Enzymatic 'Charging' of Synthetic Polymers

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General Methods and Materials

Poly(ethylene glycol) methyl ether acrylate (PEGMA, M_n = 480), Cu(I)Cl, *N*,*N*,*N*',*N*'',*N*''-pentamethyldiethylenetriamine (PMDETA), ribonucleic acid (from torula utilis, 5000-8000 g/mol), enzyme DT-Diaphorase human (D1315-1MG, Sigma-Aldrich), ethyl α-bromoisobutyrate, and *β*-nicotinamide adenine dinucleotide phosphate reduced tetra(cyclohexylammonium) salt (NADPH) were purchased from commercial sources. NMR spectra were recorded on a Bruker AV300 MHz spectrometer, using $CDCl_3$, D_2O, and DMSO-d_6 as the solvent. GPC measurements were carried out using a PL-GPC 220 instrument with a 2x PL-Gel Mix-BLS column set equipped with refractive index, viscosity, and light-scattering detectors (0.1% LiBr in DMF as eluent at 45 °C). Transmission electron microscope (TEM) (Philips, CM 12) operating at an accelerating voltage of 100 kV was employed for imaging. The samples were prepared by mounting a drop (~ 15 μL) of a solution on the carbon coated Cu grids and allowing the samples to dry under ambient conditions. The UV/Vis measurements were carried out on a Lambda 20 double beam UV/Vis-spectrophotometer from Perkin-Elmer. The particle size was characterized by dynamic light scattering (DLS) at a fixed angle of $\theta = 173^o$ using a Zetasizer Nano (Malvern, Worcestershire, UK) with a laser beam wavelength of 633 nm.

Synthesis of Azo monomer 2: To a solution of 4-nitrophenyl 4-(phenyldiazenyl)benzyl carbonate (0.1 g, 0.26 mmol), TEA (0.11 mL, 0.79 mmol), and DMAP (0.064 g, 0.53 mmol) in DCM (3.0 mL) at 0 °C was added 2-aminoethyl methacrylate hydrochloride (0.087 g, 0.53 mmol) in one portion. The reaction was stirred at 0 °C for 2 h. After this time, the reaction mixture was diluted with DCM and washed with water. The organic layer was dried over sodium sulfate, reduced under low pressure, and purified by silica gel column chromatography using Hex:EtOAc (80:20) as eluent to give 0.075 g of the product as an orange solid (yield = 77 %). ^{1}H-NMR (δ, ppm, 300 MHz, $CDCl_3$): 7.84 (m, 4H), 7.43 (m, 5H), 6.04 (m, 1H), 5.51 (m, 1H), 5.11 (s, 2H), 4.99 (br s, 1H), 4.18 (t, J = 4.18 Hz, 2H), 3.47 (q , J = 5.43 Hz, 2H), 1.86 (s, 3H); ^{13}CNMR (δ, ppm, 75 MHz, $CDCl_3$): 167.44, 156.33,

152.77, 152.52, 139.44, 136.08, 131.25, 129.24, 128.79, 126.23, 123.17, 123.03, 66.44, 63.79, 40.49, 18.43; ESI (observed: 368.16 M+H, calc. for $C_{20}H_{22}N_3O_4$ (M+H): 368.15).

Synthesis of Copolymer 3: PEGMA **1** (290 mg, 0.6 mmol), Azo-monomer **2** (110 mg, 0.3 mmol), PMDETA (7.0 mg, 0.04 mmol), Cu(I)Cl (2.0 mg, 0.02 mmol), ethyl α-bromoisobutyrate (4.0 mg, 0.02 mmol), and anisole (0.5 mL) were taken in a schlenk tube and degassed by three freeze-pump-thaw cycles. The reaction mixture was then stirred under N_2 at 70 °C for 20 h. After this time, the reaction mixture was cooled to room temperature and then precipitated into diethyl ether thrice. The obtained orange sticky solid was then dried under high vacuum conditions (350 mg). ^{1}H NMR (δ, ppm, 300 MHz, $CDCl_3$): 7.86 (br s, Ar), 7.45 (br s, Ar), 5.14 (br s, CH_2OCON), 4.02 (br s, CH_2OCO), 3.70-3.44 (br m, CH_2CH_2O), 2.19-0.63 (br m, CH_3CHCH_2). GPC (DMF): M_n = 142000, M_w = 166200, PDI (M_w/M_n) = 1.17. Please also see Figure S2. From NMR, the ratio between the monomers was found to be 3:1, in favour of monomer **1**.

Enzymatic transformation and subsequent formation of nucleic-acid-based nanoparticles: Copolymer **3** (0.1 mg) was dissolved in 100 µL of deionized water. Then, 50 μL of DT-Diaphorase solution (30 μM, pH 7.0 in phosphate buffer) was added. The enzymatic reaction was initiated by the addition of NADPH (0.5 mg) and the mixture was incubated at 37 ^{o}C under stirring conditions. The solution was monitored with the UV-Vis spectroscopy. A colorless solution formed after 8 h of the enzymatic reaction. This indicated complete cleavage of the azobenzene chromophore as also indicated by the UV-Vis spectroscopy. The reaction mixture was then dialyzed against deionized water for 1 day to remove NADPH and phosphate salts (for ^{1}H-NMR, this purified polymer was used). After this time, a solution of RNA (0.05 mg) in water (10 μL) was added under ice cooling and the resulting mixture was stirred for 12 h at room temperature. The resulting material was then analyzed by TEM, UV-Vis, and DLS techniques.

To study the enzymatic cleavage by ^{1}H-NMR, the dialyzed material (before addition of the RNA) was used. ^{1}H NMR (δ, ppm, 300 MHz, D_2O): 4.16 (br s, CH_2OCO), 3.81-3.12 (br m, CH_2CH_2O), 2.13-0.67 (br m, CH_3CHCH_2). For studying the polymer structure in DMSO, the aqueous sample was freeze-dried and then dissolved in deuterated DMSO. ^{1}H NMR (δ, ppm, 300 MHz, d_6-DMSO): 7.97 (br s, NH_3^+), 4.32 (br s, CH_2OCO), 3.72-3.46 (br m, CH_2CH_2O), 2.22-0.61 (br m, CH_3CHCH_2).

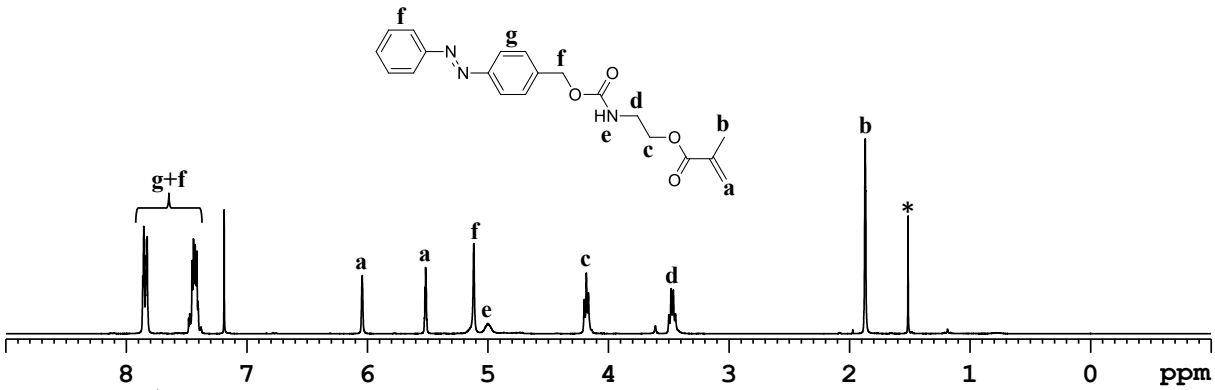

Figure S1. ^{1}H-NMR of the azo monomer **2**.

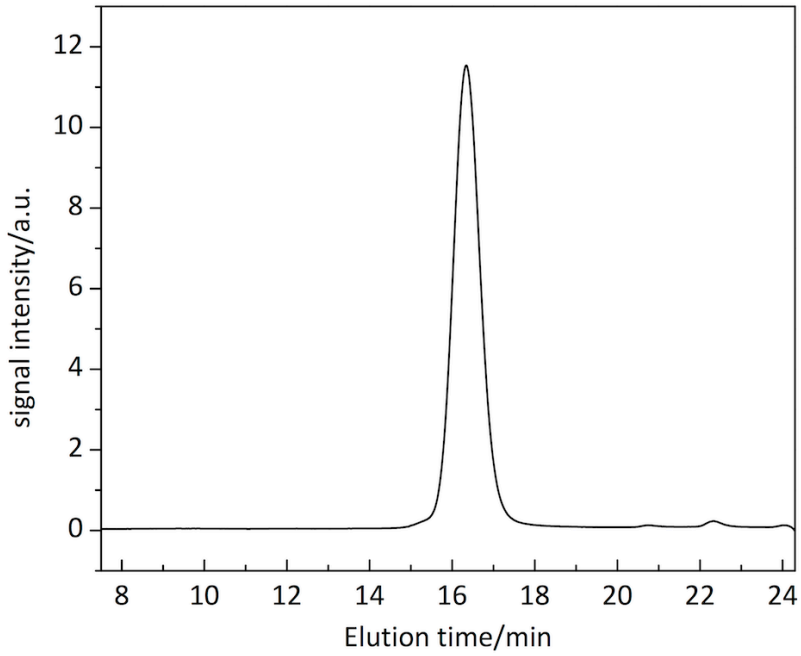

Figure S2. GPC chromatogram of polymer **3** in DMF.