Electronic Supplementary Material (ESI) for Polymer Chemistry

A Fluorescently Labeled, hyperranched Polymer Synthesized from DE-ATRP for the Detection of DNA Hybridization

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Supporting Information

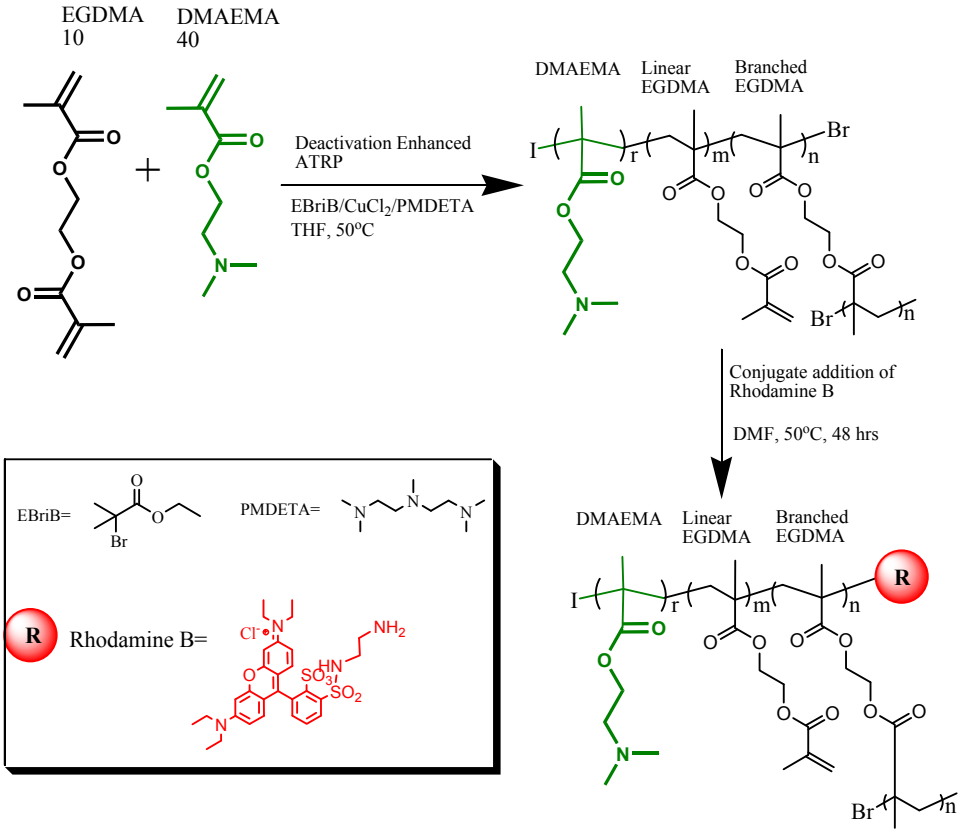
Materials:

2-(Dimethylamino) ethyl methacrylate (DMAEMA, M_n=157.22 g/mol) and Ethylene glycol dimethacrylate (EGDMA, M_n=198.22) (macro) monomers were purchased from Sigma-Aldrich. Ethyl-α-bromoisobutyrate (Aldrich) was used as the initiator. Bis(2-dimethylaminoethyl)methylamine (PMDTA, 99%, Aldrich), Copper (II) Chloride ($CuCl_2$, 97%, Aldrich) and [1,2-dihydroxyethyl]-3,4-dihydroxyfuran-2-one, L-Ascorbic acid (Sigma) were used as received. Tetrahydrofuran (HPLC grade, 99.9%, Sigma-Aldrich) was used as the solvent. The single stranded DNA probe (ssDNA probe, sequence: 5'-TAACACTGTCTGGTAAAGATGG-3'), single stranded DNA complementary target (cDNA, sequence: 5'-CCATCTTTACCAGACAGTGTTA-3') and single stranded non-complementary target (NC-target, sequence: 5'-CCATC**GGG**ACCAG**TA**AGTGTTA-3') were purchased from Eurofins MWG Operon (Germany).

Polymer synthesis and purification:

The polymer was prepared in tetrahydrofuran (the volume ratios of total monomers to solvent = 1:2) at 50˚C using a Schlenk line system to bubble argon through the solutions to remove oxygen. Liquids were transferred by means of septa and syringes while under argon. A typical reaction procedure is described: The DMAEMA (6.288 g, 40 mmol), EGDMA (2 g, 10 mmol), initiator (0.195 g, 1 mmol), PMDTA (0.0216 g, 0.125 mmol), $CuCl_2$ (0.01675 g, 0.125 mmol) and tetrahydrofuran (30 ml) were transferred to a round-bottom flask fitted with a two-way stopcock. Argon was bubbled through the solution for 15 minutes to remove the oxygen. L-Ascorbic acid was added into the flask to start the reaction which was kept in an oil bath at 50˚C and stirring at 600 rpm. Samples were taken at different time points for GPC analysis to monitor the monomer conversions by comparing the peak areas for monomers and polymers. The reaction was stopped when the desired monomer to polymer ratio was obtained. After polymerization, the obtained polymers were precipitated by adding the solution drop-wise into a large excess of hexane to remove EGDMA. To remove the DMAEMA monomer, the precipitated mixture of polymer and monomer was dissolved in acetone and protonated by adding HCl

drops to the solution until a pH 5 was obtained. The protonated polymer was then purified by dialysis (Spectra/Por dialysis membrane, molecular weight cut off 1000) in deionised water which was changed on a regular basis for 72 hours in the dark at room temperature. The final polymer solution was freeze-dried and weighed.



Scheme S1. Synthesis of DMAEMA and EGDMA into hyperbranched polymer *via* DE-ATRP method.

Polymer characterization:

The resultant polymer was characterized by gel permeation chromatography (GPC) and proton nuclear magnetic resonance (^{1}H NMR). Number average molecular weight (M_n), weight average molecular weight (M_w), and polydispersity (M_w/M_n) were obtained by GPC (920-LC Liquid Chromatograph, Varian) with a refractive index detector, column heater and evaporative light scattering (ELS) detector supplied by Varian. The columns (300×7.5 mm PolarGel-M Column, two in series) were eluted using DMF and calibrated with poly (methyl methacrylate) standards. All calibrations and analyses were performed at 40 °C and a flow rate of 1 ml/min. ^{1}H NMR was carried out on a 400 MHz JEOL NMR with DELTA processing software. The chemical shifts were referenced to the lock D_2O.

Polymer labelling:

Hyperbranched polymer was labelled with Lissamine™ Rhodamine B ethylenediamine (1.5 mg, 2.5 µmol) by reacting the Rhodamine with the terminal bromine functions of the pD-co-E polymer (3 mg, 0.25 µmol). The extent of labelling was semi-quantified by optical determination of the fluorescence intensity provided by a standardised amount of polymer (spectral scanning multimode reader: Thermo Scientific, Varioskan® Flash, US). Briefly; 3mg of DMAEMA and 1.5mg of rhodamine were dissolved in 5 ml of DMF and placed in a 25-ml-flask equipped with a magnetic stirring bar. The flask was then fitted with a rubber septum and the content degassed by purging argon for 15 min to remove oxygen. The reaction mixture was stirred at 50˚C for 48 hours. Unreacted rhodamine was removed by dialysis against distilled water. The purified product was obtained as a pink powder after freeze-drying.

UV Visible spectroscopy:

The pD-co-E/ssDNA probe complexation at different ratios was initially analysed by UV-Visible absorption, wherein; 20 µl total volume solution containing pD-co-E+ssDNA at 0:1, 0.5:1, 1:1, 2:1, 5:1, 10:1, or 1:0 were used.

A solution with a total volume of 60 µl containing 8.3 µM of PD-co-E and 10 µM of ssDNA probe was incubated at room temperature for one hour to obtain the duplex. After the incubation period, 20 µl of the solution (which by now has formed the duplex) was drawn out and added to 20 µl of 10 µM cDNA target, or 20 µl of 10 µM ssDNA non-complementary target (negative control). For each sample, 5 µl were drawn out and UV-visible absorption was measured using the NanoDrop™ 2000N (Thermo Scientific).

NOTE: The volumes were significantly higher (i.e 60 µl) than what is required (5 µl) to allow for easier handling. The same stocks were also used to obtain the gel electrophoresis results.

Gel electrophoresis:

The electrophoretic mobility of the pD-co-E/DNA complexes at different pD-co-E/DNA ratios were determined by gel electrophoresis using a 7% and 15% agarose gel in a buffer consisting of 45mM Tris-borate and 1 mM EDTA at pH 8.0. Experiments were run at 80V for 90 min. DNA was visualized under UV illumination by staining the gels with Syber®Safe DNA gel stain at room temperature.

Fluorescence Spectroscopy:

A solution of total volume of 200 µl containing 8.3 µM of pD-co-E, or 8.3 µM pD-co-E and 10 µl of ssDNA probe (Duplex), or 8.3 µM pD-co-E, 10 µM of ssDNA probe and 10 µM of cDNA target (Triplex), or 8.3 µM PD-co-E, 10 µM of ssDNA probe and 10 µM of ssDNA non-complementary target (Negative control), was added to a 96-well plate for fluorescent measurement. Fluorescence was

measured using Varioskan Flash multi-reader (Thermo Scientific) using the SkanIt RE 2.4.3 software using the an excitation of 570nm and emission of 590nm.

For fluorescent measurements in horse serum (sterile-filtered, Sigma Aldrich) the target sequences (complementary and non-complementary) were diluted with horse serum rather than phosphate buffer saline.

Fluorescent microscopy:

Solutions were prepared as describe in the previous section but with ten times the concentration of each reagent. 5 µl of the final prepared solution was added to a glass slide then dispersed by adding a cover slide on top. Images were taken with Texas Red filter functionalized Olympus Ix81 inverted microscope.

Supporting information figures and tables:

Table S1. Ratio of components used for polymer synthesis.

Reactant	**DMAEMA**	**EGDMA**	**EBriB**	**$CuCl_2$**	**PMDETA**	**AA**
Ratio	40	10	1	0.125	0.125	0.0125

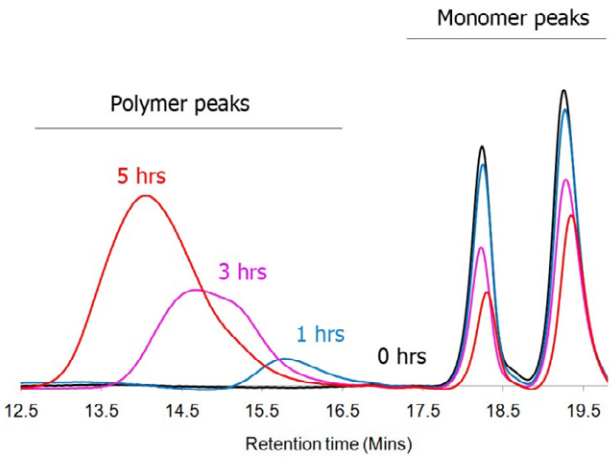


Figure S1. Chromatogram view (GPC) of pD-co-E polymer synthesis at different time periods of the reaction. The peak shift to the left over time is clearly seen where the retention time shifts to less than 14.5 minutes towards the end of the reaction.

Table S2. GPC peak analysis showing the time points of the reaction at which a sample was taken and measured. Results of the number average molecular weight (M_n), weight average molecular weight (M_w), polydispersity index (PDI, M_w/M_n) and percentage of monomer conversion (yield) are also shown for each time-point.

Reaction Time (hours)	M_n ($gmol^{-1}$)	M_w ($gmol^{-1}$)	**PDI**	**Yield (%)**
1	1230	1550	1.23	8
5	7200	10550	1.47	60

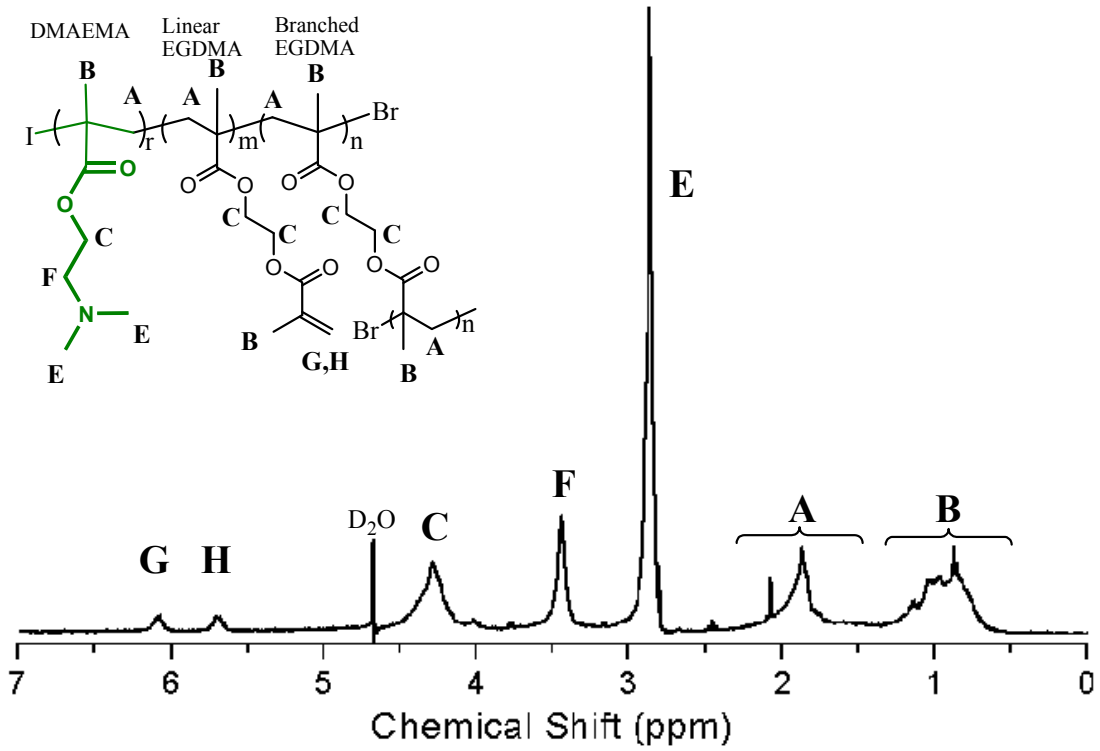


Figure S2. ^{1}H NMR analysis spectrum of the pD-co-E polymer showing peaks representative of the polymer's structure. Peaks (A) and (B) represent the polymer's backbone, while the resonances of proton E and F indicates the presence of the DMAEMA units. The vinyl functional groups are also clearly indicated by resonance of proton G and H.

The branching degree, vinyl content and DMAEMA content were calculated using the following equation:

DMAEMA unit= (Integral of F)/2 **(Eq. S1)**

Linear EGDMA unit= Integral of G **(Eq. S2)**

Branched EGDMA unit= All EGDMA unit-Linear EGDMA unit

= (Integral of C- Integral of F)/4- Integral of G **(Eq. S3)**

$$\text{Degree of branching} = \frac{\text{Branched EGDMA unit}}{\text{Linear EGDMA+DMAEMA+Branched EGDMA}} \quad \textbf{(Eq. S4)}$$

$$\text{Vinyl content} = \frac{\text{Linear EGDMA unit}}{\text{Linear EGDMA+DMAEMA+Branched EGDMA}} \quad \textbf{(Eq. S5)}$$

$$\text{DMAEMA content} = \frac{\text{DMAEMA unit}}{\text{Linear EGDMA+DMAEMA+Branched EGDMA}} \quad \textbf{(Eq. S6)}$$

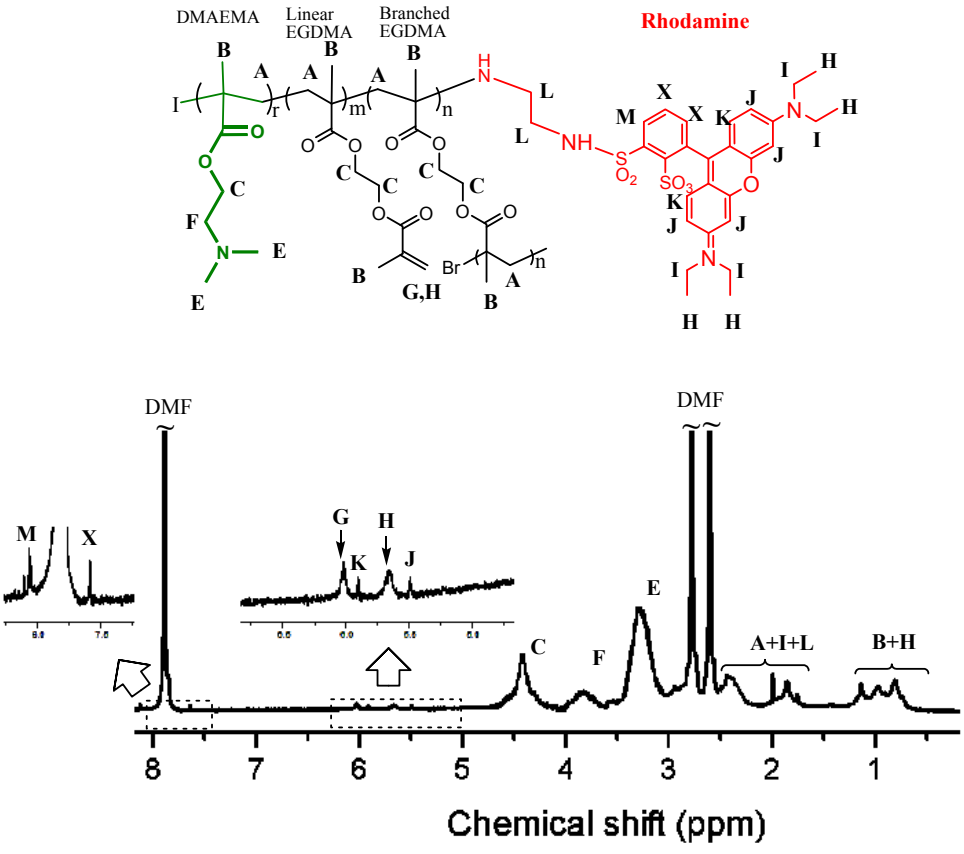


Figure S3. ^{1}H NMR analysis spectrum of the pD-co-E polymer modified by Rhodamine dye in DMF showing peaks representative of the molecular structure. The Rhodamine functional groups are clearly indicated by resonance of proton J, K, M and X.

Rhodamine unit= (integral of X)/2 **(Eq. S7)**

$$\text{Rhodamine contents} = \frac{\text{Rhodamine unit}}{\text{Linear EGDMA+DMAEMA+Branched EGDMA+Rhodamine}} \quad \textbf{(Eq. S8)}$$

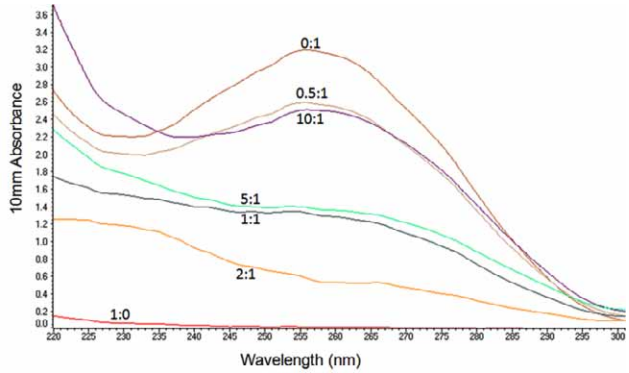


Figure S4. The UV-visible spectra for pD-co-E/ssDNA probe complexation (duplex) at different weight/weight ratios of pD-co-E/ssDNA probe. The best complexation was obtained at 2:1, but since the N/P (0.98:1) ratio is very close to the w/w, the 1:1 ratio was used for the sake of cDNA target detection.

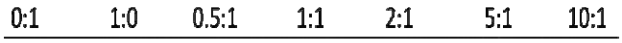


Figure S5. Agarose gel electrophoresis of pD-co-E with plasmid DNA (green fluorescent protein expressed) at different ratios of polymer to DNA.

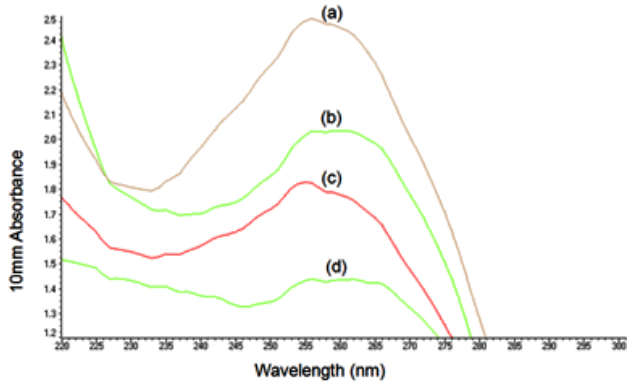


Figure S6. UV-visible spectra of (a) ssDNA probe alone, (b) Duplex+ NC target, (c) Triplex and (d) Duplex showing clear reduction in absorbance signal after triplex formation (c) compared to the Duplex+ NC target.