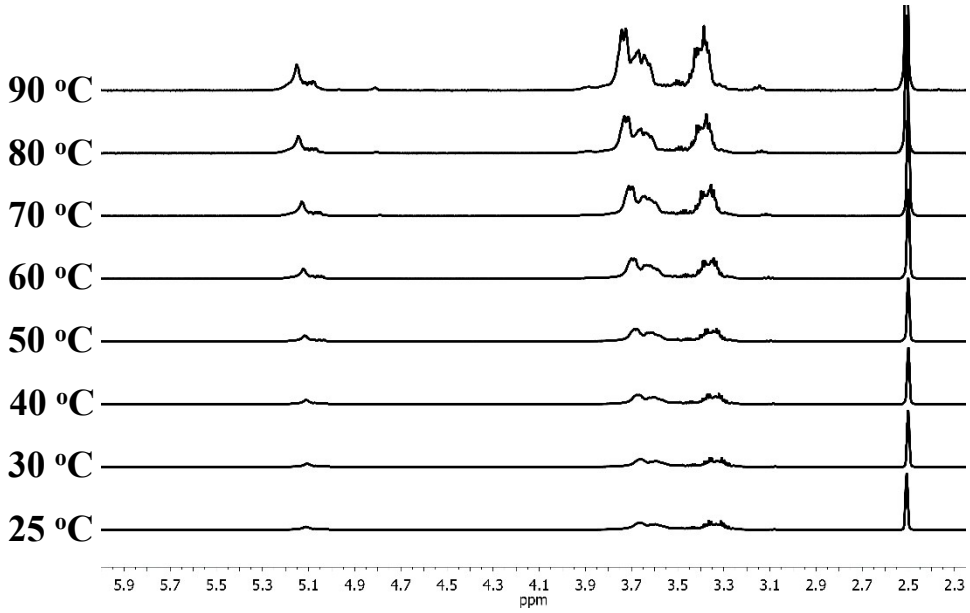

Figure S2: ^{1}H NMR spectra for native CWS with 25 µL TFA at 25 °C, 30 °C, 40 °C, 50 °C, 60 °C, 70 °C, 80 °C, and 90 °C in DMSO-d_6.

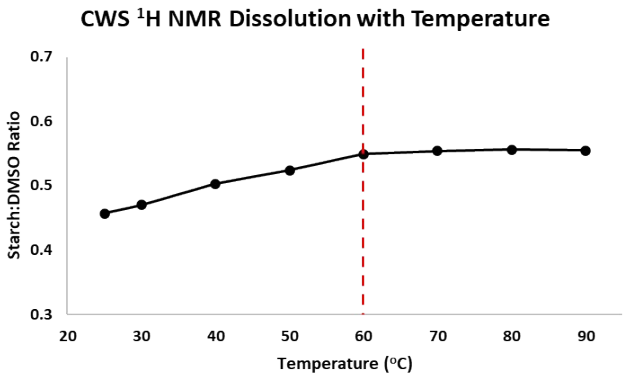

Figure S3: CWS ^{1}H NMR dissolution integral ratio (starch:DMSO) with temperature in DMSO-d_6.

^{1}H NMR Analysis

The addition of 25 µL TFA to the CWS samples was effective in shifting the hydroxyl peaks of the starch backbone allowing the effective integration of the α-(1-4) and α(1-6) anomeric protons (Fig. S4). A concern in adding TFA to the starch samples was the acid hydrolysis of the starch backbone which may alter the integration signals in determining the degree of substitution (DS). Tizzotti et al. (2011) found the method could induce acid hydrolysis to a small but negligible extent. In this work, an integration procedure was used by which the area from 4.1 ppm to 5.7 ppm was integrated for the starch. Any acid hydrolysis and production of short starch chains would create reducing end protons with shifts from 4.5 ppm to 4.7 ppm,[3] or at 4.91 ppm and 4.28 ppm, respectively,[1] which would be accounted for by integrating this region. The α(1-4) proton shift occurred at 5.12 ppm and the α(1-6) proton shift occurred at 4.78 ppm (Fig. S4). The remainder of the CWS backbone protons showed resonances from 3.0 to 4.0 ppm. For native starch, the addition of 25 µL TFA gave no visible appearance of small signals between 4.5 ppm and 4.7 ppm, or at 4.91 ppm and 4.28 ppm, indicating that acid hydrolysis had not formed reducing ends to a large extent and validating the use of the method.

Using this method, the degree of branching (DB) was obtained according to equation S1. where DB is a percentage, and $I_{1\text{-}4}$ and $I_{1\text{-}6}$ are the ^{1}H NMR signals of the α(1-4) and the α(1-6) protons, respectively.

$$DB = 100\left(\frac{I_{1-6}}{I_{1-4} + I_{1-6}}\right) \quad \text{(S1)}$$

Using Equation S1 the DB was calculated to be 4.61 ± 0.26% (n = 3). Comparing the DB and the ratio of α(1-4) to α(1-6) bonds ($I_{1\text{-}4}$:$I_{1\text{-}6}$), which was found to be 20.83, to waxy maize amylopectin standards with a $I_{1\text{-}4}$:$I_{1\text{-}6}$ of 19-21[4,5] showed that the CWS sample was almost purely amylopectin. At the same time, considering the α(1-6) bonding fraction of 0.046, the primary hydroxyl group content was found to be 5.88 ± 0.02 mmol g^{-1} (n = 3).

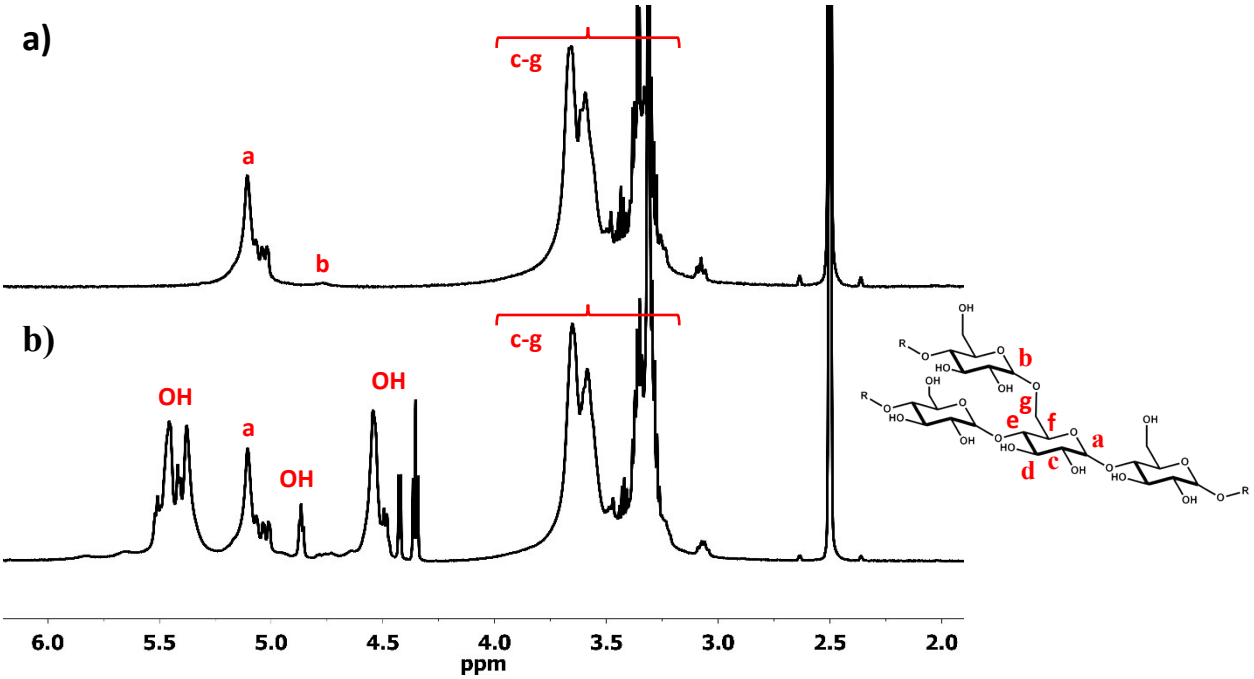

Figure S4: ^{1}H NMR spectra of CWS in DMSO-d_6 at 60 °C a) without the addition of TFA; b) with the addition of TFA.

^{1}H NMR measurement at 60 °C and with 25 µL TFA allowed the accurate calculation of the degree of substitution for the VBC functionalised CWS (VB-CWS). The DS was calculated according to equation S2 where $I_{7.215\text{-}7.530}$ was the integral of the VB-CWS aromatic proton signals and $I_{4.100\text{-}5.700}$ was the integral of the starch linkage protons, potential reducing end protons, one VB vinyl proton, and the VB methylene protons. The VB vinyl proton and methylene protons were subtracted out of the integral ($I_{7.215\text{-}7.530}/2$ and $I_{6.685\text{-}6.780}$). The entire value was divided by four to account for the four aromatic protons present in the VB-CWS. For one of the VB-CWS spectra, the values were $I_{7.215\text{-}7.530}$ = 0.217, $I_{4.100\text{-}5.700}$ = 1.000, and $I_{6.685\text{-}6.780}$ = 0.010. Note that the vinyl protons were not in direct proportion to the aromatic protons because their intensity diminished at high temperature. The calculation was performed as shown below, giving a DS = 0.062.

$$DS = \frac{1}{4}\left(\frac{I_{(7.215-7.530)}}{I_{(4.100-5.700)} - \frac{I_{(7.215-7.530)}}{2} - I_{(6.685-6.780)}}\right) \quad \text{(S2)}$$

$$DS = \frac{1}{4}\left(\frac{0.217}{1.000 - \frac{0.217}{2} - 0.010}\right)$$

$$DS = 0.062$$

Acid Hydrolysis of Grafted CWS

FTIR measurements were made on the methyl methacrylate-*co*-styrene grafted CWS (CWS-*g*-P(MMA-*co*-STY)) before and after acid hydrolysis. The presence of decreased intensity residual starch signals (Figure S5) suggested that the acid hydrolysis and purification was mostly successful in removing the CWS from

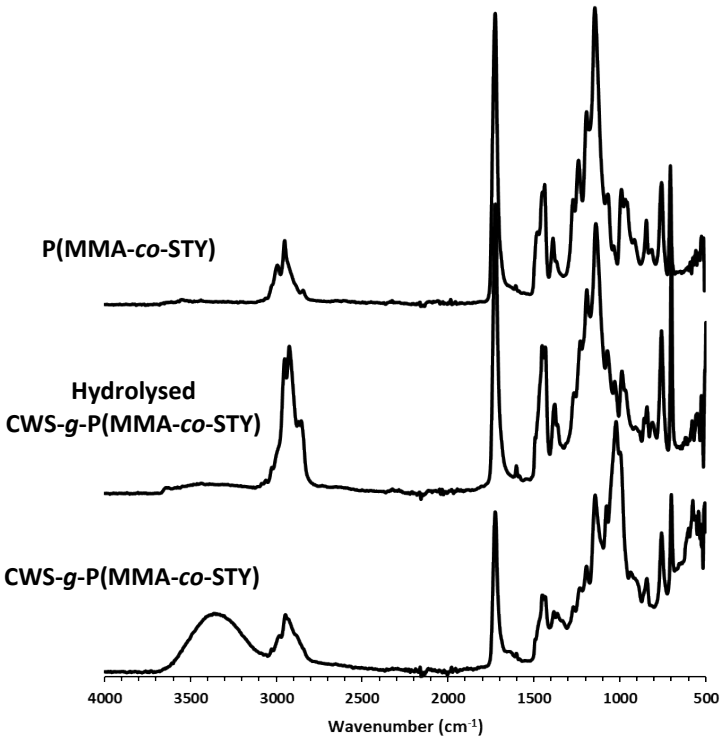

Figure S5: FTIR spectra of CWS-*g*-P(MMA-*co*-STY) before and after acid hydrolysis and compared to a P(MMA-*co*-STY) copolymer spectrum.

the grafted polymer. The spectra showed decreased intensity of the starch C-O-C ring bonds at 1073 cm^{-1} and 929 cm^{-1}, as well as showing the same bonding signals as the pure P(MMA-*co*-STY) spectrum.

P(MMA-*co*-STY) Synthesis and Acid Hydrolysis Validation

P(MMA-co-STY) was synthesised via nitroxide mediated polymerisation (NMP) in bulk. 19 mL of methyl methacrylate (MMA) and styrene (STY) with 9 mol% STY was added to a 50 mL three neck round bottom flask. BlocBuilder® MA (BB-MA) was added such that the ratio of monomer:BB-MA was 200:1. In accordance with previous methods on the NMP of methacrylates, 9 mol% free SG1 based on the BB-MA was added to the reaction to aid in the overall control.[6] The flask was purged with nitrogen for 30 min with magnetic stirring and a reflux condenser. The reactor was then immersed in an oil bath at 90 ^{o}C for two hours under continued nitrogen purging. After two hours the reactor was removed and the reaction

was quenched in an ice bath. Samples were withdrawn at periodic time intervals for conversion and GPC analysis. The copolymer was concentrated in a Rotovap Vacuum for 1 hour at spinning rates of 90 rpm to 160 rpm. The polymer was then dried under vacuum at 85 ^{o}C for 120 hours to remove any residual monomer.

The P(MMA-*co*-STY) was characterised and subjected to the same acid hydrolysis procedure as the grafted CWS to verify there were no changes in the polymer composition or average molecular weight. Figure S6 shows the FTIR spectra of the before and after hydrolysis with strong bands visible for: C-H stretching vibrations (ν, C-H) from 2800 cm^{-1} to 3080 cm^{-1}, CH/CH_2 bending vibrations from 1320 cm^{-1} to 1520 cm^{-1}, and C-O stretching vibrations at 1165 cm^{-1} and 1258 cm^{-1}. The spectra also showed a strong band at 1725 cm^{-1} due to the C=O methacrylic units in the polymer backbone. There was no observed change in the spectra before and after hydrolysis. No new bonding peaks appeared suggesting that there was no effect on the functional groups of the polymers. This included no hydrolysis of the methacrylic groups in the

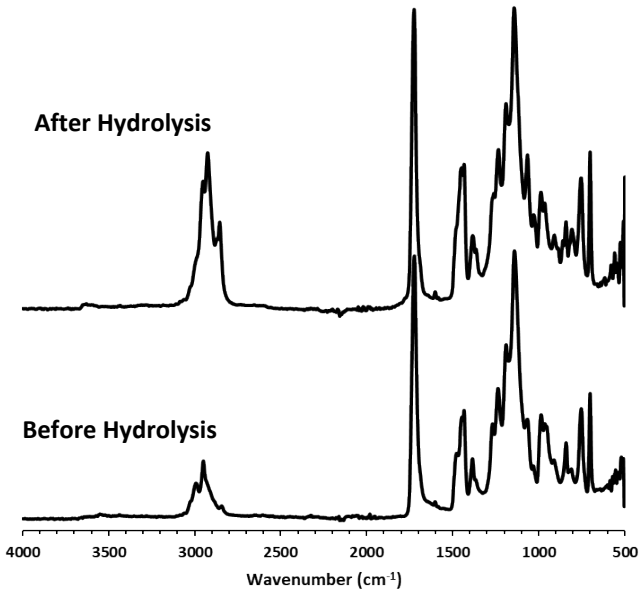

Figure S6: FTIR spectra of P(MMA-*co*-STY) before and after acid hydrolysis.

P(MMA-*co*-STY) to the corresponding carboxylic groups.

^{1}H NMR measurements of the pure and hydrolysed materials showed no significant changes in polymer structure as well. The P(MMA-*co*-STY) (Figure S7) in deuterated chloroform ($CDCl_3$) showed impurity signals at 5.00 ppm, 3.74 ppm, 1.42 ppm, and 0.06 ppm after hydrolysis. These peaks were sharp and well defined compared to the broad polymer resonances. As with the grafted CWS copolymers, the ^{1}H NMR spectra of P(MMA-*co*-STY) showed strong signals for the STY aromatic groups (*m*, 6.75 ppm to 7.65 ppm), MMA methyl groups (*m*, 3.59 ppm), STY backbone methylene groups (*m*, 2.94 ppm), MMA backbone methylene groups (*m*, 1.76 ppm) and MMA backbone methyl groups (*m*, 0.40 ppm to 1.52 ppm).

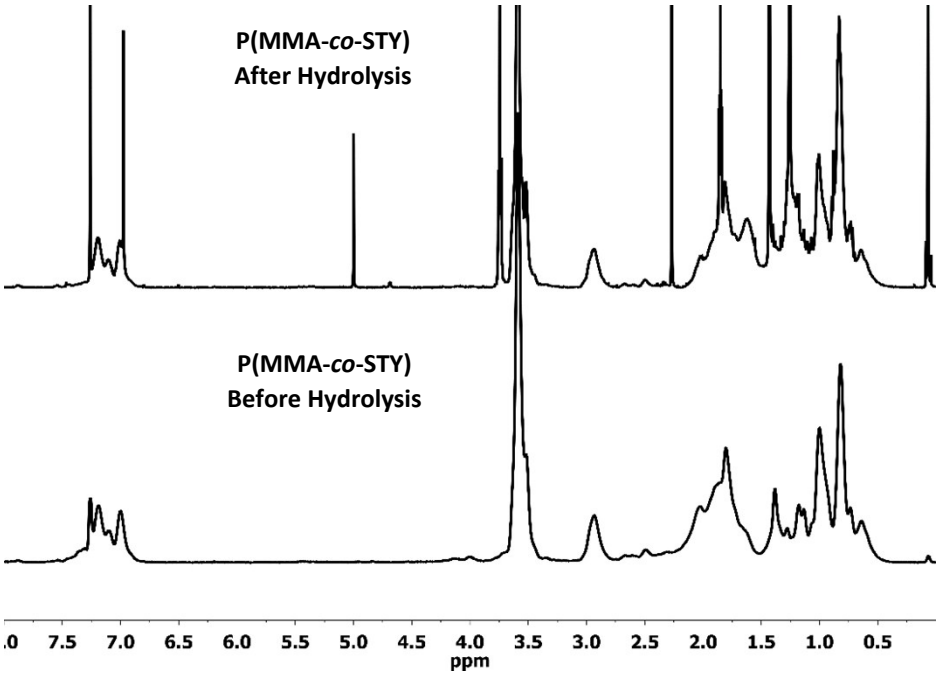

Figure S7: ^{1}H NMR spectra of P(MMA-co-STY) copolymer in $CDCl_3$ at 30 ^{o}C before and after acid hydrolysis.

GPC analysis of the P(MMA-*co*-STY) before and after hydrolysis showed that there were small changes in molecular weight but no significant difference in the traces (Figure S10). When the traces were analysed for M_n, a small shift in the average M_n to higher molecular weight was observed after acid hydrolysis. It was expected that the hydrolysis would cause a decrease in M_n due to the breaking of large chains or dissociation of the SG1 at the high hydrolysis temperatures. The GPC traces have similar populations of eluting chains at all molecular weights, so the increase in M_n was most likely due to subtle variations in the averaging of the elution profile. It was also possible that radicals would form with the harsh hydrolysis conditions. The high temperature could cause the dissociation of the SG1 end cap on the polymer chains which could lead to inter-chain radical coupling. This, however, should lead to populations at a much higher molecular weight of approximately 2x M_n (faster elution), which was not observed. The Đ of the polymer chains before and after was almost identical, suggesting that the increase in M_n was not due to radical coupling which would significantly increase the value. This also suggested that overall, the hydrolysis had very little effect on the polymer chains.

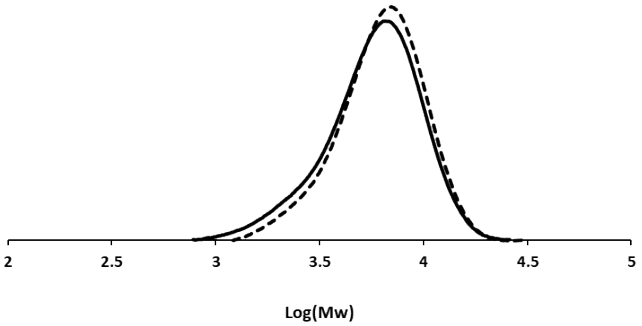

Figure S8: GPC traces of P(MMA-co-STY) before (solid) and after (dashed) acid hydrolysis.

Table S2: GPC analysis (M_n and Đ) of P(MMA-co-STY) before and after acid hydrolysis.

Polymer	Conv. (%)	M_n Theo. (g mol^{-1})	Initiation Efficiency (%)	Before Hydrolysis M_n (g mol^{-1})	Đ	After Hydrolysis M_n (g mol^{-1})	Đ
P(MMA-*co*-STY)	43	4340	97	4460	1.24	4910	1.25

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