Supplementary Information

A facile methodology using quantum dot multiplex labels for tracking co-transfection

Jessica A. Kretzmann, RuiLu Feng, Alaa M. Munshi, Diwei Ho, Anna M. Ranieri, Massimiliano Massi, Martin Saunders, Marck Norret, K. Swaminathan Iyer,* Cameron W. Evans*

* swaminatha.iyer@uwa.edu.au, cameron.evans@uwa.edu.au

1. Chemical synthesis and characterization

1.1. General instrumental characterization methods

[1]H spectra were measured using a Bruker 500 MHz spectrometer, using CD_3OD as the solvent for copolymers, azido-polymer and dendronized polymer. The chemical shifts were referenced to CD_3OD solvent peak, δ = 3.31 ppm. Gel permeation chromatography (GPC) was used to determine the molecular weight and polydispersity index of the copolymer (Waters Styragel HR 4 DMF 4.6 × 300 mm column, 5 μm). Agilent Technologies 1100 Series GPC and Agilent GPC software were used for measurements and data analysis respectively. Measurements were taken using DMF as the eluent at a flow rate of 0.3 mL/min at 50 °C, and calibrated against poly(methyl methacrylate) (PMMA) standard. Elemental analysis was conducted at the Campbell Microanalytical Laboratory, University of Otago.

1.2. Polymer synthesis

1   2   3
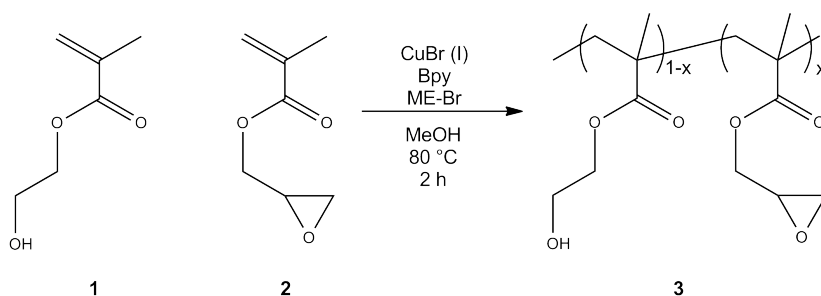
The morpholine-based initiator for ATRP polymerization was synthesized as by Weaver *et al*.[1] Polymer (**3**) was synthesized by methods described previously.[1,2] Inhibitors for hydroxyethyl methacrylate (HEMA, **1**) and glycidyl methacrylate (GMA, **2**) were removed using a basic alumina column and dissolved in methanol (MeOH) at a 1:3 ratio (monomer : MeOH). Monomer solutions were degassed and backfilled with nitrogen gas. Copper (I) bromide (CuBr, 100 mg, 0.70 mmol) and 2,2'-bipyridine (bpy, 392 mg, 2.5 mmol) were added to the flask before monomer solutions were added at ratios of 1:3 (4 ml GMA/MeOH and 12 ml HEMA/MeOH). ATRP initiator 2-(4-Morpholino)ethyl 2-bromoisobutyrate initiator (ME-Br, 210 μL, 1 mmol) was added and the reaction proceeded at 80 °C for 2 h under standard Schlenk conditions. Reaction was opened to air, and addition MeOH (15 ml) added. Product was collected and purified using repeated precipitations in diethyl ether. Solid product was dried overnight under high vacuum. Yield *ca*. 60%. Copolymer composition was determined by ^{1}H NMR (500 MHz, CD_3OD), where peaks at δH 2.70 (1H, br) and 2.87 (1H, br) correspond to the epoxide moiety; ^{1}H integration gives 16.3 mol % GMA. Polymer molecular weight (21.5 kDa) and PDI (1.30) of the polymer was measured using GPC.

1.3. Click chemistry

Azido functionalization: Copolymers were functionalized with azido groups as described previously.[2]

Propargyl dendron synthesis: Synthesis for PAMAM dendrons was adapted from methods presented by Lee *et al.* and Lin *et al*.[3,4]

Click reaction: Attachment of propargyl poly(amido amine) dendrons was achieved using previously described methods,[2] which were adapted from Zhao *et al.*[5]

Table S1: Experimental elemental analysis of fifth generation dendron-functionalized copolymer.

%C	%H	%N
42.26; 42.47	8.19; 7.99	16.07; 15.96
46.31; 46.51	8.02; 8.16	17.29, 17.22
46.71; 46.43	7.27; 7.62	17.13; 17.06
(50.24)*		(20.77)*

* Corrected for counterion and water content – sample was hygroscopic; measurements were conducted three times in duplicate with repeated drying, before being normalized to average carbon percentage previously reported.

1.4. ^{1}H NMR spectrum of dendronized polymer
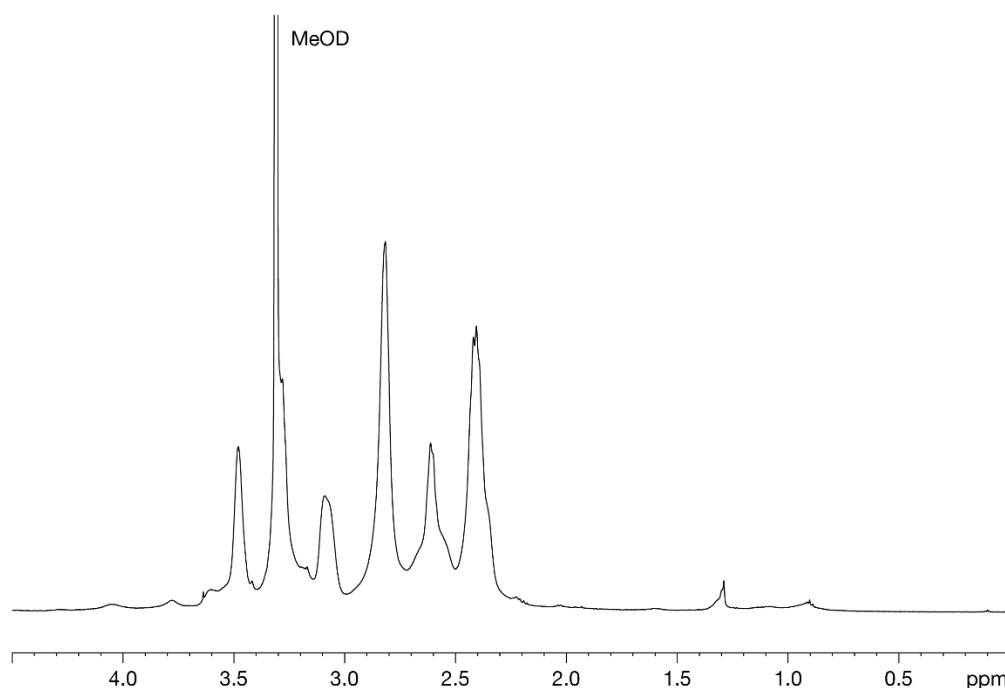
1.5. QD synthesis

QDs were synthesized according to the method of Zou *et al.*[6]

Te powder (50.8 mg, 0.4 mmol) was combined with $NaBH_4$ (*ca.* 40 mg) in water (10 ml) and heated to 80 °C under Ar for 30 min to obtain a deep purple solution.

Preparation of QDs: 40 ml of a colourless aqueous solution containing Cd^{2+} (from $Cd(NO_3)_2 \cdot 4H_2O$, 0.20 mmol) and 3-mercaptopropanoic acid (MPA, 0.34 mmol) was adjusted to pH 11.9 using 1 M NaOH. The solution was carefully degassed using the freeze–pump–thaw method before introduction of NaHTe solution (0.50 ml, 0.02 mmol) dropwise via syringe. The mixture turned orange. The flask was fitted with a condenser, and heated to reflux under a blanket of Ar for 3 h. The colour of the reaction mixture changed from orange to red over the course of heating. Aliquots were taken at various timepoints during heating.

2. Supplemental data
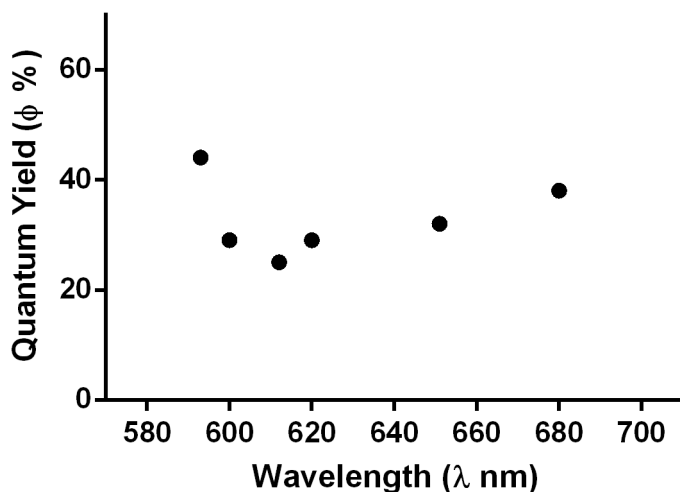
2.1. QD characterization

Figure S1: QDs samples were taken at various timepoints through synthesis and their quantum yields versus their emission wavelength measured for selected samples.
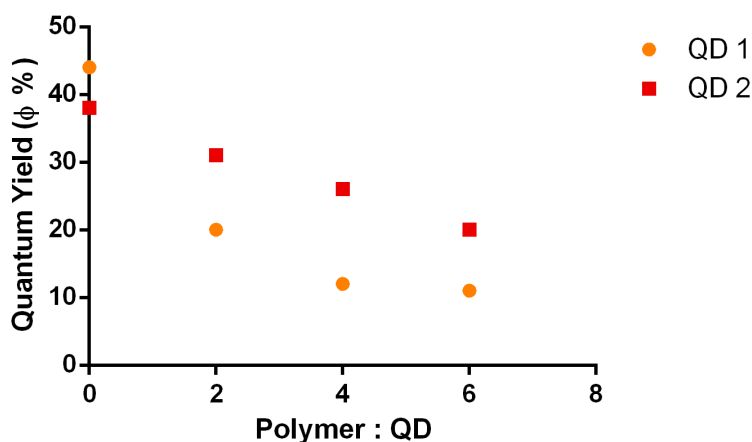

Figure S2: Quantum yield was measured for the selected quantum dots with increasing polymer : QD ratios.

2.2. Spectroscopic characterization

Absorption spectra were recorded at room temperature using a Perkin-Elmer Lambda 35 UV-Vis spectrometer. Uncorrected steady-state emission and excitation spectra were recorded on an Edinburgh FLSP980 spectrometer equipped with a 450 W Xenon arc lamp, double excitation and single emission monochromators, and a Peltier-cooled Hamamatsu R928P photomultiplier tube (185–850 nm). Emission and excitation spectra were corrected for the source intensity (lamp and grating) and emission spectral response (detector and grating) by using a calibration curve supplied with the instrument. Quantum yields (Φ) were determined using the optically dilute method of Demas *et al*.[7] at excitation wavelengths obtained from absorption spectra on

a wavelength scale (nm)and compared to the reference emitter by using the following equation:

$$\Phi = \Phi_r \left[\frac{A_r(\lambda_r)}{A_s(\lambda_s)} \frac{I_r(\lambda_r)}{I_s(\lambda_s)} \frac{n_s^2}{n_r^2} \frac{D_s}{D_r} \right]$$

Where A is the absorbance at the excitation wavelength (λ), I is the intensity of the excitation light at the excitation wavelength (λ), *n* is the refractive index of the solvent, *D* is the integrated intensity of the luminescence, and Φ is the quantum yield. The subscripts *r* and *s* refer to the reference and the sample, respectively. An air-equilibrated water solution of quinine sulphate in 0.1 M H_2SO_4 (Φ_r = 0.546) was used as the reference.[8] The quantum yield determinations were performed at identical excitation wavelengths for the sample and the reference, therefore deleting the $I(\lambda_r)/I(\lambda_s)$ term in the equation.

2.3. Fluorescence lifetimes

Emission lifetimes (τ) were determined by the single photon counting technique (TCSPC) with the same Edinburgh FLSP980 spectrometer using a pulsed picosecond LED (EPLED 295 or EPLED 360, fhwm <800 ps) as the excitation source, at repetition rates between 1 kHz and 1 MHz, and the above-mentioned R928P PMT as the detector.

The best fit was assessed by minimizing the reduced χ^2 function and by visual inspection of the weighted residuals. Experimental uncertainties were estimated to be ±8% for lifetime determinations, ±20% for quantum yields, and ±2 nm and ±5 nm for absorption and emission peaks, respectively.
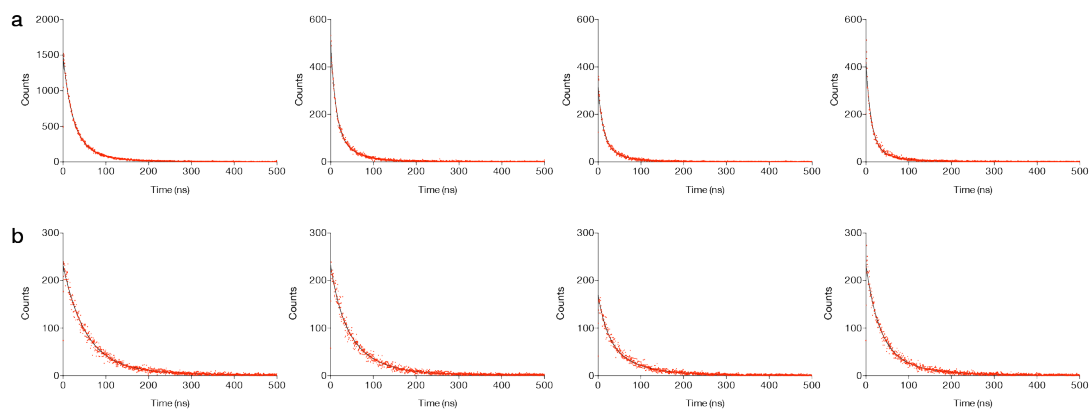
Sample	λ_{max} (nm)	τ (ns)	ϕ (%)
QD590	593	21 (51%), 59 (49%)	44
QD590	595	11 (36%), 43 (64%)	20
QD590	595	10 (30%), 38 (70%)	12
QD590	596	13 (40%), 44 (60%)	11
QD690	680	45 (54%), 88 (46%)	38
QD690	693	20 (12%), 62 (88%)	31
QD690	686	53	26
QD690	693	21 (22%), 61 (78%)	20

Figure S3: Fitted lifetime plots for (a) QD590 and (b) QD690 samples. Data and fits are shown in the table.

2.4. Cytotoxicity

MCF-7 mammary epithelial adenocarcinoma cells were seeded at a density of 5,000 cells per well in a 96-well plate. After 24 h, cell media was aspirated and replaced with 20 μl Opti-MEM. Polyplexes were prepared as described in the 'Experimental' section of the paper, using a range of polymer concentrations. Polyplexes (10 μl) were added, and after a further 4 h, 70 μl MEMα media containing 10% FBS, 1x GlutaMAX, and 1x antibiotic/antimycotic were added. After a further 24 h incubation, viable cells were assayed by MTS (CellTiter 96 Aqueous One, Promega G3581) by adding 20 μl reagent per well, incubating 3 hours at 37 °C and 5% CO_2, and then measuring absorbance at 490 nm. Values were corrected for blank absorbance and normalised relative to control.
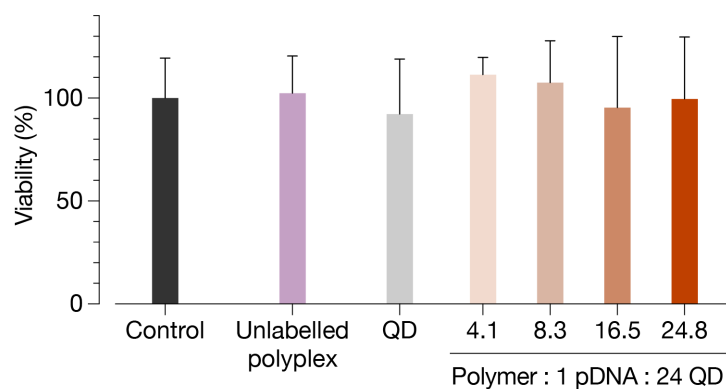

Figure S4: Cytotoxicity was assessed in MCF-7 cells after 24 h by MTS assay. No significant change in viability was observed relative to untreated control ($p > 0.05$) for all groups.

3.   References

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