Biotin Functionalized Poly(sulfonic acid)s for Bioconjugation: *In Situ* Binding Monitoring by QCM-D

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ABSTRACT: We describe the synthesis of biotin end functionalized poly(sulfonic acid)s via living radical polymerization (LRP) for conjugation to Avidin. Quartz crystal microbalance (QCM-D) and competitive binding studies were used to confirm this conjugation. A biotin initiator for copper-mediated LRP was used to provide acrylamide and methacrylate based polymers with the functional end group. This investigation revealed that 2-acrylamido-2-methyl-1-propanesulfonic acid was not a suitable monomer in its acid form but was successfully used in its sodium salt form. A second monomer, 3-sulfopropylmethacrylate as the potassium salt was also studied and both monomers produced polymers with polydispersities <1.3 and 1.4, respectively. Evolution of molecular weight with respect to time indicated that the polymerization of the acrylamide polymer is controlled. Quartz crystal microbalance with dissipation monitoring was used to confirm that the biotinylated polymers were able to bind to Avidin *in situ*. The gold surface of a quartz crystal was chemically modified resulting in a stable monolayer of Avidin; the biotinylated polymers were passed over the functionalized surface and their grafting ability was examined. A competitive binding evaluation was undertaken with 2-(4-hydroxyphenylazo)benzoic acid (HABA) dye to provide visual verification of conjugation. © 2011 Wiley Periodicals, Inc. J Polym Sci Part A: Polym Chem 49: 1163–1173, 2011

KEYWORDS: biotin–avidin; heteroatom-containing polymers; living polymerization; water-soluble polymers

INTRODUCTION Molecular recognition is of major importance in that it plays a part in many biological interactions. The avidin–biotin interaction is an excellent model for studying biorecognition due to the ultra-high binding affinity between them. Avidin is a homotetrameric protein composed of four identical sub-units, each of these units can bind with one biotin molecule; this interaction is considered to be one of the strongest non-covalent biological interactions known.[1] Bioconjugation of functional polymers to proteins results in bioconjugates with new properties, which can provide potential benefits. A number of reviews discuss the advantages of increased biological activity and stability; both of importance for therapeutic applications.[2–11] The biotin–avidin interaction is of high interest in biological applications and hence the ability to incorporate biotin into polymers is of use.

Biotin has been incorporated into polymers in a number of ways. Polymers made via atom transfer radical polymerization, LRP, have incorporated biotin by both an initiator and via post polymerization modification.[12,13] Biotinylated polymers have also been synthesized via reversible addition-fragmentation chain transfer, RAFT, with a biotinylated RAFT chain transfer agent or post polymerization modification.[14–16] Post polymerization modification with commercially available polyester (PLGA) polymers, from the McCullough Grignard metathesis reaction and chemical vapor deposition polymerization using Huisgen click chemistry for the modification has been reported.[17–19]

LRP of methacrylate and acrylamide based monomers containing sulfonic acid salts are reported in this article with biotin incorporated into the initiator. The use of copper(0) as a source of copper as opposed to copper(I) salts has emerged as en effective system for the polymerization of vinyl monomers in polar solvents such as water.[20,21] Sulfonic acids are of interest since they have been shown to form excellent hydrophilic polymers with no toxicity and to interact with fibroblast growth factor (FGF), thus inhibiting the intracellular signaling.[22,23] In terms of monomer design, acrylamide monomers are useful in a biological sense due to their thermoresponsive properties however it is well reported in literature that their polymerization via ATRP can be challenging.[24] Sulfonic acid containing monomers are generally polymerized by free radical polymerizations.[22,23,25–28] In addition, sulfonic acids can be introduced via post polymerization modification.[29,30] The quartz crystal microbalance (QCM) technique measures a mass per unit area by measuring the change in the quartz crystal resonator. The resonance is disturbed by the addition or removal of a small mass due to oxide growth/decay or film deposition at the

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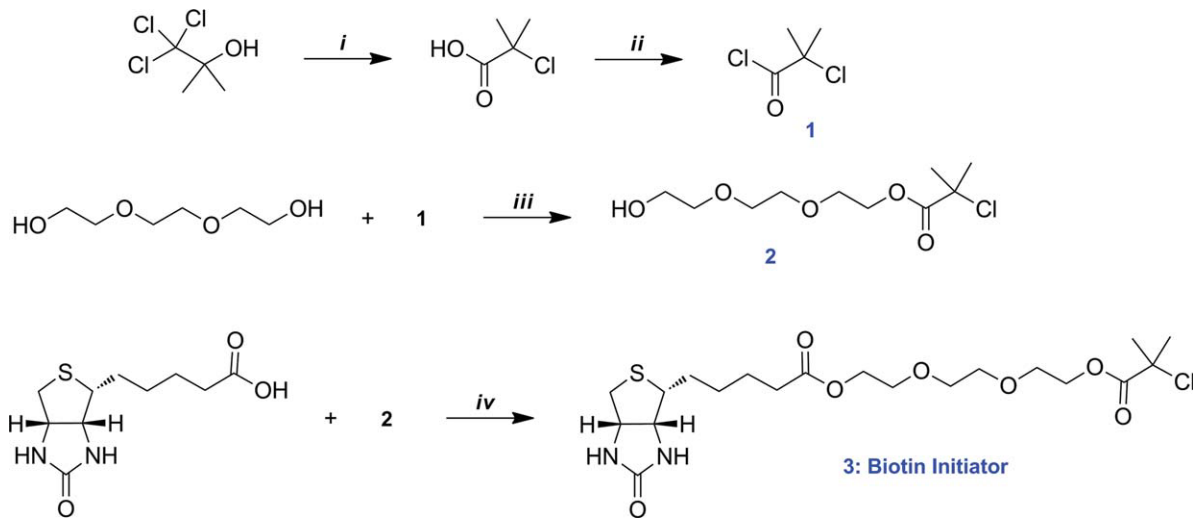


SCHEME 1 Synthesis of biotin initiator: (i) Kundiger method[32]: H_2SO_4, from 0 °C to 25 °C; (ii) $SOCl_2$, from 0 °C to 25 °C; (iii) TEA, THF, from 0 °C to 20 °C; (iv) Yamaguchi procedure: TEA, DMF, from 0 °C to 20 °C. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

surface of the acoustic resonator. The dissipation quantifies the damping in the system, and gives information on the sample's viscoelastic properties.

RESULTS AND DISCUSSION

Previous studies have demonstrated that biotinylated dendrons can be synthesized for bioconjugation to avidin.[31] Biotin was modified into a non-symmetrical initiator, which was transformed via a Michael addition with methyl acrylate and amidation with an excess of ethylenediamine to double the number of surface amine groups. This was repeated until the required dendron size was reached. This present study reports the modification of biotin to an initiator suitable for living radical polymerization (LRP) to polymerize sulfonic acid monomers to polymers with a comb-like architecture. After synthesis, the polymers biological activity was investigated using quartz crystal microbalance with dissipation monitoring, QCM-D.

Initiator Synthesis

The polymerization initiator was synthesized via modification of the valeric acid side chain of biotin via a Yamaguchi esterification reaction, Scheme 1. Standard DCC coupling provided low yielding products and EDCI coupling gave higher yields but also resulted in halogen exchange taking place.

The esterification method has been used by Wullschleger et al. for the synthesis of an *E*-configured macrolactone cyclization product whereas Yamaguchi et al. developed the procedure for the synthesis of large-ring lactones.[33,34] The reaction proceeds via the formation of biotin-2,4,6-trichlorobenzoic mixed anhydride, followed by the nucleophilic attack of the alcohol, bearing the initiator function, to the biotin carboxylic group. Maynard et al. have shown the synthesis of biotin initiators via an amide linkage for increased hydrolytic stability; however, in this report, the stability of the ester link was sufficient over the duration of the reactions.[13,15]

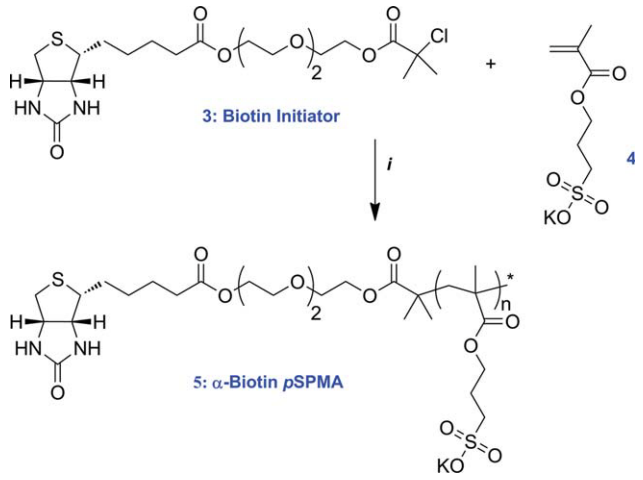
Polymer Synthesis

α-Biotin Poly(3-sulfopropylmethcrylate, potassium), α-Biotin pSPMA

α-Biotin *p*SPMA **(5)**, Scheme 2, was synthesized via LRP using 3-sulfopropylmethacrylate, potassium. Polymerization was carried out in a H_2O/MeOH/DMSO solvent mixture at 40 °C to ensure complete solubilization of all reagents. After 19 h, a conversion of 77% was reached and the polymerization was stopped. Purification by precipitation and dialysis led to a polymer with M_n = 22,000 g/mol (Table 1).

α-Biotin Poly(2-acrylamido-2-methyl-1-propanesulfonic acid, sodium), α-Biotin pAAMPSA

Following on from this synthesis, acrylamide monomers were investigated; some containing protected sulfonic



SCHEME 2 (i) Polymerization of α-biotin poly(3-sulfopropylmethcrylate, potassium) by LRP: *N*-(*n*-ethyl)-2-pyridylmethanimine, CuBr, $CuBr_2$, H_2O/MeOH/DMSO, 40 °C. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

TABLE 1 Data for the Polymers Synthesized

Biotin Polymer	Conv. (%)	DP	$M_{n(theory)}$[a] (g/mol)	$M_{n(NMR)}$ (g/mol)	$M_{n(GPC)}$[b] (g/mol)	M_W/M_n
5: *p*SPMA	77	90	25,100	22,600	20,700	1.4
13A: *p*AAMPSA	75	77	23,400	18,000	57,800	2.3
13B: *p*AAMPSA	72	74	23,400	17,500	36,600	1.3

[a] At 100% conversion.
[b] Characterization of polymers by ^{1}H NMR (D_2O) and by GPC ($NaNO_3/NaH_2PO_4$ in water, PEG calibration).

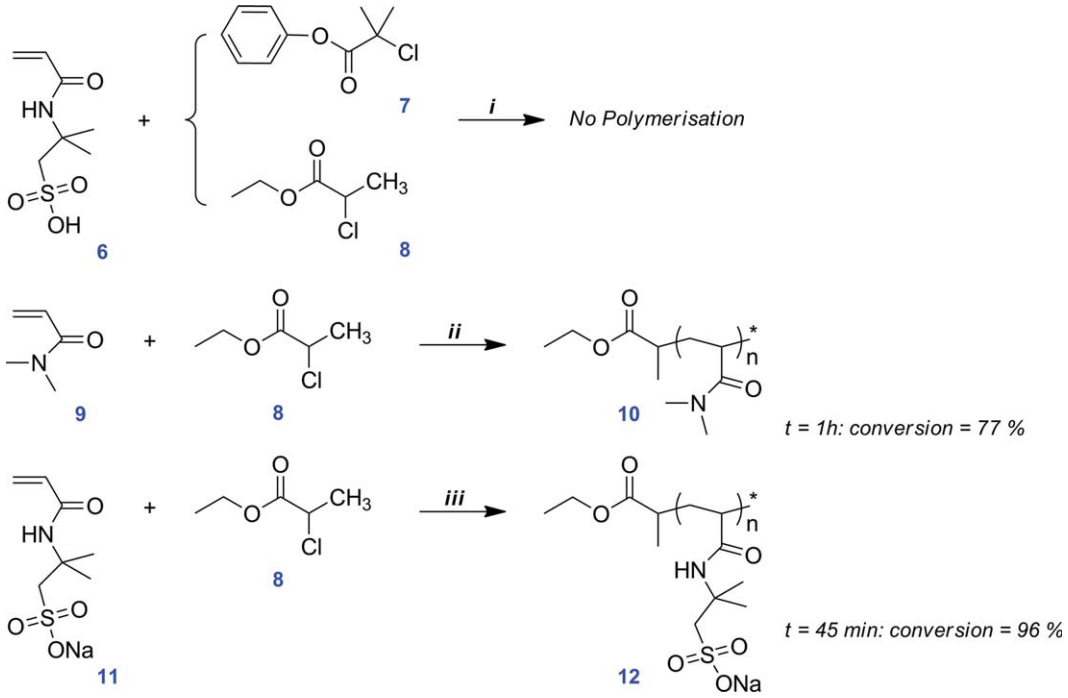
groups, Scheme 3, with the sodium salts giving the best control with standard initiators. Polymerizations of *N,N*-dimethylacrylamide were studied in DMSO at 30 °C, using Me_6-TREN and CuCl(**ii**, Scheme 3).[35]

The polymerizations in **i**, Scheme 3, reveal that the sulfonic acid monomer **(6)** is difficult to polymerize however, fast polymerization was achieved when using the commercially available monomer which is supplied in 50 wt % aqueous solution (**11**); once lyophilized to use the monomer in its powder form. The first investigation of the salt monomer, **iii**, Scheme 3 does not follow first order kinetics but formed an ill defined polymer in DMSO/water solution.

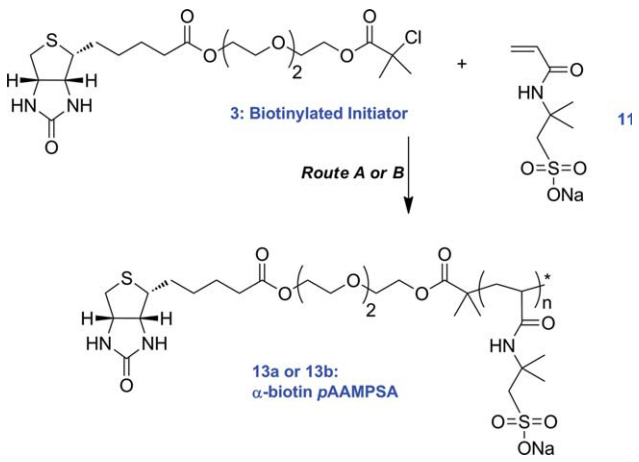
Route A (Scheme 4) by LRP was also investigated. In this case, the polymerization of (**11**) was carried out at 30 °C in a solvent mixture of MeOH/DMSO/H_2O with CuCl/*N*-(*n*-ethyl)-2-pyridylmethanimine as the catalyst. This polymerization was slower and allowed for sampling during the reaction to investigate the kinetics of the reaction. The kinetic plot (a) Figure 1 is relatively linear however, the evolution of M_n with time (b) is typical of a conventional free radical polymerization as opposed to a controlled/living polymerization and we were not able to determine if the Cl remained on the polymer terminus.

After 7 h, a conversion of 75% was reached and the polymerization was stopped. Purification by precipitation and dialysis led to a polymer with M_n =18,000 g/mol (Table 1) with a PDI = 2.3, thus this route is not an ideal way to obtain a controlled polymerization of this monomer.

Route B was finally undertaken, repeating polymerization **iii** (Scheme 3) but without the presence of water. In this case, curvature can be seen in the kinetic plot Figure 2 this can be attributed to catalyst deactivation or termination events.[13,35] After 5 h and 20 min of polymerization, a conversion of 72% was reached and the polymerization was stopped. Purification by precipitation and dialysis led to a polymer with a M_n = 18,000 g/mol (Table 1). A fairly narrow PDI of 1.3 was



SCHEME 3 Polymerization tests: (i) Polymerization of 2-acrylamido-2-methyl-1-propanesulfonic acid by LRP: Me_6-TREN, CuCl, DMSO, 30 °C; (ii) *N,N*-dimethylacrylamide by LRP: Me_6-TREN, CuCl, DMSO, 30 °C; (iii) 2-acrylamido-2-methyl-1-propanesulfonic acid, sodium salt by LRP: Me_6-TREN, CuCl, H_2O/DMSO, 30 °C. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.].



SCHEME 4 Polymerization of 2-acrylamido-2-methyl-1-propanesulfonic acid, sodium salt, Route A by LRP *N*-(*n*-ethyl)-2-pyridylmethanimine, CuCl, H_2O/MeOH/DMSO, 30 °C; Route B by LRP: Me_6-TREN, CuCl, DMSO, 25 °C. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

obtained showing that this procedure is a better route for the polymerization of this acrylamide.

Although the large molecular weight polymers complicate the ^{1}H NMR spectra analysis of the small biotin end-group signals, the theoretical M_n values (calculated at 100%) are reasonably close in value to the actual M_n values showing the initiator is fairly efficient (Table 1). For polymer **5**, M_n results from NMR and GPC are close; however, this is not the case for polymers **13A** and **13B**. This is due to the different nature of the poly(sulfonic acid) and its self-organization in water which leads to higher M_n values in aqueous GPC. An added effect on the M_n values by GPC is caused by the difference in structure between the sulfonic acid polymers and the PEG polymers used to calibrate the aqueous GPC system.

Quartz Crystal Microbalance with Dissipation Monitoring

QCM with dissipation monitoring is a technique developed by Kasemo et al. used to study molecular interactions and molecular adsorption at surfaces.[36–38] It determines the mass of very thin surface bound layers and simultaneously gives information about their structural and viscoelastic properties. It is a useful technique as the sensor mimics real-life applications, giving real-time interface characterization and evaluation of kinetics.[39]

The gold surface of the QCM sensors was chemically modified to provide a firmly bound avidin surface Scheme 5.[40] After which frequency and dissipation measurements relating to the interaction between the avidin and biotinylated polymers were collected. When Avidin was applied via adsorption, the binding was not as strong as the Avidin reaction with the biotinylated polymer. This resulted in the polymer "stripping" the Avidin from the surface which could be seen from an unusual increase in frequency.

Avidin in PBS buffer, pH 7, was passed over the QCM crystal *in situ* to ensure maximum surface coverage of Avidin and a change in frequency (Fig. 3) showed surface adsorption onto the crystal surface and a steady state was reached indicated after ~500 s. Following a buffer rinse, slight increase in frequency corresponding to the removal of unbound Avidin a steady state was reached after ~2000 s. On addition of α-Biotin *p*SPMA, a sharp decrease in frequency was observed. The rate of the Avidin-α-Biotin *p*SPMA binding is related to the gradient until a plateau is reached, Figure 3. No polymer was removed from the surface with a buffer rinse cycle, showing that the biotinylated polymer is firmly bound to the Avidin surface.

The same sequence was carried out with α-Biotin *p*AAMPSA (**13b**) and a sharp decrease in frequency was observed as with α-Biotin *p*SPMA. A very steep gradient is seen as α-

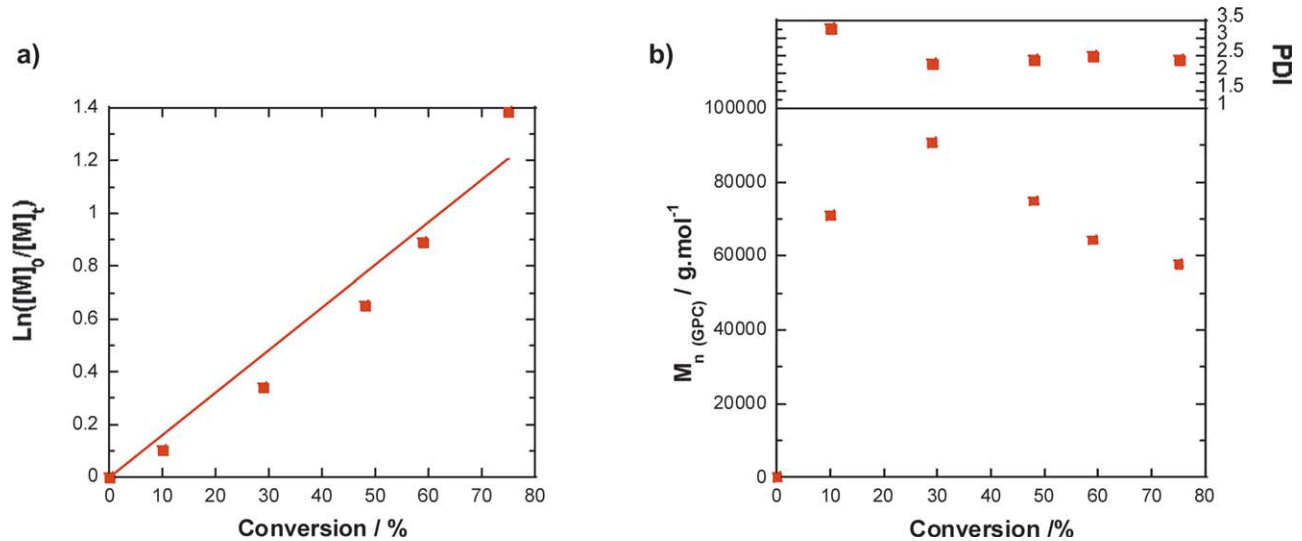


FIGURE 1 Kinetic plot of *p*AAMPSA (a) and evolution of M_n and polydispersity index (PDI) with time (b). Synthesis conditions: Route A by LRP: *N*-(*n*-ethyl)-2-pyridylmethanimine, CuCl, H_2O/MeOH/DMSO, 30 °C. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

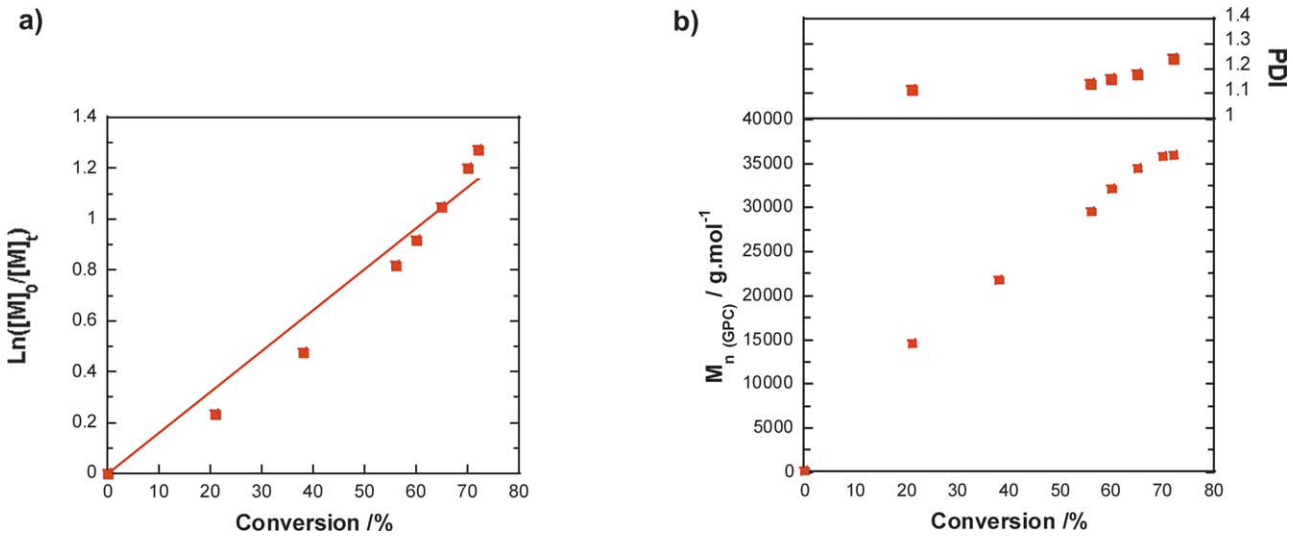
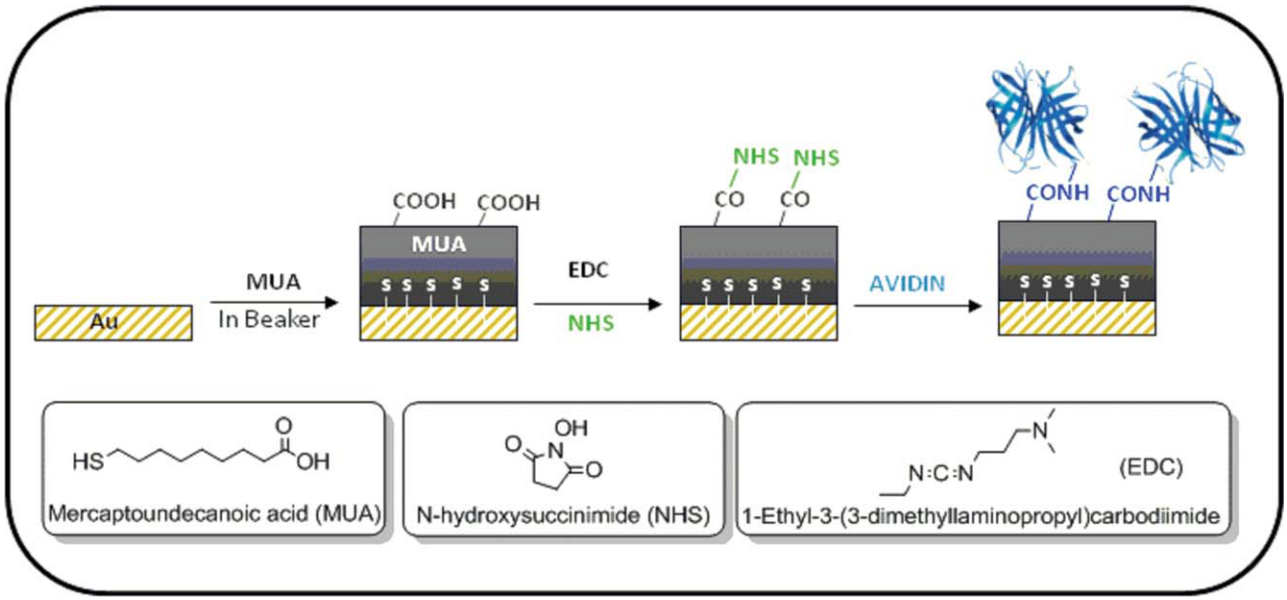


FIGURE 2 Kinetic plot of *p*AAMPSA (a) and evolution of M_n and PDI with conversion (b). Synthesis conditions: Route B by LRP: Me_6-TREN, CuCl, DMSO, 25 °C. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

Biotin *p*AAMPSA is adsorbed on the Avidin surface (Fig. 4) indicating a very fast rate of reaction. No change in frequency was observed during the final buffer rinse indicating that this polymer is also strongly bound to the Avidin surface.

A comparison of the two polymers shows that the binding rate to the Avidin surface is almost identical. The overall change in frequency following deposition of α-Biotin *p*SPMA and α-Biotin *p*AAMPSA = 8 Hz and 10 Hz, respectively. This small difference of 2 Hz indicates similar amounts of mass adsorbed on the surface; however, this is dependent on the molecular weight as well as the structure. Mass calculation of rigid films (which have a negligible dissipation trace) involves the Sauerbrey relation, which correlates the frequency of the oscillating crystal with the mass deposited on it. However, if the film is viscoelastic and the polymer film interacts with the solution being flown over it then the mass calculated is underestimated and a model must be applied instead.[39,41,42] The data was subjected to both methods to investigate the mass of biotinylated polymer on the avidin functionalized surface and give an estimated surface mass (Table 2; Fig. 5).

The Avidin-biotinylated polymer interaction was investigated under acidic conditions. Initially, PBS buffer pH 7 was passed



SCHEME 5 Gold surface functionalization. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

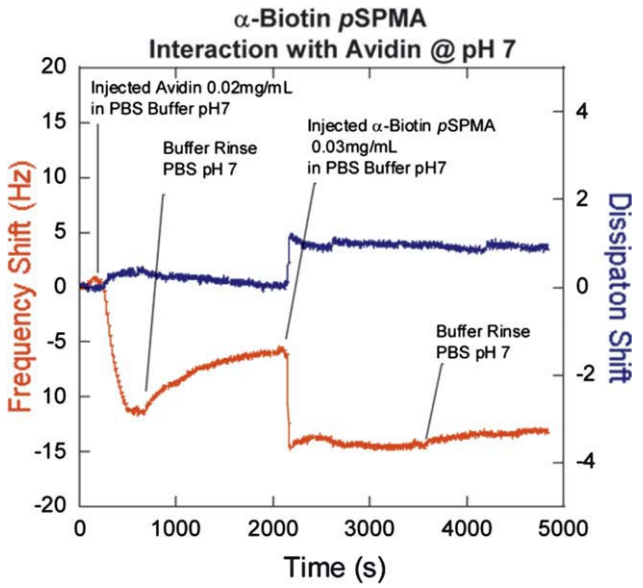


FIGURE 3 QCM-D Plot for α-Biotin *p*SPMA (**5**). [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

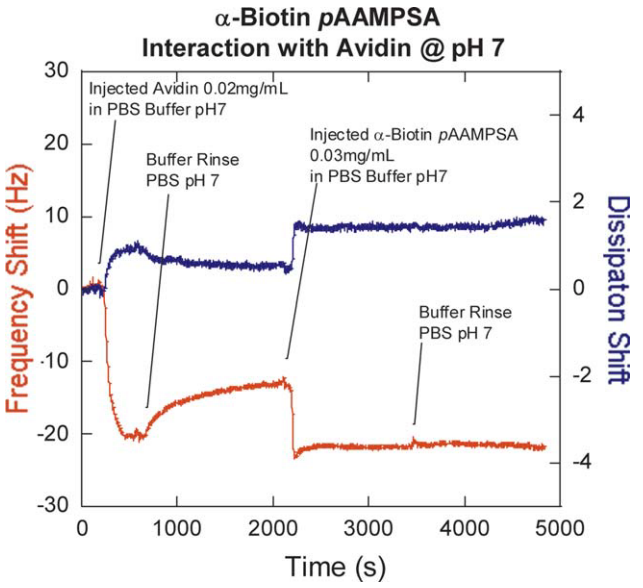


FIGURE 4 QCM-D of α-Biotin *p*AAMPSA (**13b**). [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

over the Avidin pre-treated crystal and on changing to acetate buffer at pH 4 an increase in frequency was observed, however, a steady state was not reached. This increase is ascribed to the acidic conditions removing Avidin from the surface. A similar trend has been seen previously with Concanavalin A, where on lowering the pH of the solution caused a removal of the protein from a polystyrene coated quartz crystal.[43]

A solution of Avidin in acetate buffer, pH 4, was passed over the QCM crystal to ensure maximum surface coverage of Avidin. A change in frequency was observed showing mass was adsorbed on the surface of the crystal and a steady state was reached indicated by the plateau around 2000 s, Figure 6. After a buffer rinse at pH 4, the frequency increased showing Avidin was not bound. A steady state was not reached again nevertheless the overall frequency change at 3500 s was still lower than the initial stage around (1500 s) before Avidin was passed over the surface. An initial decrease in frequency indicated adsorption of the polymer onto the surface however the frequency increase confirmed that the Avidin is not bound to the surface under these conditions. After the final buffer rinse at pH 4 all of the Avidin remaining on the surface was completely removed. Thus, Avidin does not stay bound to the surface at pH 4.

HABA Analysis

A competitive binding evaluation was undertaken as an additional method to confirm the binding ability of the biotinylated polymers to the protein receptor (Scheme 6). 2-(4-Hydroxyphenylazo)benzoic acid (HABA) binds to Avidin in a similar way to biotin and has a maximum UV absorbance at 350 nm. Once complexed to Avidin, the UV absorbance shifts to 500 nm and a red/orange color is observed in solution. Avidin's affinity for HABA is much weaker ($K_d = 10^{-6}$ M) in comparison to biotin ($K_d = 10^{-15}$ M) hence HABA should be replaced by the biotinylated polymers when they are added to the Avidin-HABA complex.

In both cases (α-Biotin *p*SPMA and α-Biotin *p*AAMPSA), when the biotinylated polymer was added to the Avidin-HABA complex solution, the red/orange color disappeared along with the peak belonging to the Avidin-HABA complex at $\lambda =$ 500 nm and a new peak appeared at $\lambda = 350$ nm correlating

TABLE 2 Mass Data

Polymer	pH	Sauerbrey's Relation Polymer Mass on Surface (ng/cm^2)	Model		
			$\bar{x}$ [Excess Avidin Mass on Surface] (ng/cm^2)	$\bar{x}$ [Polymer + Avidin Mass on Surface] (ng/cm^2)	$\bar{x}$ [Polymer Mass on Surface] (ng/cm^2)
α-Biotin *p*SPMA(**5**)	7	230	118.54 ± 1.65	2296.50 ± 44.80	2180
α-Biotin *p*AAMPSA (**13b**)	7	418	1353.18 ± 14.01	2338.73 ± 39.77	985

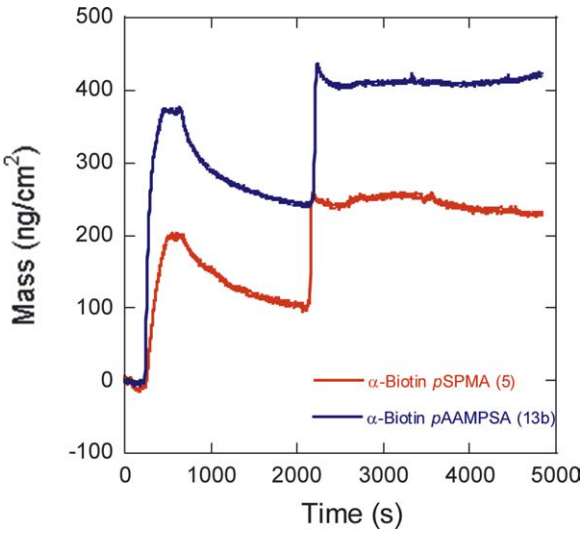


FIGURE 5 Estimated film mass over time using Sauerbrey's relation. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

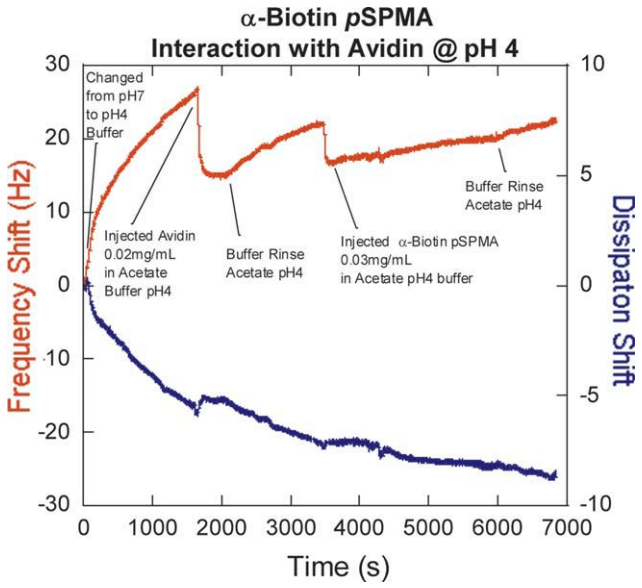


FIGURE 6 QCM-D analysis of α-biotin *p*SPMA (**5**) at pH 4. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

to native HABA, indicating that the biotinylated polymers have complexed with Avidin (Figs. 7 and 8).

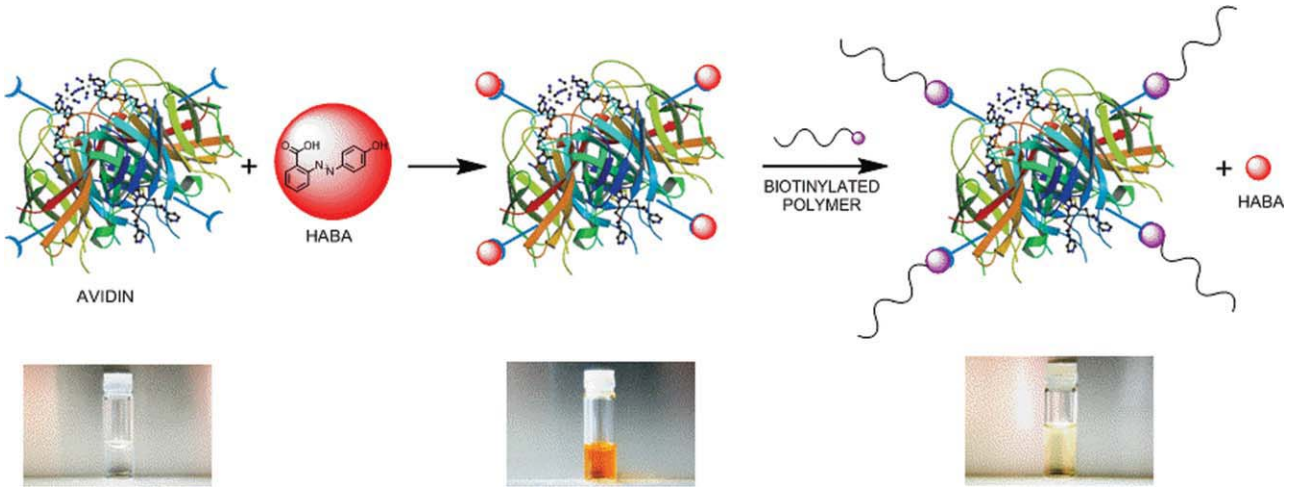
Experimental

Reagents

Biotin (Sigma-Aldrich, 99.0%), Avidin (Sigma-Aldrich, 98%), and 3-sulfopropylmethacrylate, potassium (Sigma-Aldrich, 98%) were used as received, 2-Acrylamido-2-methyl-1-propanesulfonic acid sodium salt solution (Sigma-Aldrich, 50 wt % in H_2O) was lyophilized prior to use and all other reagents and solvents were obtained at the highest purity available from Aldrich chemical company and used without further purification unless stated.

Synthesis of α-Biotin Poly(3-sulfopropylmethacrylate, potassium) (pSPMA)

SPMA (2 g, 8.1 mmol), biotin initiator (0.039 g, 0.0812 mmol), were charged in a Schlenk tube with H_2O/DMSO/MeOH (3/1.5/0.5 v/v/v) as solvent. The tube was sealed with a rubber septum and *N*-(*n*-ethyl)-2-pyridylmethanimine ligand (0.023 mL, 0.16 mmol) was injected via a syringe. The Schlenk solution was subjected to five freeze-pump-thaw cycles. This solution was then cannulated under nitrogen into a second Schlenk tube, previously evacuated and filled with nitrogen, containing Cu(I)Br (0.0058 g, 0.0406 mmol) and Cu(II)Br_2 (0.009 g, 0.0406 mmol). The temperature was adjusted to 40 °C with constant stirring. Samples were removed periodically using a degassed syringe for conversion ($t = 0$). At the end of the polymerization, the mixture was bubbled with air for 2 h. The polymer was subsequently precipitated twice into methanol, dissolved in deionized water and dialyzed (Spectra/Por 6 standard, regenerative cellulose membrane, MWCO 8 kDa) for 3 days and finally freeze-dried. ^{1}H NMR (D_2O): The conversion was



SCHEME 6 HABA schematic.

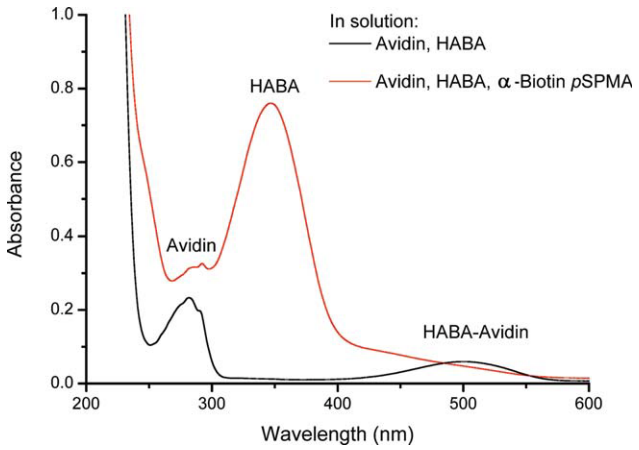


FIGURE 7 UV absorbance spectra of HABA analysis of α-Biotin *p*SPMA. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

calculated by comparing the integral of the O—C***H***$_2$—CH_2—CH_2—SO_3K group signals shifting during polymerization from 4.31 to 4.17 ppm. Conversion = 77% after 19 h of polymerization, DP_n = 90, M_n = 22,600 g/mol. M_n (GPC, 0.2 M $NaNO_3$, 0.01 M NaH_2PO_4, PEG calibration) = 20,700 g/mol, M_w/M_n (GPC) = 1.4.

^{1}H NMR (700.13 MHz, D_2O, 298 K), (Fig. 9) δ (ppm): 4.61–4.58 (dd, 1H, NHC***H***CHS), 4.44–4.40 (dd, 1H, NHC***H***CH_2S), 4.26–4.01 [br polymer hump, C***H***$_2$OC(O); masked by peak: 2H, C***H***$_2$OC(O)], 3.82–3.61 (m, 8H, C***H***$_2$O), 3.11–3.06 (m, 1H, SC***H***), 3.05–2.93 (br polymer hump, C***H***$_2SO_3$K), 2.77 (d, J = 13.20 Hz, 1H, SC***H***H), 2.70 (br s, 1H, SCH***H***), 2.41–2.37 [*t*, J = 7.48 Hz, 2H, C***H***$_2$C(O)O], 2.20–2.06 (br polymer hump,C***H***$_2CH_2SO_3$K), 2.05–1.75 (br polymer hump, backbone C***H***$_2$), 1.75–1.70 (sextet, 2H, SCHC***H***$_2$), 1.66–1.56 [m, 8H, 2 × C***H***$_3$; 2H, CH_2C***H***$_2CH_2$C(O)O], 1.46–1.41 [quintet, 2H, C***H***$_2CH_2CH_2$C(O)O], 1.31–0.80 (br polymer hump, backbone C***H***$_3$).

^{13}C NMR (176.04 MHz, D_2O, 298 K) δ (ppm): 179.70 [1C, CH2CH2***C***(O)O], 179.47 [polymer, CH2CH2***C***(O)O], 178.85 [1C,

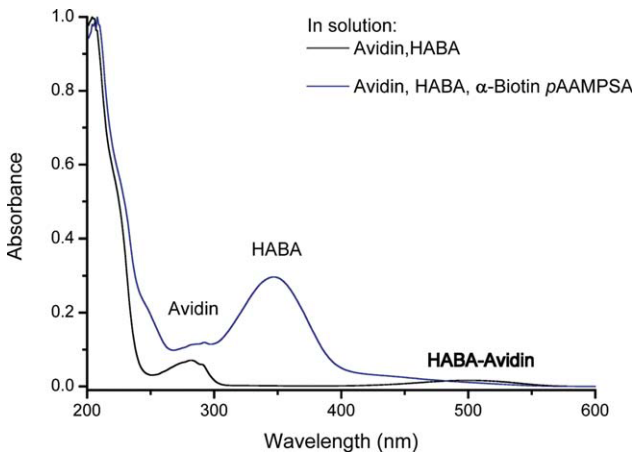


FIGURE 8 UV absorbance spectra showing HABA analysis of α-biotin *p*AAMPSA. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

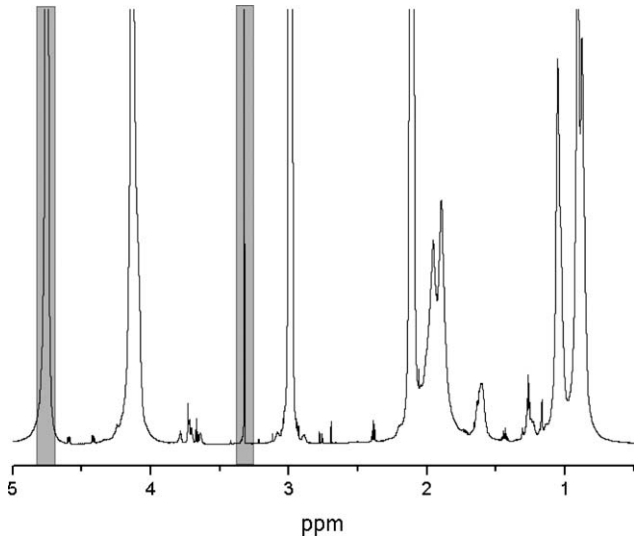
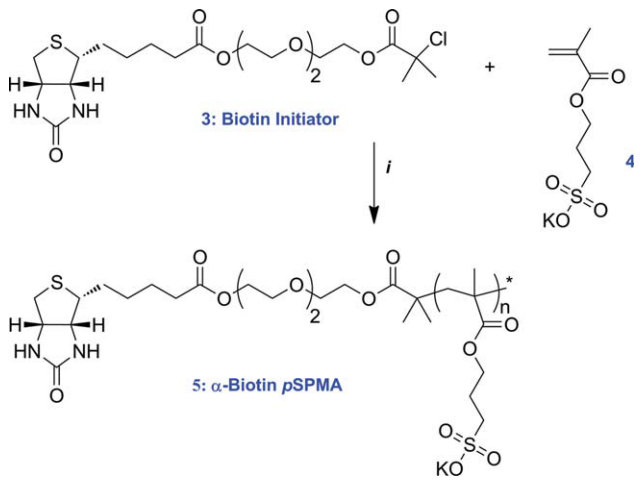


FIGURE 9 ^{1}H NMR spectrum of *p*SPMA in D_2O.

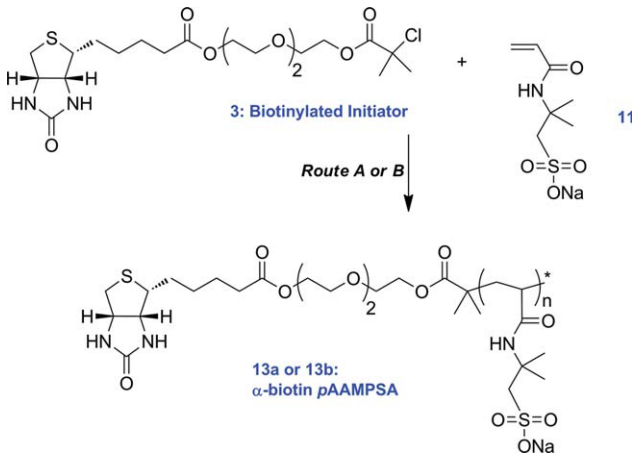
O***C***(O)C(CH3)2], 159.04 (1C, NH***C***ONH), 71.75 (1C, ***C***H2O), 69.79 (1C, ***C***H2O), 69.51 (1C, ***C***H2O), 66.55 (1C, ***C***H2O), 64.47–64.33 [polymer, ***C***H2C(O)O], −62.04 [1C, ***C***H2C(O)O], 60.42 [1C, ***C***H2C(O)O], −60.25 (1C, NH***C***HCHS), 60.15 (1C, NH***C***HCH2S), 55.26 (1C, S***C***H), −53.31 [polymer backbone ***C***(CH_3)Br], 47.95–47.84 (polymer, ***C***H2SO3K), 45.15–44.83 [polymer backbone ***C***H_2C(CH_3)Br], 39.68 (1C, S***C***H2), 35.05 [1C, (CH3)2***C***C(O)O], 33.62 [1C, ***C***H2C(O)O], 27.87 (1C, SCH***C***H2CH2), 27.61 (1C, SCHCH2***C***H2), 24.11 [1C, ***C***H2CH2C(O)O] [polymer, ***C***H2CH2C(O)O], 23.42–23.37 [2C, (***C***H3)2CC(O)O], 23.35 (polymer, ***C***H3 backbone), −18.60 (polymer, ***C***CH3 backbone), −17.19 (polymer, ***C***H2 backbone).

Synthesis of α-Biotin Poly(2-acrylamido-2-methyl-1-propanesulfonic acid, sodium) pAAMPSA (13b)

AAMPSA (1 g, 4.36 mmol), biotin initiator (0.021 g, 0.0436 mmol), were charged in a Schlenk tube with 5 mL DMSO as solvent. The tube was sealed with a rubber septum and



SCHEME 7 Synthesis of α-biotin *p*SPMA (**5**). LRP: *N*-(*n*-Ethyl)-2-pyridylmethanimine, CuBr, $CuBr_2$, H_2O/MeOH/DMSO, 40 °C. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]



SCHEME 8 Polymerization of 2-acrylamido-2-methyl-1-propanesulfonic acid, sodium salt. Route a by LRP: *N*-(*n*-Ethyl)-2-pyridylmethanimine, CuCl, H_2O/MeOH/DMSO, 30 °C; Route b by LRP: Me_6-TREN, CuCl, DMSO, 25 °C. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

Me_6TREN ligand (0.023 mL, 0.0872 mmol) was injected via a syringe. The Schlenk solution was subjected to five freeze-pump-thaw cycles. This solution was then cannulated under nitrogen into a second Schlenk tube, previously evacuated and filled with nitrogen, containing Cu(I)Cl (0.0086 g, 0.0872 mmol). The temperature was adjusted to 25 °C with constant stirring. Samples were removed periodically using a degassed syringe for conversion ($t = 0$). At the end of the polymerization, the mixture was air bubbled for 2 h. The polymer was then precipitated twice into methanol/Acetone (30/70), dissolved in deionized water, subsequently dialyzed (Spectra/Por 6 standard, regenerative cellulose membrane, MWCO 8 kDa) for 3 days and freeze-dried. ^{1}H NMR (D_2O): The conversion was calculated by comparing the integral of the OC—HN—C(C***H***$_3$)$_2$—CH_2—SO_3Na group signals of the polymer against the monomer during polymerization. Conversion = 72% after 6 h of polymerization, $DP_n = 74$, $M_n =$ 17,500 g/mol. M_n (GPC, 0.2 M $NaNO_3$, 0.01 M NaH_2PO_4, PEG calibration) = 36,600 g/mol, M_w/M_n (GPC) = 1.3.

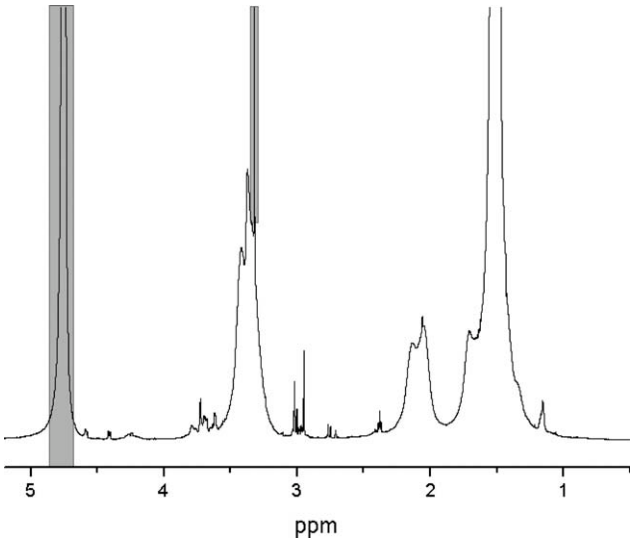


FIGURE 11 ^{1}H NMR spectrum of *p*AAMPSA in D_2O.

^{1}H NMR (700.13 MHz, D_2O, 298 K) δ (ppm) 4.60–4.58 (dt, 1H, NHC***H***CH_2S), 4.42–4.40 (dd, 1H NHC***H***CHS), 4.27–4.24 [m, 2H, C***H***$_2$OC(O)], 3.79–3.64 (m, 8H, C***H***$_2$O), 3.55–3.34 (br polymer hump C***H***$_2SO_3Na$), 3.02–2.95 (m, 3H, SC***H***, SC***H***$_2$), 2.39–2.37 [*t*, J = 7.48 Hz, 2H, C***H***$_2$C(O)O], 2.26–1.95 [br polymer hump, polymer backbone; s, (masked by polymer peak) 6H, 2 × C***H***$_3$], 1.79–1.28 [br polymer hump, (O)CNHC2(C***H***$_3$); completely masked by polymer peak: 2H,SCHC***H***$_2$], 1.21–1.10 [m, 4H, C***H***$_2$CH$_2$C(O)O; C***H***$_2$CH$_2$CH$_2$C(O)O].

^{13}C NMR (176.04 MHz, D_2O, 298 K) δ (ppm): 176.27–175.47 [1C, CH2CH2***C***(O)O, polymer, CH2CH2***C***(O)O], 1C, O***C***(O)C (CH3)2, 159.55 (1C, NH***C***ONH), 71.67–68.45 (6C, ***C***H2O), 62.03 (1C, NH***C***HCHS), 60.39 (1C, NH***C***HCH2S), 60.25 (1C, S***C***H), 58.22 (***C***H$_2SO_3Na$), 55.25 (polymer backbone ***C***H$_2$Br), 52.59–52.32 [polymer (CH$_3$)$_2$***C***NH], 43.61–43.36 (polymer

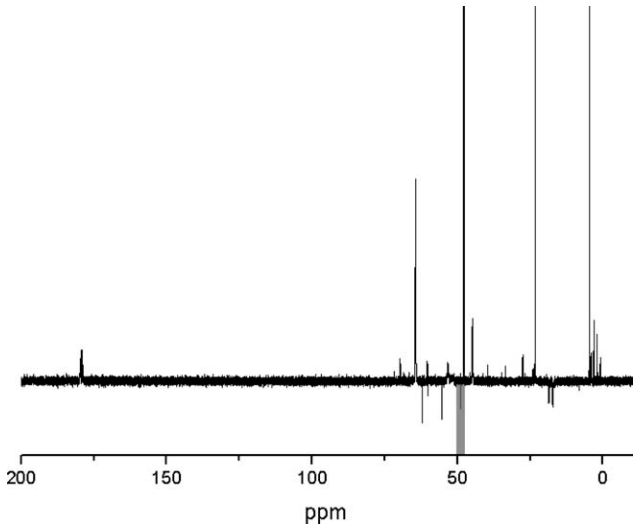


FIGURE 10 ^{13}C NMR spectrum of *p*SPMA in D_2O.

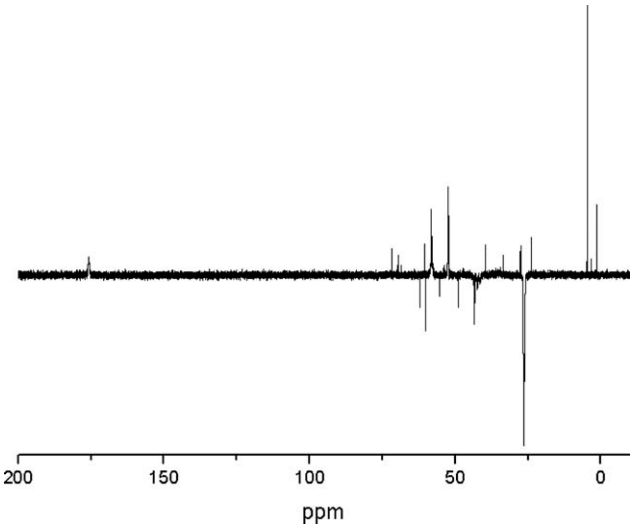


FIGURE 12 ^{13}C NMR spectrum of *p*AAMPSA in D_2O.

backbone $\boldsymbol{C}H_3CH_2Br$), 42.49 [O(O)C$\boldsymbol{C}$(CH_3)$_2$], 42.36 (1C, S$\boldsymbol{C}$H2), 39.67 [SCH(CH_2)$_3\boldsymbol{C}H_2$], 33.57 (SCH$\boldsymbol{C}H_2$), 27.82 [SCH(CH_2)$\boldsymbol{C}H_2$], 27.57 [$\boldsymbol{C}H_2CH_2C(O)O$], 26.44–26.16 [polymer ($\boldsymbol{C}H_3$)$_2$CNH], 24.07 [O(O)C($\boldsymbol{C}H_3$)$_2$].

GPC

For aqueous GPC, the system was equipped with two PL Aquagel-OH 8 μm mixed columns (300 $\times$ 7.5 mm) and a guard column, with differential refractive index detection using water (containing 0.2 M $NaNO_3$, 0.01 M NaH_2PO_4) at 1.0 mL/min as the eluent. Poly(ethylene glycol) (PEG) standards (1.6×10^5–1.0×10^3 g/mol) were used for calibration.

QCM-D

The Q-Sense E4 System: QE 401 Electronics Unit, QCP 401 Chamber Platform, QFM 401 Flow Module with Ismatec IPC-N Pump. Au coated quartz sensor [Frequency: 4.95 MHz $\pm$ 50 kHz; Cut: AT; Diameter: 14 mm; Thickness: 0.3 mm; Finish: Optically polished, surface roughness of electrode $<$3 nm (RMS); Electrode layer: 10–300 nm].

Surface Functionalization

The Au surface of a QCM chip was functionalized with Avidin following a similar prep to Krysiński et al.[44] Initially, the Au surface was reacted with 11-mercaptoundecanoic acid (MUA; 0.1 g, 0.0005 M, 218.36 g/mol) in 20 mL acetonitrile (ACN) in a sealed beaker and left overnight to react. The QCM-chip was rinsed with ACN and the carboxylic groups of the MUA layer were activated by reacting with EDC (0.31 g, 0.002 M, 155.24 g/mol) in 50 mL ACN for 3 h and *N*-hydroxysuccinimide (NHS) (0.28 g, 0.002 M, 115.09 g/mol) in 50 mL ACN. After activation and rinsing, Avidin (0.002 g in 10 mL PBS Buffer, pH 7) was attached to the activated NHS-ester surface to form a stable peptide bond between the amino groups of the protein and the activated ester surface.

Typical Experimental Conditions

A solution of Avidin (0.02 mg/mL) in PBS buffer, pH 7, was passed over the QCM crystal inside the flow module to ensure maximum surface coverage of Avidin and the change in frequency was monitored. Once a plateau was reached, a buffer rinse was carried out to remove any unbound Avidin until a flat baseline was achieved. After which biotinylated polymer (0.03 mg/mL) was injected and changes in frequency were monitored. Once a plateau was reached after this injection a buffer rinse was carried out to remove unbound polymer. The pump rate was set at 0.2 mL/min. Modeling data was obtained out using QTools software.

HABA Analysis

Saturated HABA (0.1 mL) aqueous solution was added into an Avidin aqueous solution (1 mg/mL): the colorless Avidin solution turned red/orange in color immediately, and the UV-Vis spectrum was recorded. Subsequently, 0.1 mL of Biotin polymer solution (1 mg/mL) was added into the red/orange solution of the Avidin-HABA complex. The strong color of the solution disappeared instantaneously, and an UV-Vis spectrum was recorded again.

SUMMARY

In summary, α-Biotin sulfonic acid polymers have successfully been polymerized via LRP. The biotin end group was used to bind with an Avidin surface and QCM-D data indicates the success of this. UV results of the HABA test provide further evidence of the biotin–avidin interaction.

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