One-step synthesis of low polydispersity, biotinylated poly(*N*-isopropylacrylamide) by ATRP†

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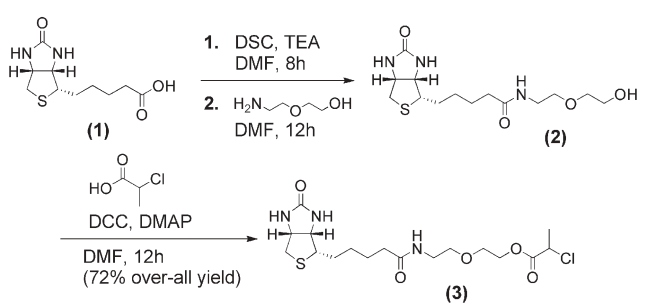
Low polydispersity poly(*N*-isopropylacrylamide) with a biotin end-group was obtained in one step from a biotinylated initiator for atom transfer radical polymerization and interacted with streptavidin to generate the thermosensitive polymer–protein conjugate.

Poly(*N*-isopropylacrylamide) (pNIPAAm) is a well-known temperature-sensitive polymer that exhibits a lower critical solution temperature (LCST) in water at ~32 °C.[1] Recent literature focuses on use of this responsive polymer in the high-impact fields of targeted drug delivery,[2] nanotechnology,[3] and microfluidics.[4] Conjugates of pNIPAAm and proteins have been proposed and investigated in biotechnology.[5] The strong affinity between biotin and naturally occurring proteins avidin and streptavidin ($K_d \sim 10^{-15}$ M) is also extensively exploited.[6] In particular, the ability of the proteins to bind four molecules of biotin has led to their widespread use as adapters to readily combine different biotinylated molecules into a single entity. For example, drug delivery systems,[7] as well as enzyme–polymer conjugates,[8] have been prepared using this technology. Therefore, the combination of a biotinylated pNIPAAm and streptavidin or avidin represents a versatile building block to confer thermosensitivity to biotinylated molecules such as drugs or antibodies or to surfaces and particles with potential application in drug delivery, biotechnology and surface engineering. We previously described the *in situ* synthesis of streptavidin–pNIPAAm conjugates by polymerization from a protein macroinitiator.[9] In this communication, we report a one-step procedure to synthesize low polydispersity pNIPAAm with a biotin end-group and demonstrate conjugate formation with the protein streptavidin.

Our approach uses a biotinylated initiator to directly generate the temperature sensitive pNIPAAm with a biotin moiety at the chain-end. This approach should be faster than post-polymerization modification[10] and would ensure that each polymer chain contains the desired functionality. Several examples of the direct synthesis of biotinylated polymers have been described. Chaikof and co-workers used a biotinylated arylamine initiator to start the cyanoxyl-mediated free-radical polymerization of glycomonomers.[11] The polymers obtained interacted with streptavidin and were employed for surface engineering.[11a] In a recent report, Wooley's group described the synthesis of a biotinylated initiator for atom transfer radical polymerization (ATRP) which allowed for the preparation of poly(acrylic acid)-*b*-poly(methyl acrylate).[12] The polymer displayed a narrow molecular weight distribution and was employed for the preparation of shell cross-linked nanoparticles (SCKs) that bind avidin. The latter technique, ATRP, is a versatile method to polymerize a variety of monomers in a controlled fashion.[13] α-Functional polymers are readily prepared by this method.[14] Recently, our group and others have used initiators for ATRP that also react with amino acid side chains.[15] The result is end-functionalized polymers amenable to coupling to proteins without any post-polymerization modification.[15a,b] Thus, biotin modified with an initiator for ATRP was employed to synthesize pNIPAAm that binds to streptavidin.

Narrow molecular weight distributions and predictable molecular weights are valuable features for those applications where well-defined macromolecular architectures are required. Appropriate selection of the initiator and experimental conditions for ATRP results in polymers with low polydispersity and defined molecular weights for many monomers.[13] However, the polymerization of acrylamides by ATRP is challenging, and many studies have been reported that investigate this problem.[16] Catalyst inactivation, low values of ATRP equilibrium constants, and displacement of the terminal halide have been recognized to complicate the polymerization.[16a,b] However, the combination of a chloropropionate-functionalized initiator and tris(2-dimethylaminoethyl)amine (Me_6TREN) as the ligand was found to be an effective system for ATRP of acrylamides[16c–e] For this reason, we used initiator **3** in conjunction with Me_6TREN as the ligand for the polymerization of NIPAAm.

Initiator **3** was obtained in two steps by modification of a literature procedure[12] as outlined in Scheme 1. Biotin (**1**) was reacted with *N*,*N*′-disuccinimidyl carbonate (DSC) in the presence of triethylamine (TEA) in DMF for 8 hours, followed by the addition of 2-(2-aminoethoxy)ethanol to yield the biotinylated alcohol **2**. After purification by recrystallization in ether–methanol,



Scheme 1 Synthesis of the biotinylated initiator for ATRP.

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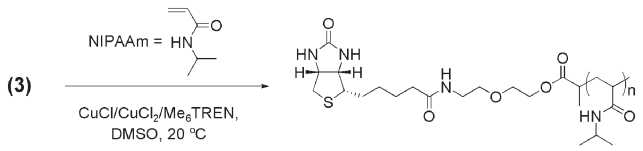

Scheme 2 Synthesis of biotinylated pNIPAAm.

the alcohol was esterified with 2-chloropropionic acid in the presence of 1,3-dicyclohexylcarbodiimide (DCC) and 4-(dimethylamino)pyridine (DMAP). The initiator **3** was purified by column chromatography (dichloromethane:methanol = 9:1) resulting in an overall yield of 72%.

The polymerization of NIPAAm was undertaken in DMSO to allow for solubilization of all components (Scheme 2). Kinetic monitoring of the reaction with an initial NIPAAm to **3** ratio of 240 to 1 ($[NIPAAm]_0$:$[\mathbf{3}]_0$:$[CuCl]_0$:$[CuCl_2]_0$:$[Me_6TREN]_0$ = 240:1:1.6:0.4:2) was conducted in deuterated DMSO at 20 °C.[17] Curvature was observed in the kinetic plot (Fig. 1a), as well as some tailing in the GPC traces at higher conversions (see ESI†), which suggests the occurrence of termination events and/or catalyst deactivation.[16c,d] Nevertheless, a conversion of approximately 80% was reached in less than 8 hours. GPC analysis showed a linear increase of M_n with conversion and the polydispersity index (PDI) remained low throughout the polymerization (Fig. 1b). The high molecular weight of the polymer complicated the analysis of the ^{1}H NMR spectra; nevertheless it was possible to identify the signals from the biotin chain-end at 2.8 ppm and 3.1 ppm (Fig. 2). The number-average molecular weight (M_n) of the polymer was calculated by comparison of these signals with the polymer peak at 3.8 ppm. Although some error was associated with the integration of the small biotin signals, the M_n calculated by NMR was comparable to the target molecular weight at each time point (Fig. 1b) indicating that the initiator is efficient.

Two different protocols were followed for the isolation and purification of the polymer. In one case, the polymerization solution was diluted with water and subjected to dialysis to remove unreacted monomer and catalyst. The polymer was then recollected after freeze-drying as a white solid. In the second procedure, the polymerization solution was diluted with THF and filtered over alumina to remove the catalyst. The solvent was then evaporated under high vacuum, the residue dissolved in THF, and the polymer subjected to precipitation cycles into hexanes. Both procedures were effective in removing residual monomer and catalyst. Although the second procedure is more labor-intensive, the polymer is isolated more quickly. The M_n of the resulting polymer calculated from ^{1}H NMR was 23 000 and the PDI was 1.17.

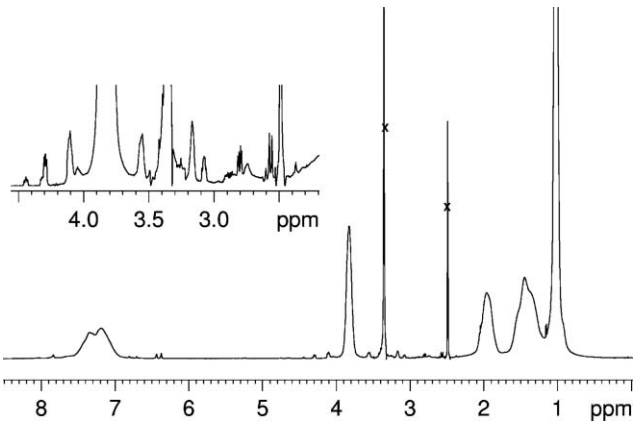

Fig. 2 ^{1}H NMR (DMSO-d_6) of the biotinylated pNIPAAm. Peaks arising from the biotin end-group are enlarged for easier visualization.

The biotinylated pNIPAAm was conjugated to streptavidin by simply dissolving both components in cold water (4 °C) and incubating the solution at room temperature for 1 hour. The shift of the complex in the SDS-PAGE gel and lack of detectable unmodified protein (Fig. 3) indicates efficient interaction of the streptavidin and polymer.

The HABA assay was used to quantify the number of chains conjugated to the protein.[18] In this assay, the dye HABA [2-(4′-hydroxyazobenzene)benzoic acid] is first incubated with streptavidin to saturate the biotin binding pockets of the protein. Addition of biotin or of a biotinylated compound causes displacement of HABA. The release of the dye can be monitored spectroscopically allowing one to calculate the number of biotinylated molecules per streptavidin. By this technique, it was

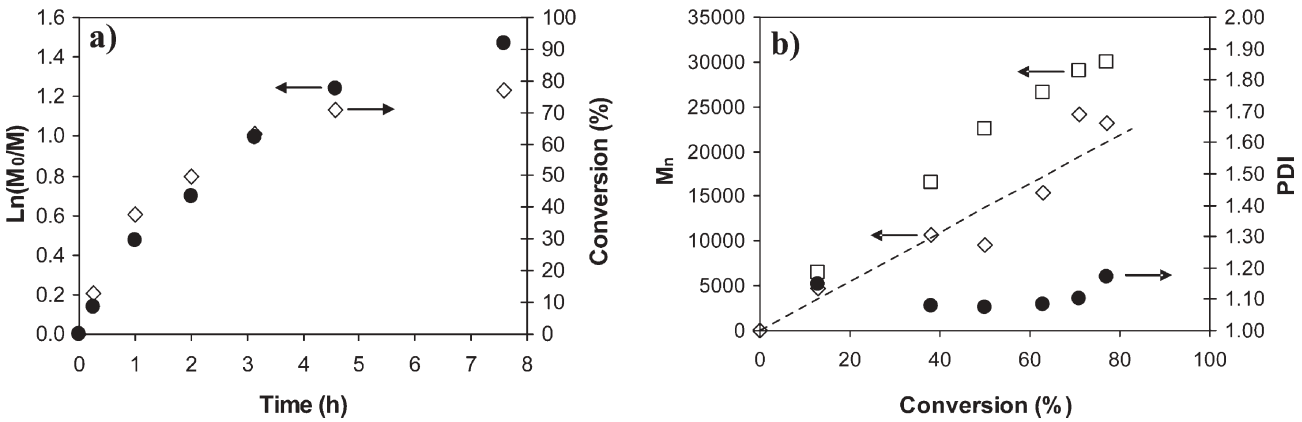

Fig. 1 Kinetic plot (a) and evolution of M_n and polydispersity with conversion (b). Synthesis conditions: $[NIPAAm]_0$ = 4 M in DMSO-d_6, $[NIPAAm]_0$:$[\mathbf{3}]_0$:$[CuCl]_0$:$[CuCl_2]_0$:$[Me_6TREN]_0$ = 240:1:1.6:0.4:2, 20 °C. Conversion obtained by ^{1}H NMR spectroscopy, M_n obtained by GPC (□) or ^{1}H NMR (◇), PDI by GPC (DMF containing 0.1M LiBr, 40 °C, two Polymer Laboratories PLgel 5 μm mixed D columns, RI detector, PMMA standards). The dotted line represents the theoretical molecular weight.

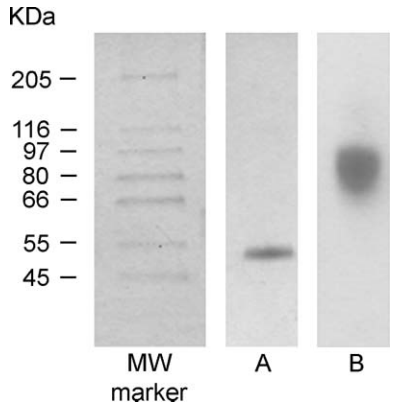

Fig. 3 SDS-PAGE gel of streptavidin (lane A) and the streptavidin–pNIPAAm complex (lane B) compared to the molecular weight marker.

determined that streptavidin interacts with 3.6 molecules of biotinylated pNIPAAm. This number is equivalent to biotin itself (3.7 biotins per streptavidin). This result demonstrates that steric hindrance does not inhibit binding of the polymer to streptavidin.

In conclusion, we have described a new strategy to synthesize α-functional pNIPAAm. The use of a biotin-functionalized initiator for ATRP allowed for the preparation of low polydispersity, biotinylated pNIPAAm in one step in good yield. The polymer chain did not hinder interaction of the biotin with the protein, and the pNIPAAm readily bound to streptavidin. The resulting streptavidin–pNIPAAm conjugate should be useful in a variety of applications.

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