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Facile synthesis of histidine functional poly(*N*-isopropylacrylamide): zwitterionic and temperature responsive materials

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N-isopropylacrylamide is copolymerised with aldehyde functional monomers to facilitate post polymer functionalisation with histidine via reductive amination. This strategy is successfully performed without the use of protecting groups with high yields, demonstrating the facile application of this synthetic strategy with high functional group tolerance. The resulting temperature responsive, histidine functionalised polymers are characterised and their responsive nature is explored. The functionalised polymers exhibit sharp Lower Critical Solution Temperatures (LCST) at histidine incorporations of up to 9%. Upon 16 and 23% incorporation, the LCST transition extends over a wider range of temperature, indicating that the LSCT is being counteracted by an increase in solubility from the histidine moieties which interact strongly with water. Dynamic Light Scattering data indicates responsive self-assembly.

Introduction

Nature has evolved a number of strategies based on the responsive properties of highly functional protein molecules to efficiently complete a range of complex functions. In fact, many biological processes can be triggered by solution conditions such as temperature[1] or pH[2] to control complex enzyme mediated reactions. The development of new synthetic mimics inspired by nature's designs will enable new advanced applications to emerge.

The most common examples of synthetic stimuli responsive materials are modulated by solution conditions such as pH and temperature. Poly(*N*-isopropylacrylamide) (PNiPAm) is the most studied thermoresponsive polymer and exhibits hydrophilic character in water below its Lower Critical Solution Temperature (LCST) as hydrogen bonds between the polymer and water molecules are responsible for its solvation.[3] When PNiPAm is heated above the LCST, the hydrogen bonds between the polymer and water molecules break and the polymer undergoes a coil to globule transition, resulting in poor solubility in water. This responsivity has been utilised to create range of advanced materials, such as nanogels and micelles for targeted drug delivery and drug release,[4–6] immunoassays,[7] functional polymeric biointerfaces,[8,9] responsive stabilisation of colloidal suspensions,[10] and self-healing coatings applications[11]. An interesting class of pH responsive materials are zwitterionic-based structures. Zwitterionic polymers, or polymers that carry equal positive and negative charges, such as sulfobetaine or carboxybetaine methacrylates (SBMA or CBMA), have anti-biofouling properties resulting from the formation of a hydration layer that prevents non-specific protein adsorption.[12,13] Nature makes regular use of zwitterions, the classical examples being amino acids and phospholipids.

The design of zwitterionic polymers utilising nature's own amino acids is an emerging field in polymer chemistry. The zwitterionic nature of amino acids stems from the relative pKa of the carboxylic acid and the pKb of the free amine. In water, this usually results in the deprotonation of the carboxylic acid and the protonation of the amine, and with both positive and negative charges present, a net neutral charge results. Polyzwitterions based on amino acids are designed such that the carboxylic acid and amine groups are left as pendant groups, using the amino acid side chain to react with a polymerisable precursor (generally methacrylates or methacrylamides).[14,15] This strategy results in interesting zwitterionic materials, however these are not a true a mimic of polypeptides as the amino acid side chains are not retained in the final structure, as their functionality is used to place them on the polymer. The use of the amino acid side chains as functional handles prevents transferring their useful and important roles in protein folding and function to synthetic polymers, and acts as a barrier to making truly biomimetic

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polymers with amino acid functionality.[16–18] The development of a synthetic strategy to create polyzwitterions based on amino acids while preserving the amino acid side chain will enable a new library of synthetic mimics, leading to a range of synthetic polymer based protein mimics.

In this work, we present a facile synthetic strategy to prepare a series of PNiPAm copolymers to which amino acid functionality can be added post polymerisation without protecting group chemistry; importantly, we have also preserved the side chain of the amino acid. The use of reductive amination to react the free amine of histidine with an aldehyde functional monomer, 4-formylphenyl methacrylate,[19] copolymerised with PNiPAm yields a histidine functional, thermoresponsive polymer. The successful attachment of histidine to the polymer without protecting group chemistry[20] is a testament to the versatility and potential that reductive amination can lend to polymer chemistry. This work explores the interesting properties that the histidine moiety adds to the thermoresponsive copolymer, including pH responsiveness and zwitterionic functionality within the appropriate pH range. Highlighted in this paper is the protective group free chemistry used to synthesise this material which can expand the ability to make a range of amino acid functionalised polymers without compromising the amino acid side chain.

Experimental

Materials

Chemicals were used as received unless otherwise stated. 4-Hydroxybenzaldehyde (98%), L-histidine (98%), 4-dimethylaminopyridine (DMAP) (>98%), methacrylic acid (99%, stabilised with 250 ppm methoxyphenol), sodium borohydride (98%), and *N*,*N*'-dicyclohexylcarbodiimide (DCC) (99%), are Alfa Aesar products obtained from VWR. Sodium hydroxide (>99%) was obtained from VWR. Potassium chloride (99%) was manufactured by ChemSupply. Hydrochloric acid (32%) was manufactured by LabScan. Glacial acetic acid was manufactured by Ajax Finechem. *N*-isopropylacrylamide (NiPAm) (98%, stabilised with MEHQ) was supplied by Tokyo Chemical Industry Co., Ltd and was recrystallised from a mixture of toluene/hexane (v/v 2:3) prior to use. 4-cyano-4-[(dodecyl-sulfanylthiocarbonyl)sulfanyl] pentanoic acid (CDP) (97% by HPLC) and azobisisobutyronitrile (AIBN) (98%) were obtained from Sigma Aldrich Company. AIBN was recrystallised in methanol prior to use. Dialysis tubing (regenerated cellulose, SnakeSkin^{TM}, 10000 MWCO) was soaked in deionised water for 15 minutes and thoroughly rinsed prior to use. Tetrahydrofuran (THF) GPR Reactapur (stabilised with BHT) and toluene (Analar Normapur) were purchased from VWR Chemicals. Diethyl ether and n-hexane (95%) were of RCI premium grade from ACI Labscan. Dichloromethane (DCM) and methanol (MeOH) were of reagent grade and obtained from Chem-Supply.

Characterisation

^{1}H NMR and ^{13}C NMR spectra were taken with a Varian 400 MHz spectrometer using the specified solvent. Fourier Transform Infra-Red (FTIR) spectra were taken with a Perkin Elmer Frontier FTIR Spectrometer using a Universal ATR Sampling Accessory. Spectra collected were taken with a resolution of 4 cm^{-1} with 64 scans. Solution pH was measured using a HACH SensION+ PH31 with a 5014T probe. Gel Permeation Chromatography (GPC) was used to determine polymer molecular weight. The polymer was dissolved in spectrometry grade DMF at a concentration of 10 mg/mL and filtered through a 0.45 µm Teflon^{TM} syringe filter. 50 µL was injected into the instrument and flowed through at a rate of 1 mL/min. The GPC instrument was equipped with a Shimadzu RID-10 refractometer (λ = 633 nm) and Shimadzu SPD-20A UV-vis detector using two Phenomenex Phenogel columns (5 µm bead size, 104 and 106 Å porosity) in series, operating at 70°C. DMF with 0.05 mol·L^{-1} LiBr (>99%, Aldrich) was used as the mobile phase. The polymer molecular weight was determined by comparison to polystyrene calibration curves. Polymer LCST measurements were taken using UV-Vis transmission (540 nm) measurements using a quartz cuvette with a 10 mm path length on a Shimazdu UVmini 1240 UV-Vis Spectrophotometer fitted with a heating cell; any resulting temperature changes were measured with an external thermocouple. Dynamic Light Scattering (DLS) measurements were taken with a Wyatt DynaPro NanoStar DLS/SLS with disposable cuvettes.

Synthesis

4-formylphenyl methacrylate 4-FPMA. Into a 150 mL round bottomed flask with magnetic stirring, 4-hydroxybenzaldehyde (3.02 g, 24.7 mmol), methacrylic acid (2.23 g, 25.9 mmol) and DMAP (0.305 g, 2.5 mmol) were added and dissolved in 5 mL of DCM. DCC (5.69 g, 27.5 mmol) was dissolved in 3 mL of DCM and added dropwise into the flask. The reaction proceeded overnight at room temperature and was filtered the next morning to isolate the filtrate. The solvent was removed by rotary evaporation and the remaining brown oil was purified by column chromatography on silica (35 Å, 40-63 µm) with DCM as the eluent. The fractions were collected and the additional solvent was removed by rotary evaporation and desiccation, yielding **4-FMPA** as a pale yellow oil (3.90 g, 78% yield). **^{1}H NMR** (400 MHz, CDCl3): δ = 10.00 (s, 1 H), 7.94 (d, J = 8.01 Hz, 2 H), 7.32 (d, J = 8.07 Hz, 2 H), 6.38 (s, 1 H), 5.81 (s, 1 H), δ = 2.07 (s, 3 H).

General procedure for the synthesis of poly(*N*-isopropylacrylamide-*co*-4-formylphenyl methacrylate) PNiPAm-*co*-PFPMA. Statistical copolymers of NiPAm and **4-FPMA** with varying incorporations of **4-FPMA** were synthesized via RAFT polymerisation in an oven dried, 25 mL Schlenk tube under nitrogen atmosphere.

The detailed synthesis for PNiPAm$_{73}$-*co*-PFPMA$_{26}$ (**P4**) is provided. The reagents, NiPAm (0.58 g, 5.1 mmol), **4-FPMA** (0.42 g, 2.2 mmol), CDP (0.010 g, 0.025 mmol) and AIBN (0.0015 g, 0.0062 mmol) were dissolved in THF (2.8 mL) ca. 40% wt/wt. Following three freeze-pump-thaw iterations, the reaction was heated to 60°C and the polymerization proceeded for 24 hours. The resulting polymer (**P4**) was

precipitated twice into diethyl ether and dried in a vacuum oven (90°C) overnight, yielding a white powder (0.77 g, 76%). **^{1}H NMR** (400 MHz, CD_3OD): δ = 9.87 (s, 0.35 H), 8.09-7.04 (broad, 1.83 H), 3.93 (bs, 0.65 H), 2.64-0.68 (broad, 6.47 H). **^{13}C NMR** (100 MHz, CD_3OD): δ = 192.91, 176.06, 135.48, 132.04, 123.82, 42.57, 22.32. **IR:** (cm^{-1}) 3303, 2972 2934 (PNiPAm CH_2), 1751 (ester), 1700 (aldehyde), 1642 (amide I), 1532 (amide II), 1458, 1387, 1367, 1208, 1156, 1131, 1092, 1013.

General procedure for the synthesis of poly(*N*-isopropylacrylamide-*co*-histidine) [PNiPAm-*co*-PFPMA]-*co*-His. The functionalisation of the aldehyde functional polymers PNiPAm-*co*-PFPMA with histidine was performed in two steps in 15 mL vials.

The detailed synthesis of [$PNiPAm_{89}$-*co*-PFPMA]-*co*-His_8 (**P6**) is provided. The reactants $PNiPAm_{89}$-*co*-$PFPMA_8$ (**P2**) (0.098 g, 0.081 mmol of PFPMA repeating units) and histidine (0.13 g, 0.86 mmol) were dissolved in ≈1 mL acetic acid and reacted for 12 hours at 50°C. The acetic acid was removed by evaporation and replaced with an equal volume of methanol. Sodium borohydride (0.036 g, 0.94 mmol) was added and left to stir overnight at room temperature. The functionalised polymer (**P6**, [$PNiPAm_{89}$-*co*-PFPMA]-*co*-His_8) was purified by dialysis. After freeze drying and drying in the vacuum oven at 100°C overnight, a pale yellow powder remained (0.087g, 84.1%). **^{1}H NMR** (400 MHz, CD_3OD): δ = 8.11-7.03 (broad, 1.35 H), 6.95 (s, 0.09 H), 4.18 (s, 0.19 H), 3.95 (bs, 0.9 H), 3.74 (s, 0.11 H), 3.17 (broad, 0.18 H), 2.48-0.46 (broad, 9.21 H). **^{13}C NMR** (100 MHz, CD_3OD): δ = 176.00, 135.27, 134.80, 132.10, 123.64, 44.19, 42.50, 37.06, 28.15, 22.83. **IR:** (cm^{-1}) 3288, 2972 2933 (PNiPAm, CH_2), 1745 (ester), 1637 (amide I), 1538 (amide II), 1458, 1386, 1367, 1202, 1169, 1101, 1018.

Polymer Titration

Polymers **P5**-**P8** were titrated under nitrogen atmosphere to quantify their pH responsive histidine functionality. Titrations were performed on 2 mg/mL samples of polymer in electrolyte (0.10 M KCl). The pH was initially adjusted to 2.5 with 0.10 M HCl. Volumetric increments (10 µL) of NaOH (0.10 M) were added until the pH approached 12. Prior to each pH measurement, the sample was stirred for 30 seconds after the addition of NaOH. The titration curves of the polymer samples were compared to the titration of an equal volume of 0.10 M KCl. All stock solutions used in titrations were purged with nitrogen for 1 hour before the titrations.

LCST measurements

The LCST of the polymers **P5**-**P8** was measured by observing the sample transmission as the temperature of the sample was changed. Polymer samples in deionised water were prepared at a concentration of 10 mg/mL. The sample pH was changed with 0.01 M KOH or 0.10 M HCl stock solutions to determine the effect of pH on the temperature response of the polymer. During pH adjustments, the concentration of the polymer solution was maintained between 9 mg/mL and 10 mg/mL.

Dynamic Light Scattering

DLS measurements were performed on polymer samples of 1 mg/mL with and without electrolyte (0.01 M KCl). Polymer solutions were filtered through 0.45 µm Teflon filters at room temperature prior to any temperature changes or measurements. Samples were made from a polymer stock solution and pH was adjusted using 0.10 M KOH and HCl solutions.

Results and Discussion

To develop a versatile platform for amino acid functionalisation a series of copolymers of PNiPAm with an aldehyde containing monomer were prepared. As commercially available aldehyde containing monomers are limited, one was synthesised by DCC coupling of methacrylic acid and 4-hydroxybenzaldehyde. The monomer, 4-formylphenyl methacrylate (**4-FPMA**), was synthesised in good yields (≈80%) and was easily purified by column chromatography. Due to the reactive nature of aldehydes, benzaldehyde was incorporated into the monomer rather than an aliphatic aldehyde to minimise polymer crosslinking reactions that are less likely to occur with the less reactive benzaldehyde group.

Controlled polymerisation was used to synthesise the copolymers of NiPAm and **4-FPMA** via Reversible Addition-Fragmentation Chain-Transfer (RAFT) polymerisation. The polymer series was synthesised with increasing amounts of aldehyde, from 4.3% in **P1** to 26.2% in **P4**. The molecular weights of the platform polymers **P1**-**P4** ranged between 43 kDa and 55 kDa with dispersities for all polymers being 1.5-1.6. The high dispersity could be due to a number of factors. The GPC trace indicated some interaction between the GPC column (measured in DMF, Fig. S1) and the polymer samples that are high in PNiPAm content. Additionally, the RAFT agent chosen was best suited to methacrylates, with some compatibility with acrylamides. Likely, a combination of GPC column interactions and RAFT agent compatibility is causing this high dispersity. The aldehydes could also be reducing the radicals in the polymerization, destroying some of the growing chains. In order to confirm that the high polydispersity is due to RAFT agent compatibility and some column interactions, we synthesized a homopolymer of PNiPAm by RAFT polymerisation using the same CTA (ESI). The shape of the GPC trace (Fig. S2) is very similar, with a polydispersity of 1.4 (for a 16 hour polymerization) which indicates that the compatibility with the RAFT agent with NiPAm is influencing the dispersity of the polymers. Better polydispersity could be achieved by smaller reaction times and through an investigation of other RAFT agents. The composition of the copolymers **P1**-**P4** was quantified by ^{1}H NMR and is summarised in Table 1.

Reductive amination is a two-step reaction with relatively mild reactions conditions. Abdel-Magid reported comprehensive work of its utility in organic chemistry covering a large scope of substrates.[21] A hydroxyamine intermediate is formed and produces an imine upon dehydration, usually in acidic conditions. The imine is subsequently reduced to form a stable amine linkage. Due to the dehydration of the

Table 1 Aldehyde functionalised, temperature responsive polymer synthesis and characterisation and their conversion to histidine functional, temperature responsive polymers

Polymer	Composition	Aldehyde %[a]	Mw (kDa)[b]	$Đ$[b]	His %[c]	Conversion of aldehydes (%)[c]
P1	$PNiPAm_{94}$-*co*-$PFPMA_4$	4.3	52	1.5	-	-
P2	$PNiPAm_{89}$-*co*-$PFPMA_9$	9.3	46	1.5	-	-
P3	$PNiPAm_{81}$-*co*-$PFPMA_{18}$	18.1	55	1.6	-	-
P4	$PNiPAm_{73}$-*co*-$PFPMA_{26}$	26.2	54	1.5	-	-
P5	[$PNiPAm_{94}$-*co*-PFPMA]-*co*-His_3	-	52	1.5	2.9	66.6
P6	[$PNiPAm_{89}$-*co*-PFPMA]-*co*-His_8	-	46	1.5	7.7	84.6
P7	[$PNiPAm_{81}$-*co*-PFPMA]-*co*-His_{12}	-	55	1.6	12.1	67.2
P8	[$PNiPAm_{73}$-*co*-PFPMA]-*co*-His_{23}	-	54	1.5	23.3	89.1

[a]Determined by ^{1}H NMR, comparing the aldehyde proton at 10 ppm and the isopropyl proton at 3.9 ppm. [b]Determined from GPC in DMF, using polystyrene standards. [c]Determined by titration.

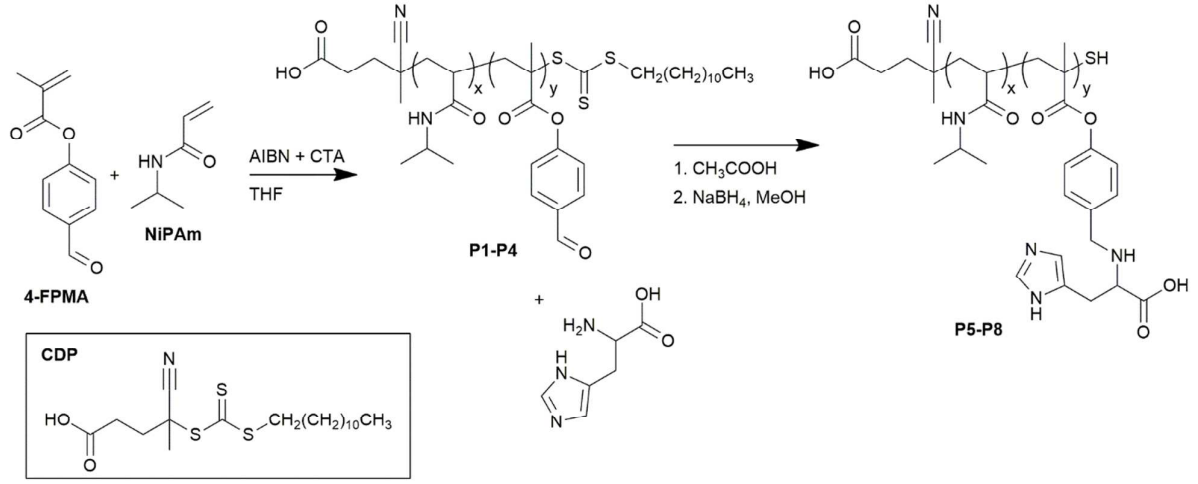
hydroxylamine intermediate to form the imine, the removal of water from the reaction to drive the equilibrium to completion is a common strategy to get high yields from this reaction. We found that the removal of water was not necessary in the system studied. Scheme 1 shows the approach we have used to functionalise the PNiPAm-*co*-PFPMA polymer series with histidine by reductive amination.

Finding an optimal solvent for the reductive amination to functionalise the PNiPAm-*co*-PFPMA polymer series with histidine was challenging; histidine is insoluble in most solvents except water, which would be a poor solvent choice for this chemistry considering the dehydration of the hydroxylamine to form the imine. Additionally, polymers **P2**-**P4** were insoluble in water due to their increased content of the hydrophobic **4-FPMA**. Abdel-Madgid reports successful reductive amination in both THF and dichloroethane,[21] and Ciszewski *et al.* report the use of reductive amination in acetic acid to synthesise monosubstituted thioureas.[22] Acetic acid was found to be a good solvent for both the polymer series and histidine. The formation of the imine is catalysed by acid which protonates the aldehyde, making it more prone to nucleophilic attack. After the imine formation, the acetic acid was evaporated and replaced with methanol for the reduction. The reducing agent used in this work was sodium borohydride.

Histidine was successfully reacted onto the aldehyde functional PNiPAm-*co*-PFPMA polymer series (**P1**-**P4)**, without any protecting group chemistry, to form histidine functional polymers (**P5**-**P8**, respectively). The conversion of polymers **P1**-**P4** to **P5**-**P8** was confirmed by ^{1}H NMR. The absence of the aldehyde peak **g** present in the ^{1}H NMR spectra of PNiPAm-*co*-PFPMA and the presence of broad peaks for methyne (**d**), methylene (**c** and **e**), and imidazole (**b**) peaks (illustrated in Fig. 1) indicate that histidine is covalently bound and that we have synthesised a histidine functional, temperature responsive polymer.

By controlling the amount of aldehyde in the temperature responsive polymer platform, we can control the histidine content of the resulting histidine functional polymers. This is demonstrated by the synthesis of 4 platform polymers **P1**-**P4** and their subsequent conversion to histidine functional polymers **P5**-**P8**. With increasing histidine content, the ^{1}H NMR spectra show increased signal and increased broadening in the methyne (**d**), methylene (**c** and **e**), and imidazole (**b**) peaks, shown in Fig. 2.

Scheme 1 Post polymer functionalisation of thermoresponsive poly(N-isopropylacrylamide) copolymer with unprotected histidine via reductive amination.



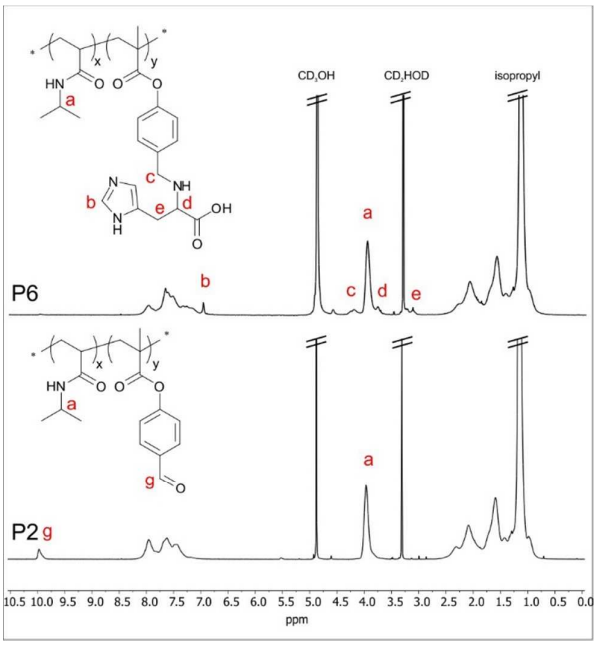


Fig. 1 The ^{1}H NMR spectra of the polymer platform **P2** (bottom) and the histidine functionalised polymer **P6** (top). The disappearance of the aldehyde peak **g** in **P6**, as well as the broadening of histidine peaks (methyne peak **d**, methylene peaks **c** and **e**, and imidazole peak **b**) indicate the presence of histidine on the polymer and the successful functionalisation of the aldehyde functional handle with histidine.

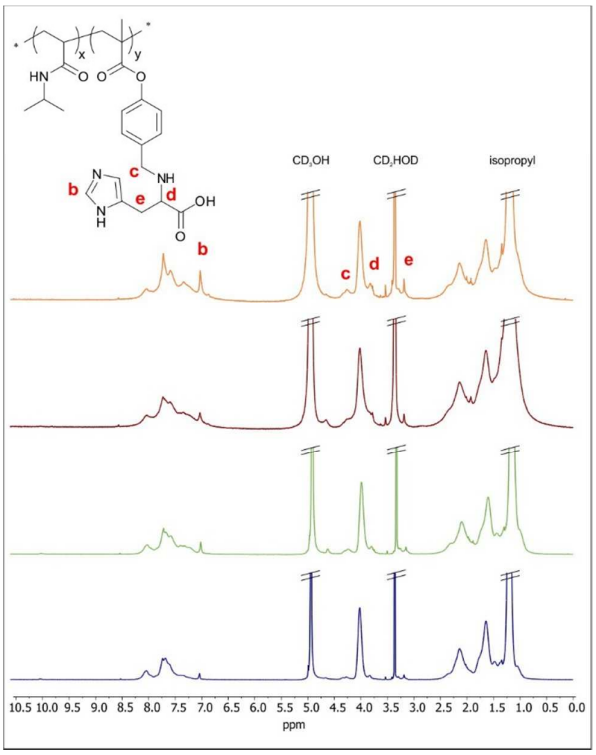


Fig. 2 Controlling the amount of aldehyde in the platform polymer series **P1**-**P4** allows us to control the amount of histidine on the histidine functional polymers **P5**-**P8**. Here, the ^{1}H NMR spectra of histidine functional, temperature responsive polymers **P5-P8** demonstrate our series of polymers with increasing histidine content.

Polymers **P5**-**P8** were titrated due to the pH responsive nature of the histidine moiety to quantify and confirm the identity of the ionisable groups. The quantification was achieved by comparing the titration curve of the polymers **P5-P8** to that of an electrolyte solution. The points of inflection of the titration curves correspond to known pKa values for carboxylic acids (pKa ≈ 4.5),[23] protonated imidazoles (pKa ≈ 7),[24] and protonated amines (pKa ≈ 9-10).[25] We achieved 65% to 90% conversion using reductive amination without the use of protecting groups. Table 1 summarises the conversions.

To corroborate the ^{1}H NMR and titration evidence of polymer functionalisation, FTIR spectra of the starting materials and functionalised polymers were compared. In the polymer platform **P4**, the peaks at 1750 cm^{-1} and 1697 cm^{-1} correspond to C=O stretches for the ester and aldehyde (respectively) present in the **4-FMPA** unit and the strong peaks at 1644 cm^{-1} and 1534 cm^{-1} correspond to amide stretches from the PNiPAm. In the functionalised polymer **P8**, the PNiPAm stretches are still present and so is the C=O ester stretch at 1750 cm^{-1} however the aldehyde stretch is absent (Fig. 3, see Fig. S3 for full spectra). This indicates the successful functionalisation of the polymer, and combined with the NMR data, demonstrating the versatility of reductive amination chemistry to functionalise polymers **P1**-**P4** with histidine.

The pH response of this material suggests that it has four charged states as the pH is increased from 2 to 12. At low pH (<3), the carboxylic acid is protonated and charge neutral while the imidazole group and the secondary amine are positively charged, yielding an overall doubly positive charge on the histidine pendant groups. Increasing the pH above 4 results in the deprotonation of the carboxylic acid, reducing the overall charge to +1. Further increasing the pH above 7.5 results in the deprotonation of the protonated imidazole group and brings our material into its zwitterionic state. And finally, in highly basic conditions with pH in excess of 10.5, the deprotonation of the secondary amine leaves a single negative charge on the carboxylic acid. Fig. 4 illustrates the different charged states as a function of pH.

LCST response of the polymers

The temperature response of PNiPAm is due to hydrogen bonding between the polymers polar groups and water. Below the LCST, water molecules are hydrogen bonded to the polymer, giving it a coil conformation which is in solution. Above the LCST, there is enough thermal energy to break the polymer-water hydrogen bonds and the polymer hydrogen bonds with itself, undergoing a coil to globule transition. The addition of hydrophilic groups to a temperature responsive polymer results in a higher LCST due to the increased hydrogen bonding of water with the hydrophilic groups.[26] As expected the addition of histidine to the PNiPAm polymers results in an increased LCST, demonstrated with **P5** and **P6** with 3% and 8% histidine, respectively, in Fig. 5. As we further increase the histidine content in samples **P7** and **P8**, to 12% and 23% respectively, the thermal response broadens. This is consistent

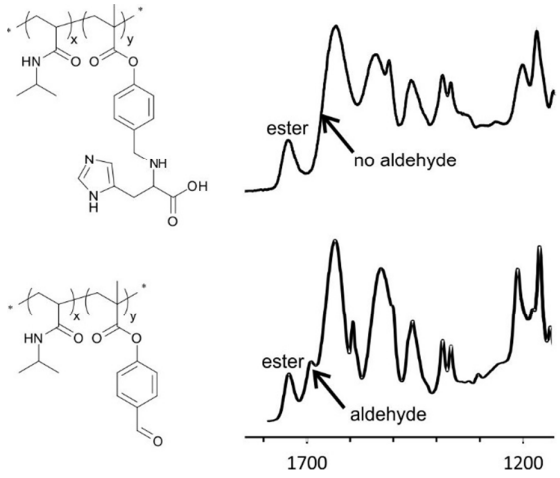


Fig. 3 The FTIR spectra of the copolymer platform **P4** (bottom) shows clear ester and aldehyde C=O stretching. While the C=O stretch from the ester is still present in the functionalised polymer **P8** (top), the absence of the aldehyde peak indicates the successful polymer functionalisation with histidine. While the FTIR spectra for Polymers **P1**-**P3** and **P5**-**P7** show the same aldehyde peak and its absence, polymers **P4** and **P8** are shown due to the higher incorporation of aldehyde, showing the most pronounced aldehyde peak.

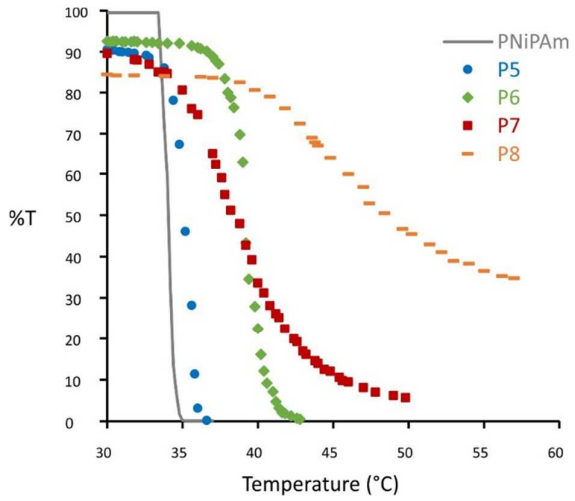


Fig. 5 The LCSTs of our histidine functional, temperature responsive polymers **P5**-**P8** relative to that of PNiPAm (measured by transmission measurements at 540 nm) with varying incorporations of histidine. The pH of the solutions ranged between 6.2 and 6.8, due to the pH responsive nature of the histidine moieties. At low incorporations of histidine, the LCST of the copolymer is increased as expected due to the addition of hydrophillic groups (samples **P5** and **P6**). At higher incorporations, the LCST is broadened and thermoresponsive nature of the PNiPAm is competing with that of the zwiterionic histidine moieties (**P7** and **P8**).

with results reported by Chang *et al.*[27] who showed that a copolymer of NiPAm with sulfobetaine methacrylate (SBMA) with molar ratio of 70:30 exhibited UCST and LCST behaviour, with broadened transitions. The broad transition might be explained by the histidine groups exhibiting zwitterionic behaviour at near neutral conditions, where the addition of heat causes an increase in solubility in the zwitterionic blocks.[28] With increased incorporations of histidine, the broadening of the LCST transition could be due to the competition between the increased solubility of the histidine groups and the decreased solubility of the PNiPAm groups.29 The incorporation of 23% histidine groups in P8 is enough to prevent the full decrease in transmission of light through the polymer solution, indicating a decrease in solubility, but not to the same extent as with less histidine incorporation.

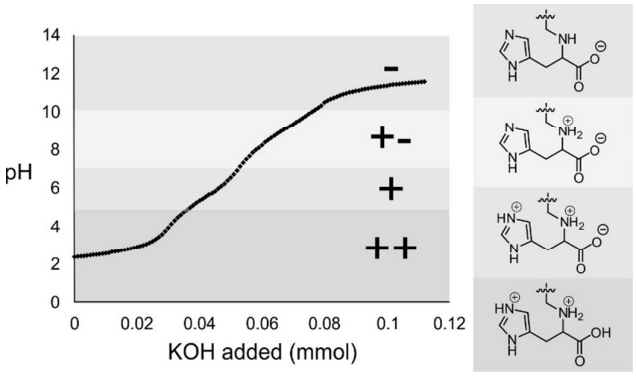


Fig. 4 The titration curve for the temperature responsive, histidine functional polymer **P8** showing the suggested charged states of the material for the range of pH values between 2 and 12.

Polymers with pH responsive moieties and temperature responsive moieties are known to have an LCST that is affected by pH.[30] The effect of pH on the LCST of the histidine functionalised polymers was measured in order to characterise the temperature response. At a pH of 6.4, the LCST of **P7** is approximately 38°C. The pH was adjusted to 3.0 and to 10.2 and in both cases, the LCST was increased, shown in Fig. 6. At a pH of 6.4 without the addition of any ions into the sample, the LCST is lower than in the charged states. The charged states of the polymer, both positive and negative, at pH 3.0 and 10.2 increase the solubility and hydrophilicity of the polymer chain. The addition of charge to the polymer increased the LCST of

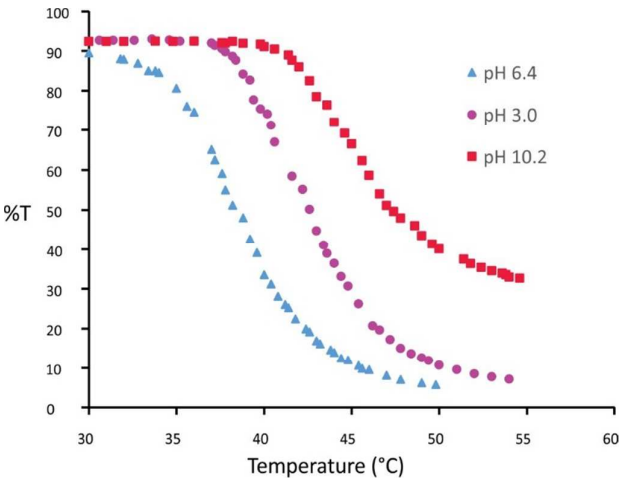


Fig. 6 The effect of pH on LCST of the 16% histidine functional polymer, **P7**. The lowest LCST occurs near neutral pH, while increased LCST is observed for acidic and basic conditions where the polymer is expected to have net positive and negative charges, respectively.

the material which results from the increase in solubility from the pH responsive charged states.

In order to further understand this pH responsive behaviour of the system, DLS was used to measure the hydrodynamic radius of **P7** as a function of both temperature and pH with (0.10 M KCl) and without added salt. At neutral pH, the temperature responsive aggregation is visible and observable as an opaque solution. The pH of the polymer solutions had a dramatic effect on the size of the hydrodynamic radii upon heating. At both acidic and basic pH, the size of the polymer aggregates at 50°C were increased from 5 nm at room temperature to 100-200 nm without any visible opacity. In 0.1 M KCl solution, the acidic pH caused smaller aggregates to form from the polymer solution, with the size increasing to only ≈ 30 nm at 50°C from 5 nm at room temperature. Fig. 7 shows these trends). The increase in charge (doubly positive charge at pH less than 3, single negative charge at pH above 10) at the lower pH, indicates a change in the copolymers self-assembling behaviour with pH. The excessively large aggregates found in the neutral pH could also be attributed to the LCST aggregation of PNiPAm followed by the swelling of the zwitterionic blocks at elevated temperatures.

Throughout all pH ranges the polymers were not observed to aggregate except upon the addition of heat. We expect that a larger molar ratio of histidine moieties would result in greater pH responsive behaviours, or that a DLS study of the hydrodynamic radius of the polymers as a function of pH would likely show signs of micellisation, to be expanded upon in a future study.

Conclusions

A simple, high yielding approach to functionalise the temperature responsive polymer PNiPAm with histidine without the need for protection group chemistry has been developed. Copolymers of NiPAm with aldehyde containing **4-FPMA** incorporations varying between 4 and 26% were synthesised and subsequently functionalised with histidine via reductive amination with 65% to 90% conversion. The strategy demonstrates the versatile and effective use of reductive amination chemistry for polymer functionalisation, with its mild reaction conditions and tolerance to many functional groups, including the imidazole of histidine. Importantly, these synthetic methods enable the design of new polymers functionalised with amino acids with the side groups of amino acids preserved. The temperature responsive histidine functional polymers synthesised exhibit interesting pH and electrolyte dependent LCST behaviour. This highly functional material exhibits multiple responsive behaviours. The facile synthesis negates the need for protecting group chemistry and demonstrates a powerful strategy to produce amino acid and other amine functional polymers.

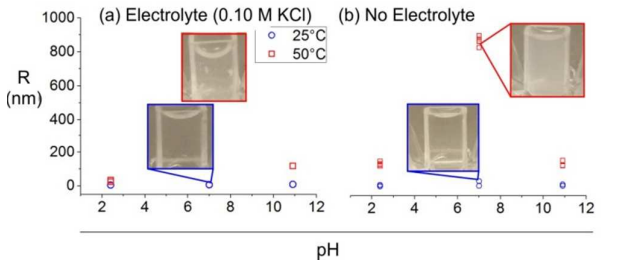


Fig. 7 DLS data exploring the size of **P7** as a function of temperature at different pH values with and without electrolyte. Photographs of the neutral pH samples are shown above for 25°C (blue circles) and 50°C (red squares) measurements. The DLS measrements did not converge, however visible aggregation is clearly seen in both cases. The aggregates clump together in the polymer electrolyte solution, shown in Fig. 7a.

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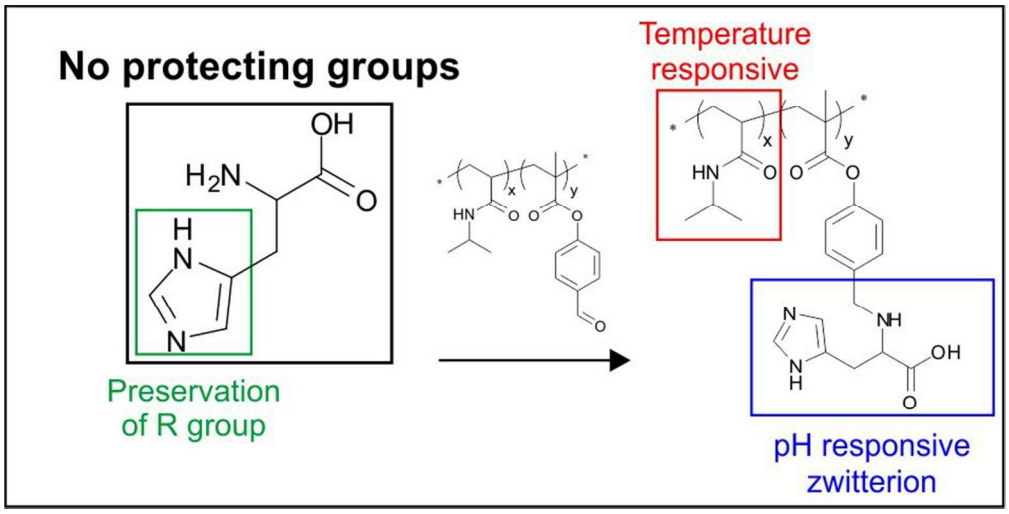

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