Electronic Supplementary Material (ESI) for Soft Matter

Supporting Information

Charge complementary enzymatic reconfigurable polymeric nanostructures

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Materials 2-isopropyl-2-oxazoline (iPrOx, Tokyo Chemical Industry) was stirred overnight with calcium hydride (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 pnitrophenylphosphate to p-nitrophenol in a total reaction volume of 1 ml at 37°C), propargyl p-toluensulfate (Fluka), 11-azido-3,6,9-trioxaundecan-1-amine (Aldrich), anhydrous N,N dimethylformamide (DMF, Aldrich), *O*-(Benzotriazol-1-yl)-*N,N,N′,N′*-tetramethyluronium hexafluorophosphate (HBTU, Aldrich), *N,N*-Diisopropylethylamine (DIPEA, Aldrich), 3.5 kDa regenerated cellulose membrane (Triple Red Ltd), 500 Da cellulose acetate membrane (Aldrich) anhydrous acetonitrile (AcN, Aldrich), tetrahydrofuran (THF, Aldrich), fluorenylmethoxyloxycarbonyl-phosphorylated tyrosine (Fmoc-*p*Y-OH) fluorenylmethoxyloxycarbonyl-lysine (tert-butyloxycarbonyl) (Fmoc-K(Boc)-OH) 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. 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}. Atomic Force Microscopy (AFM) was performed on a Veeco Innova Scanning Probe Microscopy. The tip was a Phosphorous doped Si tapping mode tip and the instrument was operated in tapping mode. Images were processed using a 2D plane fit using the software included in the instrument.

Polymer synthesis and characterization

Polymerisation Polymerisation of iPrOx was performed following a procedure reported in literature.[16] Microwave vials (2.0-5.0 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 ptoluensulfate and 4 mmol of iPrOx was made directly in the microwave vial under nitrogen atmosphere and under stirring using AcN as the solvent. Total reaction volume was 3 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 dialyzed against water for 3 days. After dialysis the solution was freeze-dried.

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 S1).

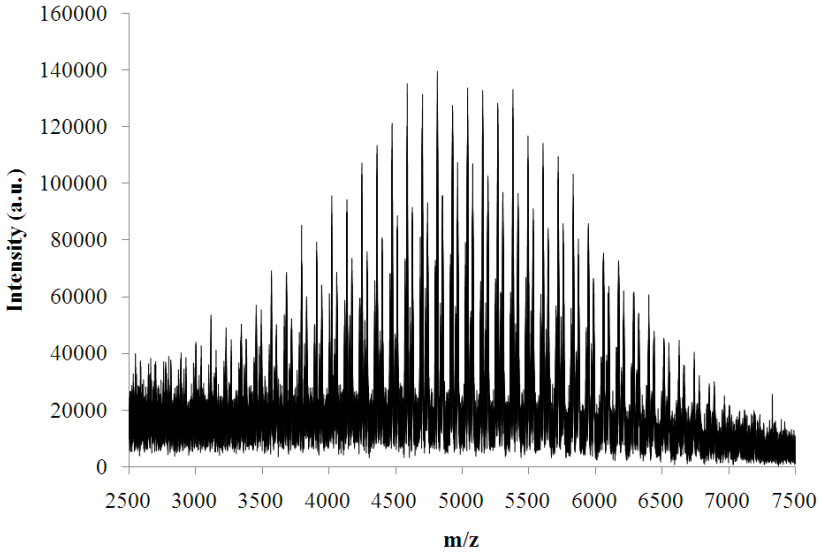


Fig. S1. MALDI-TOF mass spectrum of ST propargyl-PiPrOx-OH.

Synthesis and characterisation of Fmoc-amino acids bearing terminal N_3

Synthesis of azide functionalized Fmoc-amino acids 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 using 2 eq. of HBTU and DIPEA in DMF/DCM (1:1). A solution containing 1 eq. of 11-azido-3,6, 9-trioxaundecan-1-amine a in DMF/DCM (1:1) was added to the activated the amino acid**.** The reaction was left overnight under rotation. Dialysis was performed with a membrane of regenerated cellulose (500 Da molecular weight cut off). Finally, the material was freeze dried. Synthesis of Fmoc-K(Boc)-OH bearing a terminal azide group was performed using the same coupling procedure detailed above. Removal of Boc protecting group was performed overnight using a solution of TFA/H_2O (1:4 ratio). Solvents were removed using a vacuum pump.

Characterisation The purity of the obtained compounds was assessed by HPLC (initial flow rate 40% H_2O, 60 % AcN) dissolving 0.2 mg of either Fmoc-*p*Y-OH (control) or Fmoc-*p*Y-N_3 in 1 ml of AcN/H_2O (1:1 ratio) (Fig. S2) or Fmoc-K(Boc)-OH / Fmoc-K(Boc)-N_3 (Fig. S3). Purity of the final products was calculated to be 93% and 87% for Fmoc-*p*Y-N_3 and Fmoc-K(Boc)-N_3, respectively.

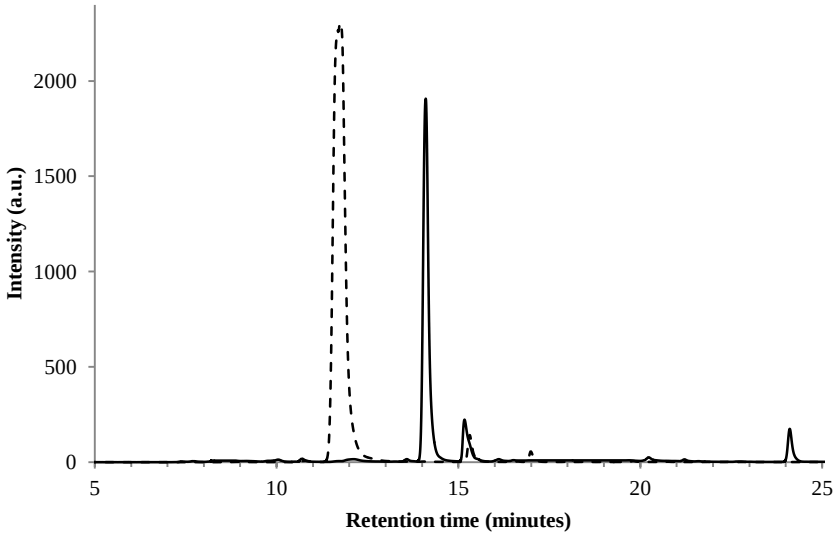


Figure S2. HPLC chromatogram obtained at 300 nm showing Fmoc-*p*Y (dotted line) and Fmoc-*p*Y-N_3 (continuous line) as the main peaks.

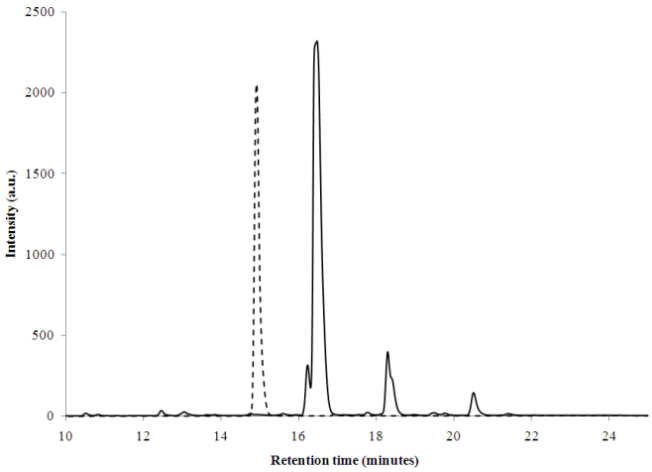


Figure S3. HPLC chromatogram obtained at 300 nm, showing Fmoc-K(Boc)-OH (dotted line) and Fmoc-K(Boc)-N_3 (continuous line) retention time.

After deprotection the Fmoc-K-N_3 was analyzed through MS to check the reaction was successful (Fig. S4).

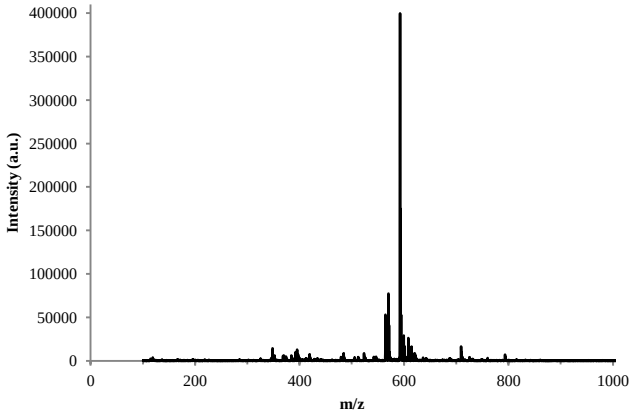


Figure S4. MS showing molecular weight profile of purified Fmoc-K-N_3 complex. The main peak has a value of 592, corresponding to the sodium adduct of Fmoc-K-N_3 (Mw=569).

"Click" coupling of Fmoc-amino acid azide and polymer

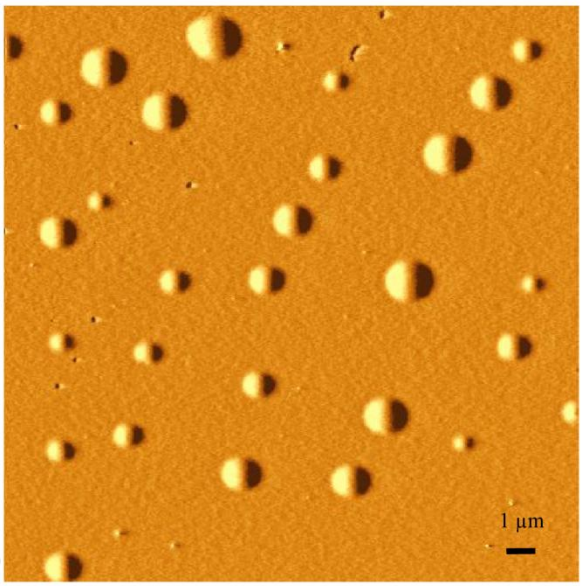
"Click" reaction (Scheme 1) was performed in water, using $CuSO_4$ as catalyst and ascorbic acid as reducing agent in a ratio propargyl group/azide group/ $CuSO_4$ 1:2:0.3. 2 eq. of azide and 0.3 eq. of $CuSO_4$ and ascorbic acid were dissolved in a solution containing the polymer in water (final volume 7 ml). The reaction was left overnight on the rotator, before purification by dialysis, using a membrane with a molecular weight cut off of 3.5 kDa and freeze drying.

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. Calibration curves were obtained for each Fmoc-amino acid by measuring the absorbance values of Fmoc-*p*Y and Fmoc-K dissolved in H_2O, at different concentrations. 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.

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 0.2°C, within a temperature range of 25-60°C. The absorbance started to increase when the phase transition temperature of the polymer was reached and transmittance values plotted into a graph.

AFM experiments

a) 

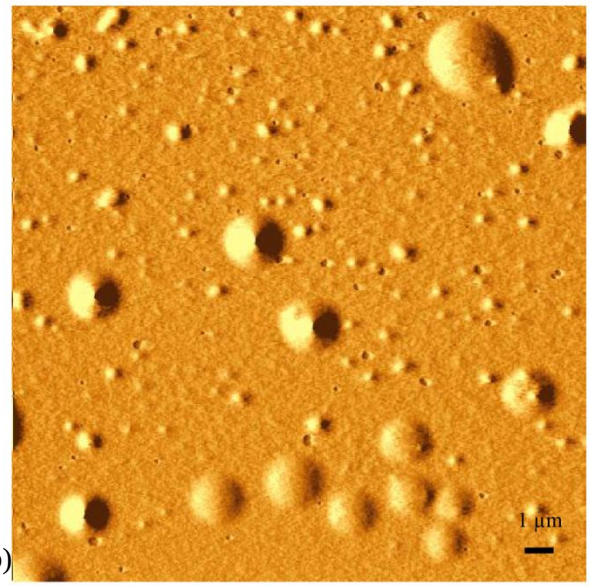
b) 

Figure S5. AFM images showing aggregates formed by Fmoc-pY-PiPrOx + Fmoc-pY-PiPrOx prior (a) and after (b) enzyme addition.

Fluorescence experiments

Aqueous solution, containing a known amount of polymers (<0.01 mg), was used to take the initial measurement. The solution was left to incubate and measurements were taken every 12 hours. When the fluorescence spectrum did not show further changes, 50 U of phosphatase (5 µL) was 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.

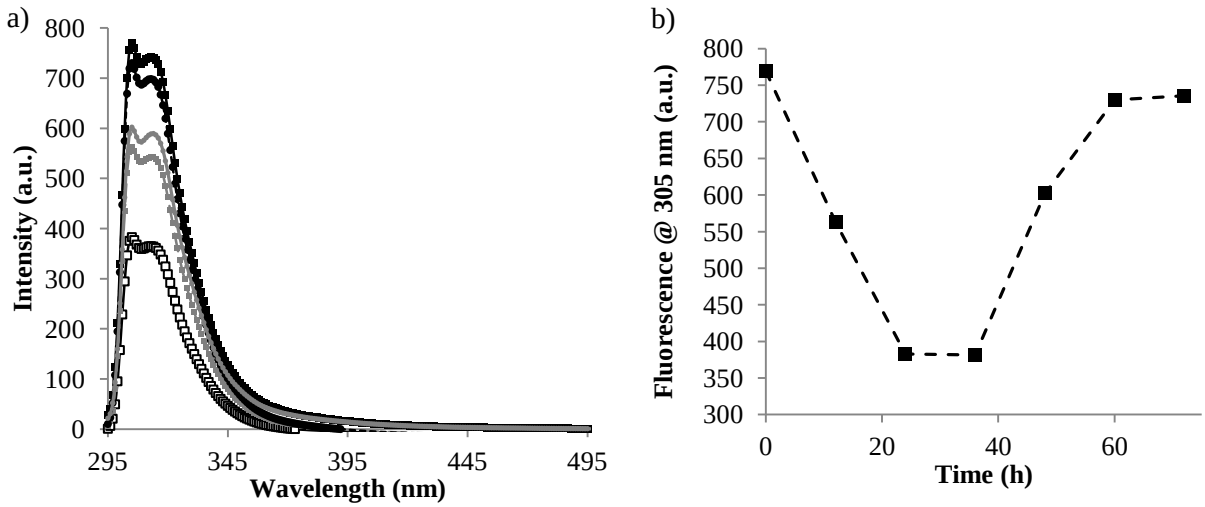


Figure S6. Fmoc-pY-PiPrOx + Fmoc-K-PiProx self-assembly kinetics followed by fluorescence exploiting the Fmoc peak at 305 nm. a) Dissolution of the polymers (■), 12 hours incubation (■), 24 hours incubation, enzyme addition (□), 12 hours after enzyme addition (●), 24 hours after enzyme addition (●); b) Fmoc intensity @ 305 nm plotted against time.

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 0.2 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 ensure reproducibility.

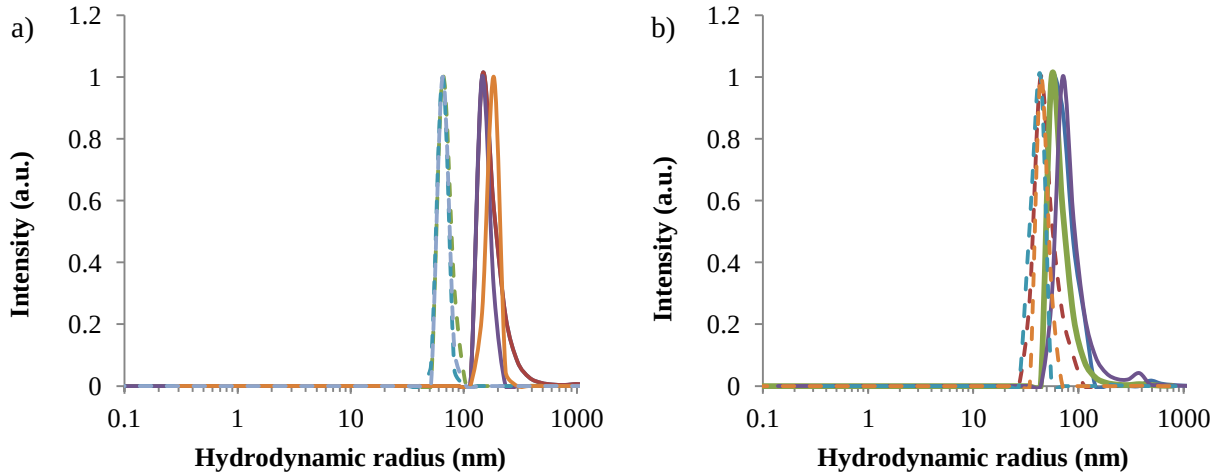


Fig. S7. DLS data for 1+3 (a) and 2+3 (b). Dotted lines show hydrodynamic radii above the LCST while continuous line below the LCST.