Supplementary Material (ESI) for Polymer Chemistry


# Supporting Information

# Phosphatase/temperature responsive poly (2-isopropyl-2-oxazoline)

Pier-Francesco Caponi, [a] Xing-Ping Qiu, [b] Filipe Vilela, [a] Françoise M. Winnik [b] and Rein V. Ulijn[a]

[a] Department of Pure and Applied Chemistry/WestCHEM, The University of Strathclyde, Glasgow G1 1XL, UK.

[b] Department of Chemistry and Faculty of Pharmacy, University of Montreal, Montreal, QC Canada H3C 3J7, Canada.

**Materials** 2-isopropyl-2-oxazoline (iPrOx, Tokyo Chemical Industry) was stirred overnight with $CaH_2$, vacuum distilled and stored under nitrogen atmosphere. Alkaline phosphatase (New England BioLabs, 10.000 U/ml, one unit is defined as the amount of enzyme that hydrolyzes 1µmol of p-nitrophenylphosphate to p-nitrophenol in a total reaction volume of 1 ml in 1 ml at 37°C), propargyl p-toluensulfate (Fluka), 11-azido-3,6,9-trioxaundecan-1-amine (Aldrich), anhydrous N,N-dimethylformamide (DMF, Aldrich), dichloromethane (DCM, Aldrich), N-hydroxysuccinimide (NHS, Aldrich), N,N-diisopropylcarbodiimide (DIC, Aldrich), 3.5 kDa regenerated cellulose membrane (Triple Red Ltd), 500 Da cellulose acetate membrane (Aldrich) and anhydrous acetonitrile (AcN, Aldrich) tetrahydrofuran (THF, Aldrich) were used as received.

**Instrumentation** Fluorescence studies were performed on a Jasco FP-6500 spectrofluorometer. UV/Vis absorbance was measured on a Beckman Coulter DU 800 spectrophotometer equipped with a Beckman Coulter High Performance Temperature Controller. $^1$H NMR spectra were recorded on a Bruker AV 400 at 292 K. DLS was performed on an AVL/LSE-5004 light scattering electronics and multiple tau digital correlator using an angle of 90°. MALDI-TOF mass spectrometry was performed on a Kratos Analytical AXIIMA CFR using dithranol matrix. High performance liquid chromatography (HPLC) was carried out on a Dionex P680 HPLC system fitted with a UVD170U detector. An aliquot sample (100 µl) was injected into a Macherey-Nagel C18 column of the following dimensions: length 250 mm; internal diameter 4.6 mm; particle size 5 µm; flow rate 1 ml min$^{-1}$.

## Polymer synthesis and characterisation

**Polymerisation** Polymerisation of iPrOx was performed following a procedure reported in literature.[16] Microwave vials (0.5-2.0 mL) were left in a heating oven (125 °C) and cooled down to room temperature under nitrogen atmosphere. A solution containing 0.067 mmol of propargyl p-toluensulfate and 4 mmol of iPrOxwas made directly in the microwave vial under nitrogen atmosphere and under stirring using AcN as the solvent. Total reaction volume was 1 ml. The vial was capped, and heated at 140 °C for 11 minutes. After the reaction, excess of $H_20$ was added to the vial, and the solution was extensively dialysed against water for 3 days. After dialysis the solution was freeze-dried.

**$^1$H NMR** The spectra were calibrated using the solvent signal ($CDCl_3$ 7.26 ppm). Polymer concentration used was 2-5 mg ml-1 (Fig. S1).

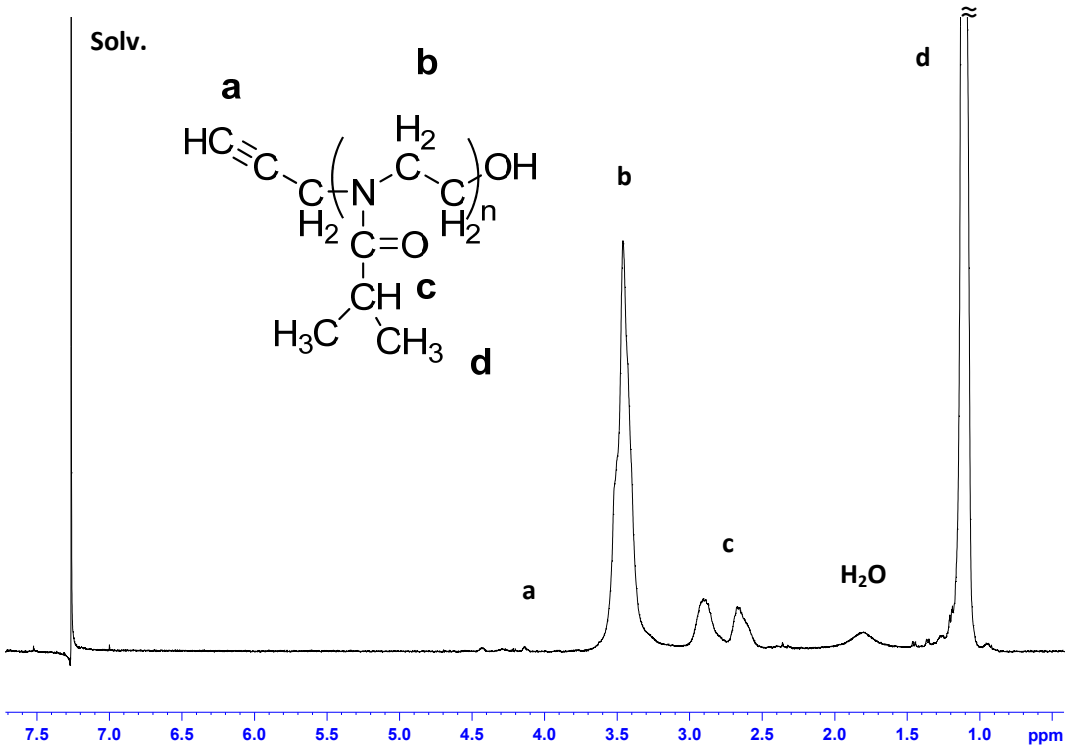


Fig. S1. $^{1}$H NMR spectra of ST propargyl-PiPrOx-OH in $CDCl_3$.

**MALDI-TOF** The samples were prepared by mixing THF solution of the polymer and matrix (20 mg $ml^{-1}$) in a ratio of 2:1 (v/v) (Fig S2).

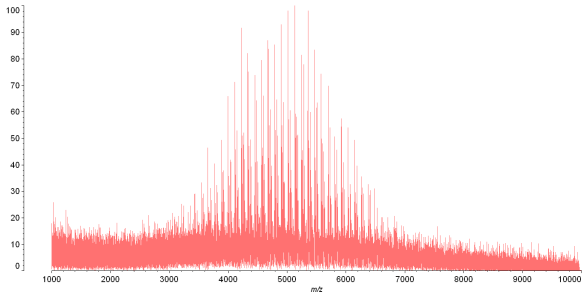


Fig. S2. MALDI-TOF mass spectrometry spectra of ST propargyl-PiPrOx-OH.

## Synthesis and characterisation of Fmoc-*p*Y bearing terminal $N_3$

**Synthesis** Synthesis of Fmoc-*p*Y bearing a terminal azide group was performed using a standard coupling procedure. The carboxylic terminus of Fmoc-*p*Y was activated during an overnight reaction in the rotator using 2 eq. of NHS and DIC in DMF/DCM (1:1). A solution containing 1 eq. of 11-azido-3,6,9-trioxaundecan-1-amine and 2 eq. of TEA in DMF/DCM (1:1) was added to the activated the amino acid**.** The reaction lasted overnight under rotation. The dialysis, which was followed from freeze-drying, was performed with a membrane of regenerated cellulose (500 Da molecular weight cut off).

**Characterisation** The purity of the obtained compound after the purification process was assessed by HPLC (initial flow rate 40% $H_2O$, 60 % AcN) dissolving 0.2 mg of Fmoc-*p*Y-OH in 1 ml of water, as a control experiment, and the same amount of Fmoc-*p*Y-$N_3$ complex (Fig. S3).

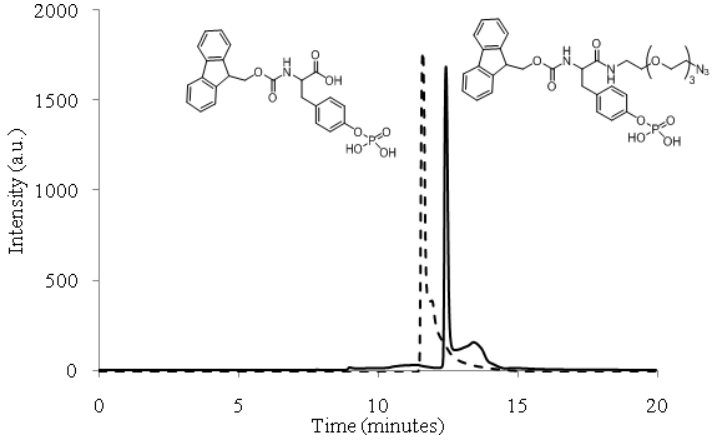


Fig. S3. HPLC data showing the switch in the retention time between Fmoc-*p*Y-OH (dotted line, 11.5 minutes) and Fmoc-*p*Y-$N_3$ complex (continuous line, 12.5 minutes).

## "Click" coupling

**"Click" reaction** (Scheme 1) was performed in water, using $CuSO_4$ as catalyst and ascorbic acid as reducing agent to convert Cu (II) in Cu (I). The ratio used is the following: propargyl group/azide group/ $CuSO_4$ 1:2:0.3. 2 eq. of azide and 0.3 eq. of $CuSO_4$ and ascorbic acid were dissolved in a solution containing the polymer in water (final volume 7 ml). The reaction was left overnight on the rotator, before going through the purification process, using a membrane having a molecular weight cut off of 3.5 kDa.

**Fmoc loading quantification** The freeze-dried polymer was used to evaluate the Fmoc loading by UV absorbance of Fmoc at 300 nm, comparing it with the concentration/absorbance dependence of a calibration curve (Fig. S4). A known amount of polymer (~0.5-1.0 mg) was dissolved in 1 ml of water. The obtained absorbance at 300 nm was used to evaluate the concentration of Fmoc, which was then divided for the number of functionalised amino termini present on the polymer to obtain the final loading. The calibration curve was obtained taking the absorbance values of Fmoc-*p*Y dissolved in $H_2O$, at different concentrations.

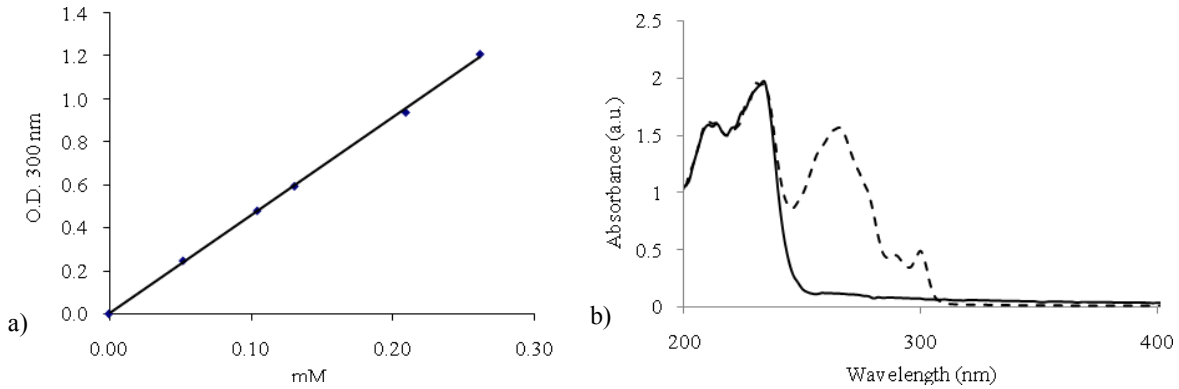


Fig. S4. a) Plot of the calibration curve used for the quantification of the Fmoc-*p*Y loading on the polymers b) PiPrOx UV spectra before (continuous line) and after functionalisation (dotted line) with Fmoc-*p*Y.

## UV/Vis experiments

**LCST** A thermostatic cell in the UV was used to evaluate the cloud point temperature of the polymers. The absorbance of a known concentration of polymer dissolved in water (1 mg/ml) was read at 600 nm, in order to have no absorbance at room temperature.
The sample was heated in the thermostatic cell with intervals of 0.5/1.0°C, with a holding time of one minute within a temperature range of 30-50°C. The absorbance started to increase when the phase transition temperature of the polymer was reached and transmittance values plotted into a graph.
Optical images were taken as further proof of the reversibility of phase transition (Fig. S5).

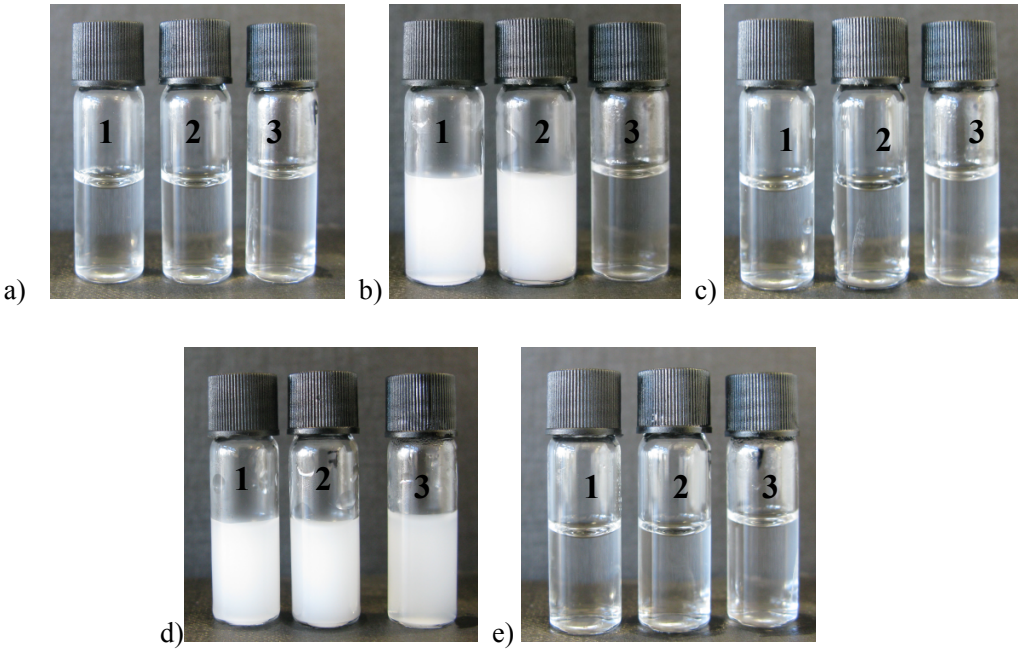


Figure S5 Optical images of 1, 2 and 3 showing the reversibility of phase transition. a) Room temperature. b) After heating at 40°C. c) After cooling at room temperature d) After heating at 50°C. e) After cooling at room temperature.

**LCST cycles** Using the same procedure used to evaluate the LCST, the reversibility of the thermal properties of the polymer was assessed. Solutions with the same concentrations of polymer were heated above and below the phase transition temperatures (Fig. S6).

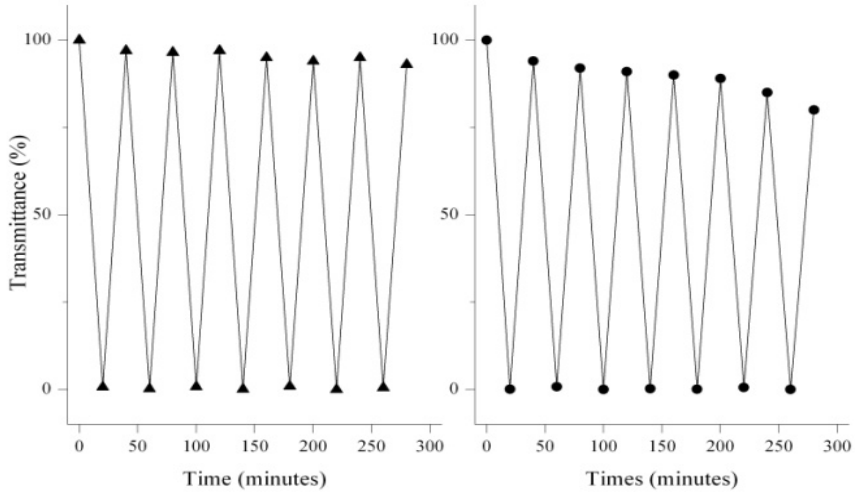


Fig. S6 LCST study for the reversibility of thermal transition for ST OH-PiPrOx-*p*Y-Fmoc (1) (▲) and ST OH-PiPrOx-Y-Fmoc (2) (●). Higher values of transmittance correspond to 35°C while lower values to 45°C.

**Fluorescence experiments**

Aqueous solution, containing a known amount of polymer (<0.01 mg), was used to take the initial measurement. In order to verify that the fluorescence spectra did not change prior to the enzymatic addition, before each experiment the sample was left 1 hour in the fluorimeter and the spectra were checked. 50 U of phosphatase (5 μL) were then added directly to the fluorimeter cuvette and changes in fluorescence spectra recorded. Used excitation wavelength 290 nm, emission range 295-500 nm, band width (Ex) 3 nm, band width (Em) 3 nm.

**DLS measurements**

Aqueous solutions of polymer (2.5 mg/ml) were used to determinate the average particle sizes before and after the enzymatic reaction. Prior to the addition in the light scattering vial, the solution was filtered (PDV 45 μm filter) to eliminate impurities. After taking a first measurement, 50 U of phosphatase (5 μL) was added directly to the vial and the sample was left overnight at room temperature, before taking a second measurement standing for the average particle size after the enzymatic conversion. Each measurement was repeated 3 times to assess the reliability of the results.