

Supporting information

Synthesis of fluorescent probes

All materials were used as received from the supplier (Aldrich, Gillingham, United Kingdom, for 2, 2'-(Ethylenedioxy) diethanethiol, propylene sulphide, ethyl bromoacetate, dimethyl sulfoxide, YPD broth, sodium hydrogen bicarbonate; Fluka, Gillingham, United Kingdom, for 1,8-Diazabicyclo [5.4.0]undec-7-ene and Limonene; Invitrogen, United Kingdom, for Calcofluor White M2R, Germ Agglutinin Alexafluor 633 conjugate and Concanavalin A Alexafluor 488 conjugate; Oxoid, Basingstoke, United Kingdom, for Phosphate Buffered Saline; MP Biomedical, Cambridge, United Kingdom, for Zymolyase (20T) 20,000 units/g).

THF was degassed by bubbling argon under inert atmosphere for 1 hour before use.

Abbreviations: Dansyl acrylate: **DA**. 2, 2'-(Ethylenedioxy) diethanethiol: **EDT**. Propylene sulfide: **PS**. 1,8-Diazabicyclo [5.4.0]undec-7-ene: **DBU**. Dimethyl sulfoxide: **DMSO**.

Molecular characterisation

¹H-NMR spectra were acquired on a 300 MHz Bruker spectrometer on CDCl₃ solutions. ATR-IR spectra were recorded on polymer films obtained by evaporation of dichloromethane polymer solutions directly on the diamond of a Bruker Tensor 27 equipped with a temperature-controlled Golden Gate ATR accessory. GPC analysis was performed in THF solution on a Polymer Laboratories GPC50 equipped with refractive index and viscosimetric detectors, using monodisperse polystyrene standards. Fluorescence spectra were acquired on a Perkin Elmer LS55 (Perkin Elmer, USA) on dichloromethane solutions.

Synthetic procedures

Thiolate-reactive fluorescent dye (DA, Dansyl acrylate). Under inert atmosphere dansyl chloride was dissolved in THF (60 ml/1 g solid). 0.95 eq. of ethanolamine and 3.0 eq. of triethylamine were dissolved in 100 ml THF and added dropwise, stirring the mixture at room temperature for 3 hrs. The reaction was cooled down at 0°C; 1.5 eq. of acryloyl chloride were dissolved in 100 ml dichloromethane and added dropwise, causing the immediate

precipitation of a white powdered precipitate of triethylammonium chloride. The mixture was stirred overnight at room temperature and then filtered. After removal of the solvent, the crude product was redissolved in CH_2Cl_2 , extracted with 5% NaHCO_3 and then water, and then dried over Na_2SO_4 . After evaporation of the solvent, the product was purified by flash chromatography (silica, hexane:ethyl acetate = 5:3), yielding a yellow crystalline solid (2.15 g, yield 45%).

$^1\text{H NMR}$ (CDCl_3): δ = 2.80 (s, 6H, dansyl $-\text{CH}_3$), 3.15 (q, 2H, $-\text{SNCH}_2\text{CH}_2\text{O}-$), 3.98-4.04 (m, 2H, $-\text{SNCH}_2\text{CH}_2\text{O}-$), 5.03-5.07 (t, 1H, $-\text{NHCH}_2-$), 5.65-5.69 (d, 1H, $-\text{OCOCHCH}_2$ *trans* to carbonyl), 5.77-5.88 (m, 1H, $-\text{OCOCHCH}_2$), 6.15 (d, 1H, $-\text{OCOCHCH}_2$ *cis* to carbonyl), 7.10 (d, 1H, naphthalene proton in **6**), 7.48 (q, 2H, naphthalene protons in **3** and **8**), 8.20 (d, 2H, naphthalene protons in **4** and **7**), 8.47 ppm (d, 1H, naphthalene proton in **2**).

ATR-IR (thin film): 3295 (ν NH), 2944 (ν_{as} CH_2), 1724 (ν C=O unsaturated), 1188 (ν_{as} C(=O)-O), 1137 (ν \square SO_2NH), 1061 (ν_{s} C(=O)-O), 938 ($\delta_{\text{o.o.p.}}$ $\text{CH}=\text{CH}_2$), 787 cm^{-1} ($\delta_{\text{o.o.p.}}$ naphthyl).

FLU (CHCl_3): λ_{exc} = 337 nm, λ_{em} = 493 nm.

DA-320. Under inert atmosphere 500 mg (1.85 mmol) of dansyl chloride were dissolved in 30 ml THF. To the reactor were first introduced 0.77 ml (5.55 mmol) of triethylamine and then 230 μl (1.75 mmol) of hexylamine (dissolved in 5 ml THF) were added to the mixture via a dropping funnel. The reaction mixture was stirred for 2 h protected from light and then directly filtered over paper filter to remove the salt. After removal of the solvent with the rotatory evaporator, the yellow crude was dissolved in 20 ml CH_2Cl_2 and extracted 3 times with 4 ml of H_2O correcting the PH to neutral with NaHCO_3 . The organic phase was then dried over Na_2SO_4 for 20 min, and after filtration and complete removal of the solvent the product was purified by flash chromatography (silica, n-hexane:ethyl acetate = 5:3), yielding after overnight drying at reduced pressure 300 mg of a yellow oil (yield 53%).

$^1\text{H NMR}$ (CDCl_3): δ = 8.47 (d, 1H), 8.20 (d, 2H), 7.48 (q, 2H, metha protons), 7.10 (d, 1H), 4.64-4.51 (m, 1H, $-\text{NHCH}_2-$), 2.90 (t, 2H, $-\text{SNCH}_2-$), 2.80 (s, 6H, $-\text{CH}_3$), 1.35-1.05 (m, 8H), 0.95 ppm (t, 3H, $-\text{CH}_3$)

ATR-IR (thin film): 3295 (ν NH), 2958 (ν_{as} CH_3), 2920 (ν_{as} CH_2), 1448 and 1371 (δ_{as} and δ_{s} CH_3 bending), 1137 (ν \square SO_2NH), 787 cm^{-1} ($\delta_{\text{o.o.p.}}$ naphthyl).

FLU (CHCl_3): λ_{exc} = 337 nm, λ_{em} = 492 nm.

DA620. 2, 2'-(Ethylenedioxy) diethanethiol (**EDT**) (200 mg, 1.1 mmol) and dansyl acrylate (76 mg, 0.2 mmol) were dissolved under inert atmosphere in degassed THF. The reaction was started by adding 1.12 ml (334 mg, 2.2 mmol) of a 300 mg/ml THF solution of DBU for a 1:1 DBU/thiol ratio. The reagents were allowed to react for 2 hours protected from light and under inert atmosphere; to the reactor was then added ethyl bromoacetate (0.55 g, 3.3 mmol) for 1.5 molar excess to the initial thiolates to terminate the remaining unprotected groups. The mixture was allowed to react for further 90 minutes, then filtered over glass wool and evaporated at the rotary evaporator. The crude product was dissolved in 20 ml CH₂Cl₂ and extracted 3-times with 4 ml of water. The organic phase was then dried over Na₂SO₄ for 20 min, and after filtration and complete removal of the solvent the product was purified by flash chromatography (silica, n-hexane:ethyl acetate = 1:1), yielding after overnight drying at reduced pressure 75 mg of a yellow oil (yield 55%).

¹H NMR (CDCl₃): δ = 8.47 (d, 1H), 8.20 (d, 2H), 7.48 (q, 2H, meta protons), 7.10 (d, 1H), 4.37–5.33 (m, 1H, -NHCH₂-), 4.15–4.20 (q, 2H, CH₃CH₂COO-), 4.09 (m, 2H, -SNCH₂CH₂O-), 3.61–3.71 (m, 8H, H of carbons on ether), 3.30 (s, 2H, -S-CH₂-COO-), 3.19 (q, 2H, -SNCH₂CH₂O-), 2.90 (s, 6H, -CH₃), 2.80 (t, 2H, -OOCCH₂CH₂-), 2.70 (m, 4H, -SCH₂CH₂O-), 2.45 (m, 2H, -OOCCH₂CH₂-), 1.25 (t, CH₃, CH₃CH₂COO-) ppm. **ATR-IR** (thin film): 3295 (ν NH), 2958 (ν_{as} CH₃), 2920 (ν_{as} CH₂), 1735 (ν C=O), 1448 and 1371 (δ_{as} and δ_s CH₃ bending), 1240 (ν_{as} C-C(=O)-O), 1137 (ν SO₂NH), 1105 (ν_{as} C-O), 787 cm⁻¹ (δ_{o.o.p.} naphthyl).

FLU(CHCl₃): λ_{exc} = 343 nm, λ_{em} = 494 nm.

DA-1100, DA1500, DA-2500 and DA-3800. The polymers used in this study were prepared in parallel experiments in an Omni 6 parallel reactor (Thermo Electron) at room temperature under argon atmosphere, to yield a series of polymers with different molecular weight but same composition. 2,2'-(Ethylenedioxy) diethanethiol (**EDT**) and propylene sulfide (**PS**) were dissolved in the reactor under inert atmosphere in degassed THF to yield mixtures with a variable ratio between monomer and initiator (e.g. containing 20 mg/ml of **EDT** and 275 mg/ml of **PS** for a 1:20 molar ratio, corresponding to a 1:10 thiol-to-monomer ratio). The polymerisation was then started by adding the required amount of a 300 mg/ml THF solution of DBU for a 1:1 DBU/thiol ratio. The reagents were allowed to react for 45 minutes, and then to each reactor was added the required amount of 50 mg/ml THF solution of dansyl acrylate

(**DA**) for a 1:4 DA/thiol ratio or 1:10 DA/thiol ratio (for DA-1100). After additional 2 hrs, a THF stock solution of ethyl bromoacetate (**EBA**) (200 mg/ml) was added to each reactor in the required quantity to provide a 1.5 times molar excess of **EBA** to the theoretical unprotected thiolates. The mixture was allowed to react for further 90 minutes, then directly filtered over glass wool and the solvent evaporated at the rotary evaporator. The viscous crude was dissolved in 30 ml CH₂Cl₂, extracted 3-times with 6 ml of water correcting the PH to neutral with NaHCO₃. The organic phase was then dried over Na₂SO₄ for 20 min, and after filtration and complete removal of the solvent the product was extracted 3 times with 5 ml MeOH. After sedimentation from the MeOH solution, the polymer was separated and dried for 24 hours under reduced pressure. A viscous yellow-green fluorescent oil (average yield 65-70%) was finally collected.

¹H NMR(CDCl₃): δ = 1.26-1.30 (t, CH₃, **CH₃CH₂COO-**), 1.30-1.34 (d, CH₃ in PPS chain), 2.51-2.61 (m, 1 diastereotopic H of **CH₂** in PPS chain), 2.73-2.79 (t, 4H, in **-S-CH₂-CH₂-O-CH₂-**), 2.75-2.93 (m, **CH** and 1 diastereotopic H of **CH₂** in PPS chain), 3.20-3.37 (m, 4H, **-S-CH₂-COO-**), 4.15-4.20 (q, 2H, **CH₃CH₂COO-**) ppm.

ATR-IR (thin film): 3295 (ν NH), 2958 (ν_{as} CH₃), 2920 (ν_{as} CH₂), 2864 (ν_s CH₃), 1735 (ν C=O), 1448 and 1371 (δ_{as} and δ_s CH₃ bending), 1260-1240 (ν_{as} C-C(=O)-O acetate), 1173 (ν C-S), 1105 (ν_{as} C-O), 787 cm⁻¹ (δ_{o.o.p.} naphthyl).

FLU(CHCl₃): λ_{exc} = 343 nm, λ_{em} = 494 nm.

Table. Characterisation data for low MW and oligo/polymeric probes

Probe	2n ^a	¹ H-NMR	GPC		Ex/Em ^c (nm)	% of probes with 2, 1 & no fluorophore ^d	Yield (%)
		\overline{M}_n ^b	\overline{M}_n	$\frac{\overline{M}_w}{\overline{M}_n}$			
DA-320	--	320	-- ^e	-- ^e	337/492	0 / 100 / 0	0.53
DA-620	0	620	-- ^e	-- ^e	343/ 494	0 / 100 / 0	0.55
DA-1100	7	1060	-- ^e	-- ^e	343/ 494	2 / 8 / 90	0.60
DA-1500	15	1580	1500	1.12	343/ 494	6.2 / 18.8 / 75	0.70
DA-2400	30	2550	2350	1.18	343/ 494	6.2 / 18.8 / 75	0.75
DA-3800	40	3680	3800	1.21	343/ 494	6.2 / 18.8 / 75	0.77

^a monomers per initiator in the feed.

^b For oligo/polymeric probes calculated as the ratio between the integral value of a PPS methyl proton (1.30 ppm) and that of a 2, 2'-(Ethylenedioxy) diethanethiol proton (2.73-2.79 ppm). For low MW probes calculated from the molecular structure as confirmed from ¹H-NMR (and IR).

^c Excitation and emission maxima measured in CHCl₃.

^d Theoretical percentage of chains with double, single and no dansyl end-capping, calculated assuming a quantitative reaction of dansyl acrylate and an independent reactivity of the two polymer termini.

^e DA-1100 was out of range of our GPC columns (PLgel 5µm MIXED-D, PLgel 5µm MIXED-C, PLgel 10 µm MIXED-B).