Electronic supporting information

Boronic acid ester with dopamine as a tool for bioconjugation and for visualization of cell apoptosis

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Experimental

Materials

3-carboxy phenyl boronic acid (Aldrich, 99%), 9-aminoacridine hydrochloride monohydrate (Aldrich, 98%), calcium chloride (Ajax Finechem, 98%), 1,8-diazabicycloundec-7-ene (DBU, Aldrich 98%), triethylamine (TEA; Ajax Fine Chem, 99%), dimethyl sulfoxide (DMSO; Ajax, 98.9%), dichloromethane (DCM; Ajax Finechem, 99%), methanol, dopamine hydrochloride (Fluka, 95.8%), phenyl boronic acid (PBA; Aldrich, 95%), fluorescein O-methacrylate (Aldrich, 97%), phosphate buffer powder (Aldrich), alizarin red S (ARS; Aldrich), *N,N*-dimethyl acetamide (DMAc; Aldrich, 99.9%), *N,N*-dimethylformamide (DMF; Aldrich), diethyl ether (Et_2O; Ajax Fine Chem, 99%), hydrochloric acid (HCl; Ajax Fine Chem, 32%), H_2O_2 (Ajax Fine Chem, 30 % w/v), (EDC; Aldrich, 98%), *N*-hydroxysuccinimide (NHS; Aldrich, 98%),%), *cis*-diammineplatinum (II) dichloride (CDDP; Sigma Aldrich, 99.9%), toluene (Aldrich; purum), calcium chloride (Aldrich, anhydrous 93%) were used without further purifications.

Oligo(ethylene glycol) methyl ether methacrylate (OEGMEMA, M_n= 300 g mol^{-1}; Aldrich), methyl methacrylate (Aldrich, 99%), *tert*-butyl methacrylate (Aldrich, 98%) were destabilized by passing them over a column of basic alumina and stored at -7°C. 2,2-Azobisisobutyronitrile (AIBN; Fluka, 98%) was purified by recrystallization from methanol. Deionized (DI) water produced by Milli-Q water purification system and has a resistivity of 17.9 mΩ/cm.

Analysis

Nuclear Magnetic Resonance (NMR) Spectroscopy

^{1}H and ^{11}B NMR spectra were recorded using a Bruker ACF300 (300 MHz) spectrometer, using $(CD_3)_2SO$, CD_3OD, D_2O or $CDCl_3$ as solvents. All chemical shifts are stated in ppm (δ) relative to tetramethylsilane (δ = 0 ppm), referenced to the chemical shifts of residual solvent resonances.

Size Exclusion Chromatography (SEC)

SEC was implemented using a Shimadzu modular system comprised of a DGU-12A degasser, LC-10AT pump, SIL-10AD automatic injector, CTO-10A column oven, RID-10A refractive index detector, and SPD-10A Shimadzu UV/vis detector. A Phenomenex 50 × 7.8 mm guard column and four Phenogel 300 × 7.8 mm linear columns (500, 10^3, 10^4, and 10^5Å pore size, 5 μm particle size) were used for the analyses. *N,N*-dimethylacetamide (DMAc; HPLC grade, 0.05% w/v LiBr, 0.05% w/v 2,6-dibutyl-4-methylphenol (BHT)) with a flow rate of 1 mL min^{-1} and a constant temperature of 50 °C was used as the mobile phase with an injection volume of 50 μL. The samples were filtered through 0.45 μm filters. The unit was calibrated using commercially available linear polystyrene standards (0.5-1000 kDa, Polymer Laboratories). Chromatograms were processed using Cirrus 2.0 software (Polymer Laboratories).

Dynamic Light Scattering (DLS)

The average hydrodynamic diameters and size distribution of prepared micelle solution in an aqueous solution at concentration of 1 mg mL^{-1} were obtained using Malvern Nano-ZS as particle size analyzer (laser, 4 mW, λ=632 nm; measurement angle 12.8° and 175° °). Samples were filtrated to remove dust using a microfilter 0.45 μm prior to the measurements and run for at least three times at 25 °C.

Fluorescence spectroscopy

Fluorescence measurements performed on Agilent Cary Eclipse Fluorescence Spectroscopy with xenon flash lamp using a 1 cm path length 4-sided quartz cuvette. All measurements were obtained at room temperature and ffluorescence spectra were recorded between 400-800 nm at λ_{ex} = 468 nm, λ_{em} = 572 nm with entrance and exit slit width of 10mm.

Synthesis and procedures

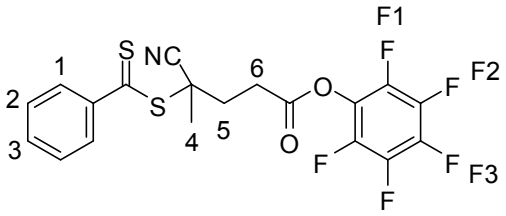
Synthesis of dopamine terminated RAFT agent (CPADBD)

The 4-cyanopentanoic acid dithiobenzoate was synthesised according to literature.[34] To a 100 mL round bottom flask was added CPADB (0.2 g, 0.72 mmol) and DCC (0.18g, 0.86mmol) were then dissolved in anhydrous DCM (30 mL). The reaction mixture was cooled to 0 ^{o}C in an ice bath and pentafluorophenol (0.145 g, 0.78 mmol) was added to the flask. In another cospak vial, DMAP (20 mg, 0.164 mmol) was dissolved in anhydrous DCM (10 mL) and was then added drop wise into the round bottom flask with fast stirring. The reaction was left to stir in an ice bath for 2 hours and then left to stir under room temperature overnight.

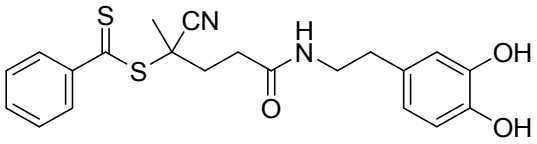
The solid was then filtered and the solvent was removed from the filtrated under reduced pressure on a rotary evaporator. The crude product was purified via gel column chromatography using chloroform as eluent. The first fraction was collected and solvent removed under reduced pressure to obtain pink/red oil and was placed in the freezer for crystalisation. Yield: 76 %

^{1}H-NMR (300MHz, $CDCl_3$) δ (ppm): 7.80 (d, 2H, H-1), 7.50 (t, 1H, H-3), 7.30 (d, 2H, H-2), 2.31 (m, 2H, H-5), 2.70 (m, 2H, H-6), 1.85 (s, 3H, H-4).

^{19}F-NMR (300MHz, $CDCl_3$) δ (ppm):: -152.5(t, 2F, F-1), -157.3(t, 2F, F-2), -161.8(t, 2F, F-3).



Dopamine was dissolved in DMF (3 mL) and TEA was slowly added to the reaction mixture which was left to stir for 10 minutes. Then CPADB-PFP crystals dissolved in DMF (2 mL) was added to the dopamine solution and left to stir at room temperature for overnight. The reaction mixture was then dripped slowly into a phosphate buffer (20 mL, pH 4.0) to obtain a pink precipitate. The solution was then centrifuged and water is decanted followed by freeze drying. The product was redissolved in acetone to precipitate out phosphate buffer salt; alternatively the product could be purified using gel chromatography using chloroform as eluent first then change to chloroform: ethylacetate (1:1) to obtain the product. Yield: ? mg (87 %)



^{1}H-NMR (300 MHz, $CDCl_3$) δ (ppm): 7.85 (d, 2H, H-1), 7.50 (d, 2H, H-2), 7.35 (t, 1H, H-3), 6.79 (d, 1H, H-10), 6.65 (d, 1H, H-12) 6.51 (dd, 1H, H-11), 5.56 (br, 1H, H-7), 2.70 (m, 2H, H-6), 2.50 (m, 2H, H-9), 2.30 (m, 2H, H-5), 2.20 (m, 2H, H-8) 1.85 (s, 3H, H-4).

Synthesis of acridine phenyl boronic acid

In solution 1, 3-Carboxy phenylboronic acid (100 mg, 0.6 mmol) and DBU (100 mg, 0.65 mmol) was dissolved in DMSO (2 mL). In another glass vial 9-amino acridine hydrochloride monohydrate (165 mg, 0.66 mmol) and TEA (0.1 mL) was dissolve din DMSO (1.5 mL) and left to stir for 1 h at room temperature before added to solution. The reaction mixture was left to stir for 48 h at 40 ^{o}C before precipitated into DI water and centrifuged at 7000 rpm for 5 minutes. The supernatent was removed by decanting and the remaining solvent was removed by freeze dry to obtain a yellow powder. The product was then purified via gel column chromatography using 7:1 dimethyl chloride: methanol as eluent. The third fraction was collected and the solvent removed under reduced pressure to obtain a yellow powder. Yield: 180 mg (53 %)

^{1}H-NMR (300MHz, DMSO D-6) δ (ppm): 8.80 (dd, 2H, H-1), 8.44 (s, 1H, H-2), 8.23 (br, 2H, H-3), 8.04-8.01 (t, 4H, H-4), 7.59 (dd, 2H, H5), 7.48 (t, 1H, H6)

RAFT polymerization of OEGMEMA using dopamine terminated 4-cyanopentanoic dithiocarbonate CTA (CPADBD)

Two separate reaction mixtures of OEGMEMA (3.00 g, 1.00 $\times 10^{-2}$ mol), CPADBD (83.0 $\times$ 10^{-3} g, 2.00 $\times 10^{-4}$ mol), and AIBN (6.50 $\times$ 10^{-3} g, 240 $\times 10^{-5}$ mol) were dissolved in 4.5 mL of toluene. The reaction mixtures were placed in an ice bath and purged with nitrogen for 30 min. The two reaction mixtures were then immersed in a preheated oil bath at 65 ^{o}C for 2 h and 4 h respectively. The polymerization was terminated by placing the samples in an ice bath for 5 min. By comparing the intensity of vinyl proton peaks (6.1 and 5.6 ppm) to that of aliphatic proton peaks (1.1-1.3 ppm), the conversion of monomer during the course of polymerization was determined. The polymer was purified three times by precipitation in petroleum spirits (boiling range of 40-60 ^{o}C). After centrifugation (7000 rpm for 15 min), the polymer was dried under reduced pressure at room temperature. The samples were stored in a freezer prior to any modifications. POEGMEMA with 15 repeating units (conversion = 30%, $M_{n(theo)}$= 4500 g mol^{-1}, $M_{n(SEC)}$= 4800 g mol^{-1}) and POEGMEMA with 32 repeating units (conversion = 58 %, $M_{n(theo)}$= 9600 g mol^{-1}, $M_{n(SEC)}$= 11000 g mol^{-1}) and were used as a so-called macro-RAFT agent to control the chain extension with MMA. The number of repeating units of POEGMEMA was calculated from the monomer conversion obtained from ^{1}H-NMR.

Chain extension of POEGMEMA with methyl methacrylate (POEGMEMA-b-MMA)

POEGMEMA macro-RAFT with 32 repeating units ($M_{n(theo)}$= 9600 g mol^{-1}, $M_{n(SEC)}$= 11000 g mol^{-1}) was used for the chain extension with tert-butyl methacrylate (tBMA). POEGMEMA macro-RAFT (0.31 g, 3.12 $\times 10^{-5}$ mol), MMA (0.31 g, 3.12 $\times 10^{-3}$ mol) and AIBN (0.5 mg, 3.05 $\times 10^{-6}$ mol) were dissolved in7.5 mL toluene. The reaction mixtures were placed in an ice bath and purged with nitrogen for 30 min was then immersed in a preheated oil bath at 70 ^{o}C for 20 h. By comparing the intensity of vinyl proton peaks (6.1 and 5.6 ppm) to that of methyl peaks (2.1 ppm), the conversion of monomer during the course of polymerization was determined. The polymer was purified three times by precipitation in petroleum spirits (boiling range of 40-60 ^{o}C). After centrifugation (7000 rpm for 15 min), the polymer was dried under reduced pressure at

room temperature. The conversion was to be determined at 71 % after 20 h with 71 repeating units of MMA ($M_{n(theo)}$= 17600 g mol^{-1}, $M_{n(SEC)}$= 21700 g mol^{-1}).

End group modification of RAFT polymers for fluorescent studies

Three different polymers **B001** ($POEGMEMA_{15}$), **B002** ($POEGMEMA_{32}$) and **B003** ($POEGMEMA_{32}$-b-MMA_{71}) each with weight of 1 g were dissolved in toluene (5 mL) and 5 times (mol %) of hexamine was then added to the solution drop wise. The reaction was left to stir overnight at room temperature and the polymers were purified by precipitating in cold diethyl ether. After centrifugation (7000 rpm for 5 minutes) the solvent was decanted and polymer was dried in vacuum oven for fluorescent binding studies.

RAFT polymerisation of OEGMEMA and fluorescein O-methacrylate (FOMA) dopamine terminated 4-cyanopentanoic dithiocarbonate CTA (CPADBD)

OEGMEMA (2.00 g, 6.67×10^{-3} mol), CPADBD (55.30 × 10^{-3} g, 1.33 ×10^{-4} mol), fluorescein O-methacrylate (5.33 mg, 1.32 × 10^{-5} mol) and AIBN (4.37 × 10^{-3} g, 2.67 ×10^{-5} mol) were dissolved in 4.5 mL of toluene. The reaction mixtures were placed in an ice bath and purged with nitrogen for 30 min where then immersed in a preheated oil bath at 65 ^{o}C for 2.5 h. The polymerization was terminated by placing the samples in an ice bath for 5 min. By comparing the intensity of vinyl proton peaks (6.1 and 5.6 ppm) to that of aliphatic proton peaks (1.1-1.3 ppm), the conversion of monomer during the course of polymerization was determined. The polymer was purified three times by precipitation in petroleum spirits (boiling range of 40-60 ^{o}C). After centrifugation (7000 rpm for 15 min), the polymer was dried under reduced pressure at room temperature. POEGMEMA(F) 28 repeating unites ($M_{n(theo)}$= 8400 g mol^{-1}, $M_{n(SEC)}$= 9800 g mol^{-1}) was used as macro-RAFT agent for the chain extension of methacrylic acid (MAA) and tert-butyl methacrylate (tBMA). The number of repeating units of POEGMEMA(F) was calculated from the monomer conversion obtained from ^{1}H-NMR.

Chain extension POEGMEMA-co-FOMA with tert-butyl methacrylate (tBMA)

POEGMEMA(F) macro-RAFT with 28 repeating units ($M_{n(theo)}$= 8400 g mol^{-1}, $M_{n(SEC)}$= 9800 g mol^{-1}) was used for the chain extension with tert-butyl methacrylate (tBMA). POEGMEMA-co-FOMA macro-RAFT (50 mg, 5.95 $\times 10^{-6}$ mol), tBMA (0.422 g, 2.97$\times 10^{-3}$ mol) and AIBN (0.2 mg, 1.22 $\times 10^{-6}$ mol) were dissolved in 3 mL of methanol. The reaction mixtures were placed in an ice bath and purged with nitrogen for 30 min where then immersed in a preheated oil bath at 70 ^{o}C for 6 h. The polymerization was terminated by placing the samples in an ice bath for 5 min. The polymer was purified three times by precipitation in petroleum spirits (boiling range of 40-60 ^{o}C). After centrifugation (7000 rpm for 15 min), the polymer was dried under reduced pressure at room temperature. By comparing the intensity of vinyl proton peaks (6.1 and 5.6 ppm) to that of *tert* butyl peaks (1.59 ppm), the conversion of monomer during the course of polymerization was determined and the polymer obtain contains additional 71 repeating units of tBMA ($M_{n(theo)}$= 18400 g mol^{-1}, $M_{n(SEC)}$= 21800 g mol^{-1})

Deprotection of tert-butyl group of POEGMEMA(F)-b-tBMA

The copolymer POEGMEMA(F)-b-tBMA (30 mg, 2.72 $\times 10^{-6}$ mol) was dissolved in 2 mL of methanol and added 2 drops of HCl (32%), the reaction mixture was then heated to 70 ^{o}C overnight. The copolymer was purified by precipitating in cold diethyl ether, centrifuged and dried under reduced pressure to give final block copolymer of $POEGMEMA(F)_{28}$-b-MAA_{175}.

Self-assembly of POEGMEMA-b-PtBMA & POEGMEMA-b-MMA

10 mg of statistical (POEGMEMA-co-FOMA)-b-PtBMA or POEGMEMA-b-PMMA polymer were dissolved in methanol (2 mL), DI water (2mL) was added dropwise over 2 hours and the solution was dialysed against DI water for 2 days with frequent water change.

Synthesis of cis,cis,trans-[$Pt(NH_3)_2Cl_2(OH)_2$] (Oxoplatin)

Oxoplatin was synthesized according to literature, [34] A mixture of cisplatin (1.0 g, 3.05 mmol) and H_2O_2 30% w/v (3.5 mL, 30.5 mmol) in an aluminum foil covered round-bottom flask was heated at 70 °C for 5 h. The heat was then removed and the reaction mixture was stirred overnight. The product was recrystallized in situ at 4 °C overnight. The product was obtained by vacuum filtration and washed with ice cold water, ethanol, and diethyl ether. After filtration, the solvent was removed under reduced pressure to give the expected product as bright yellow powder.

Attachment of oxoplatin to POEGMEMA(F)-b-MAA copolymer

30 mg of POEGMEMA(F)-b-PMAA polymer was dissolved in DMF (5mL). Oxoplatin (25 mg, 0.07 mmol), 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC) (25 mg, 1.30×10^{-4}mol) and 4-dimethylaminopyridine (DMAP) (6 mg, 4.9×10^{-5} mol) was added to the solution and it was left to stir in an oil bath at 40^{o}C for 24 h. The sample was then purified by centrifugation to remove the undissolved oxoplatin and then dialyzed against water for 48 hrs using membrane (MWCO3500) dialysis to remove free oxoplatin. The water is replaced every 6 hrs. During dialysis, micelle formation was induced since the hydrophilic PMAA takes on hydrophobic featured after oxoplatin conjugation.

Attachment of acridine-boronic acid linker to micelles

Acridine-boronic acid linker (2.5 mg,) was dissolved in DMSO (1mL) and added to MilliQ water (4 mL) to give a concentration of 0.5 mg/mL. The solution was added to the micelle solution according to 20% (mole ratio between acidine and polymer concentration) acrdine content. The solution was stirred overnight before further use.

Fluorescence binding studies

The experimental set-up for fluorescence binding studies has been described in the literature.[35] For ARS-boronic acid fluorescent measurement, a stock solution of ARS (9.0×10^{-5} M) in 0.10 M sodium phosphate mono basic buffer made fresh (within the last 7 days) and stored in the refrigerator, was diluted 10-fold with 0.10 M sodium phosphate monobasic buffer to give final concentration of 9.0×10^{-6} M ARS in 0.10 M sodium phosphate buffer at pH of 7.4 (solution A). Boronic acid (BA) was added to a portion of solution A to make a 9.0×10^{-6} M ARS, 2.0×10^{-3} M boronic acid solution in sodium phosphate buffer (solution B). The pH was checked again and corrected if necessary.

Solution B was titrated into solution A in order to make mixtures with a constant concentration of ARS and a range of concentrations of BA. Twelve different concentrations were made in order to cover as much of binding curve as possible which gives an increasing concentration of ARS-BA complex at 0.25, 0.75, 1, 2.5, 5, 7.5, 10, 30, 50, 70, 100 and 222 equiv. of BA. Each mixture was allowed to stand for 5 minutes before any measurements were taken. The intensity of the excitation was recorded at 468 nm and emission at 572 nm. The experiments were carried out in triplicates.

Competitive binding assay

A solution of 9.0×10^{-6} M ARS and 2.0×10^{-3} M of PBA in 0.10 M sodium phosphate buffer was prepared and pH adjusted (solution B). Diol of choice (dopamine, 3,4-dihydroxy benzoic acid, polymers or micelle solution) was dissolved in a portion of solution B which gives a final concentration of 0.20×10^{-3} M diol, 9.0×10^{-6} M ARS and 2.0×10^{-3} M of PBA in 0.10 M sodium phosphate buffer at pH of 7.4 (solution C). Various volume of solution C was added to solution B to make thirteen mixtures of a constant concentration of ARS and PBA and a range of concentrations of diols at 0.25, 0.75, 1, 2.5, 5, 7.5, 10, 30, 50, 70, 100, 200 and 222 equiv. of PBA. The experiment was repeated using the same concentration of all compounds in 0.10 M PBS buffer at pH 5.

The following equations calculate the equilibrium constant between boronic acid and catechol in competitive binding studies:

$$\frac{1}{\Delta I_f} = (\Delta kp_o I_o K_{eq1})^{-1} \frac{1}{[L]} + (\Delta kp_{oo} I_o)^{-1}$$

$$\frac{[S_o]}{P} = \frac{K_{eq1}}{K_{eq}} Q + 1$$

$$Q = \frac{[I]}{[IL]}$$

$$P = [L_o] - \frac{1}{QK_{eq1}} - \frac{[I_o]}{(Q+1)}$$

Keys:

I_f	Fluorescent intensity
L	Ligand (boronic acid)
$[L_o]$	Total concentration of ligand (boronic acid)
I	Indicator (ARS)
$[I_o]$	Total concentration of indicator (ARS)
Δkp_o	Constant derived from the intrinsic fluorescence and laser power
S	Substrate (diol)
K_{eq}	Association constant of the diol-PBA complex
K_{eq1}	Association constant of ARS-PBA complex
Q	Ratio between concentration of free ARS to complexed ARS

Effect of Calcium ions on the formation of boronates esters

Five different solution were set up in either DI water or PBS. A stock solution of ARS was prepared by dissolving (3 mg, 8 x 10^{-3}mmol) in 10 mL DI water. The concentration of the different compounds for the preparation of the five samples are according to the following table.

The amount of Ca ion was chosen in relation to the cell experiments carried out below. The cell study uses 5 x 10^{-6} M of polymer (which is 5 x 10^{-6} M of dopamine and also boronic acid), while the Ca^{2+} concentration in apoptopic cells can be up to 100 μM (1 x 10^{-4} M). This equates to a potential ratio of the Ca^{2+}/PBA concentration of up to 20.

The fluorescent experiment employed a ratio of 112 for the Ca^{2+}/PBA concentration to achieve a more dramatic measureable effect.

Table S 1. Concentrations of compounds in solution for fluorescent measurements

Sample	Solvent	Calcium chloride (M)	ARS (M)	PBA (M)	Dopamine (M)
A	DI water	N/A	$9x10^{-6}$	2×10^{-3}	N/A
B	DI water	N/A	$9x10^{-6}$	2×10^{-3}	4×10^{-3}
C	DI water	0.45	$9x10^{-6}$	2×10^{-3}	N/A
D	DI water	0.45	$9x10^{-6}$	2×10^{-3}	4×10^{-3}
E	PBS	N/A	$9x10^{-6}$	2×10^{-3}	N/A

Boron NMR Spectroscopy

Four solutions were prepared for the analysis of Boron in Boron NMR spectroscopy. PBA (4 mg, 3.28×10^{-5} mol) was dissolved in PBS buffer (0.1 M, 0.5 mL) and added 0.5 mL of D_2O. In another solution PBA (4 mg, 3.28×10^{-5} mol) and dopamine hydrochloride (140 mg, 73.8×10^{-4} mol) was dissolved in PBS buffer (0.1 M, 0.5 mL) and added 0.5 mL of D_2O pH is adjusted to 7.4. In the third solution same amount of PBA and dopamine was added except the pH was adjusted to 5. In the last solution PBA (4 mg, 3.28×10^{-5} mol), calcium chloride (10 mg, 90 mM) and dopamine hydrochloride (140 mg, 73.8×10^{-4} mol) was dissolved in PBS buffer (0.1 M, 0.5 mL) and added 0.5 mL of D_2O pH adjusted to 7.4.

Additional sample were prepared for the analysis of Boron NMR spectroscopy. Polymer B1 (50 mg) of approximate molecular weight of 4800 g/mol was dissolved in 30 % hydrogen peroxide solution and was left to stir at room temperature for overnight followed by freeze drying to remove the water. The polymer was then dissolved in a mixture of PBS buffer solution (0.1 M, 0.5 mL) and D_2O (0.5 mL) as well as PBA (4 mg, 3.28×10^{-5} mol).

Cell culture

Human ovarian carcinoma OVCAR-3 cells were kindly provided by Dr. Paul de Souza from the St. George Hospital, Sydney, Australia. The cell lines were grown in a ventilated tissue culture flask T-75 using Roswell Park Memorial Institute (RPMI-1640) media containing 10% foetal bovine serum (FBS) and antibiotics. The cells were incubated at 37°C in a 5% CO_2 humidified atmosphere and passaged when monolayers at around 80% confluence were formed. Cell density

was determined by counting the number of viable cells using a trypan blue dye (Sigma-Aldrich) exclusion test. For passaging and plating, cells were detached using 0.05% trypsin-EDTA (Invitrogen), stained using trypan blue dye, and loaded on the haemocytometer.

Sample preparation for laser scanning confocal microscopy

Human ovarian carcinoma Ovcar-3 cells were seeded in 35 mm Fluorodish (World Precision Instruments) at a density of 60,000 per dish and cultured for 3 days with RPMI 1640 medium supplemented with 10% fetal bovine serum. Micelles solution was loaded to Ovcar-3 cells at a working concentration of 50 μg/mL of polymer and incubated at 37 ºC for designed periods (1 hr, 6 hrs and 24 hrs). After incubation, the cells were washed thrice with phosphate buffered saline (PBS, pH 7.4). Then the cells were stained with 100 nM LysoTracker Red DND-99 (Invitrogen) for 1 min. The dye solution was quickly removed and the cells were gently washed with PBS. Finally, the cells were mounted in PBS and observed under a laser scanning confocal microscope system (Zeiss LSM 780). The system equipped with a Diode 405-30 laser, an argon laser and a DPSS 561-10 laser (excitation and absorbance wavelengths: 405 nm, 488 nm and 561 nm, respectively) connected to a Zeiss Axio Observer.Z1 inverted microscope (oil immersion ×100 /1.4 NA objective). The ZEN2011 imaging software (Zeiss) was used for image acquisition and processing.

Control experiment using added Ca^{2+} in to the cell growth media

Ovcar-3 cells were seeded at 60,000 cells per dish and cultured for 3 days. Micelles were loaded to the cells at a concentration of 50 μg/mL in serum plus RPMI 1640 medium. After incubation for 3 hrs with micelles, the cells were washed with PBS once and incubated with Hank's buffer containing 8.4 mM Ca^{2+} for another 3 hrs. The Hank's buffer also contained 50 μg/mL micelles. The control was the cells treated with normal Hank's buffer (1.3 mM Ca^{2+}) containing micelles. Finally, the samples were observed under a confocal microscope (Zeiss LSM780) after staining with LysoTracker.

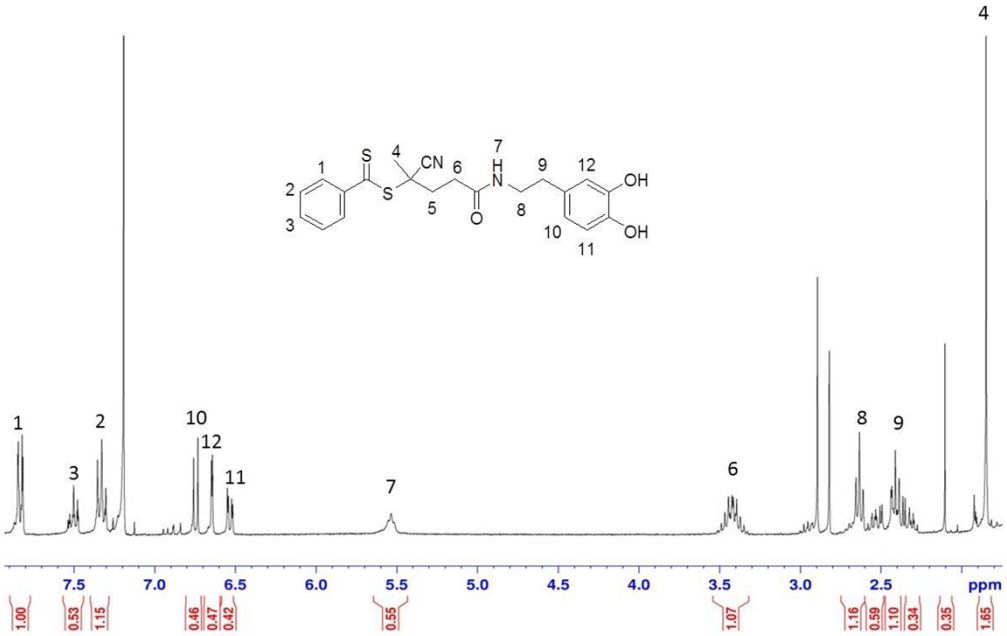


Figure S 1. [1]H-NMR spectra of dopamine terminated dithiol carbonate RAFT agent

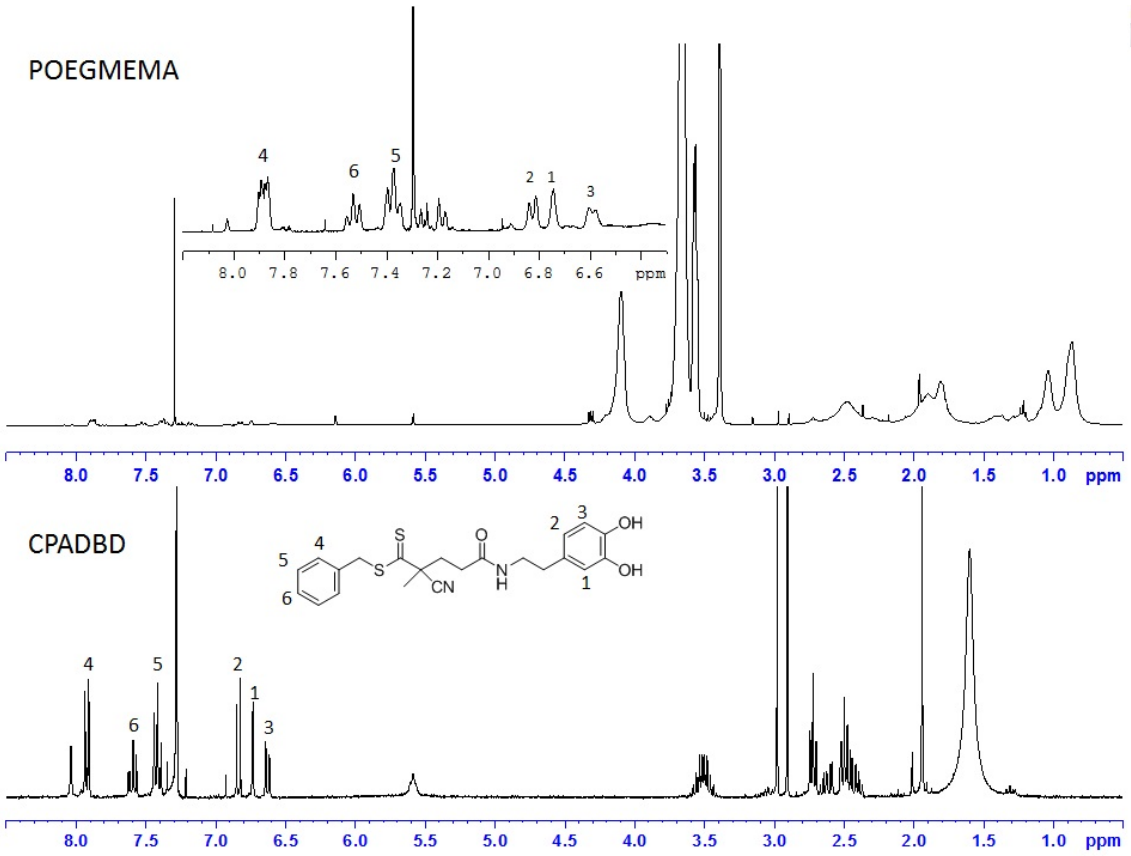


Figure S 2. ^{1}H-NMR spectra comparison of poly[oligo(ethyleneglycol) methylether methacrylates] POEGMEMA and dopamine terminated dithiol carbonate RAFT agent confirms the end group functionality of the polymer

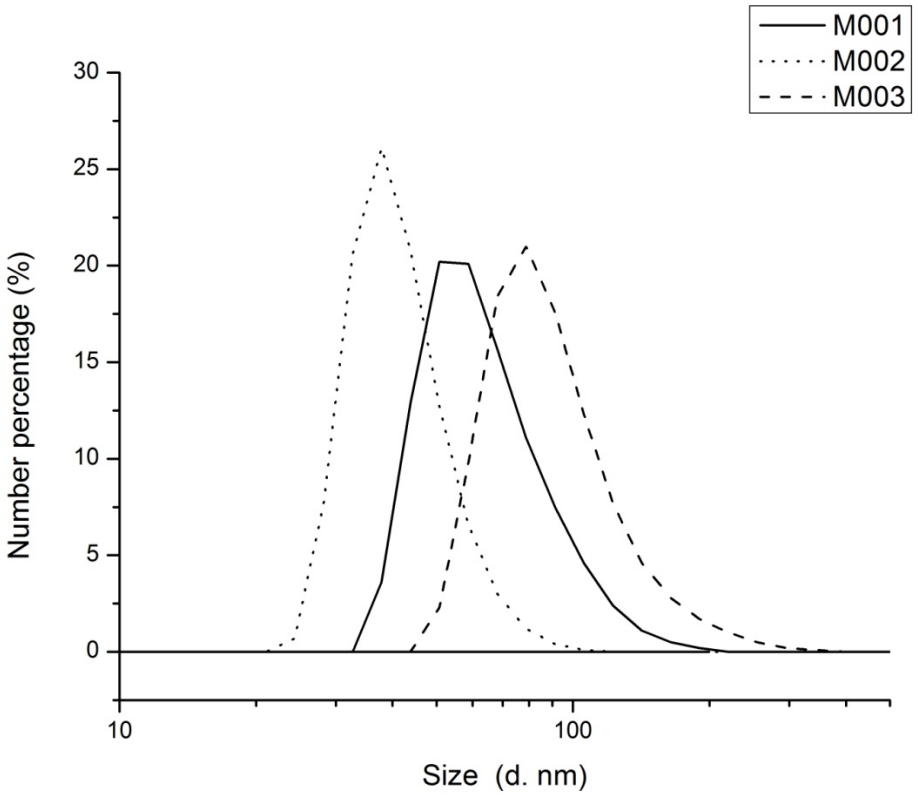


Figure S 3. Hydrodynamic diameter distribution of micelles synthesised M001 ($POEGMEMA_{32}$-b-MMA_{71}), M002 ($POEGMEMA_{28}$-b-$tBMA_{175}$), M003 ($POEGMEMA_{28}$-b-MAA_{175}-pt)

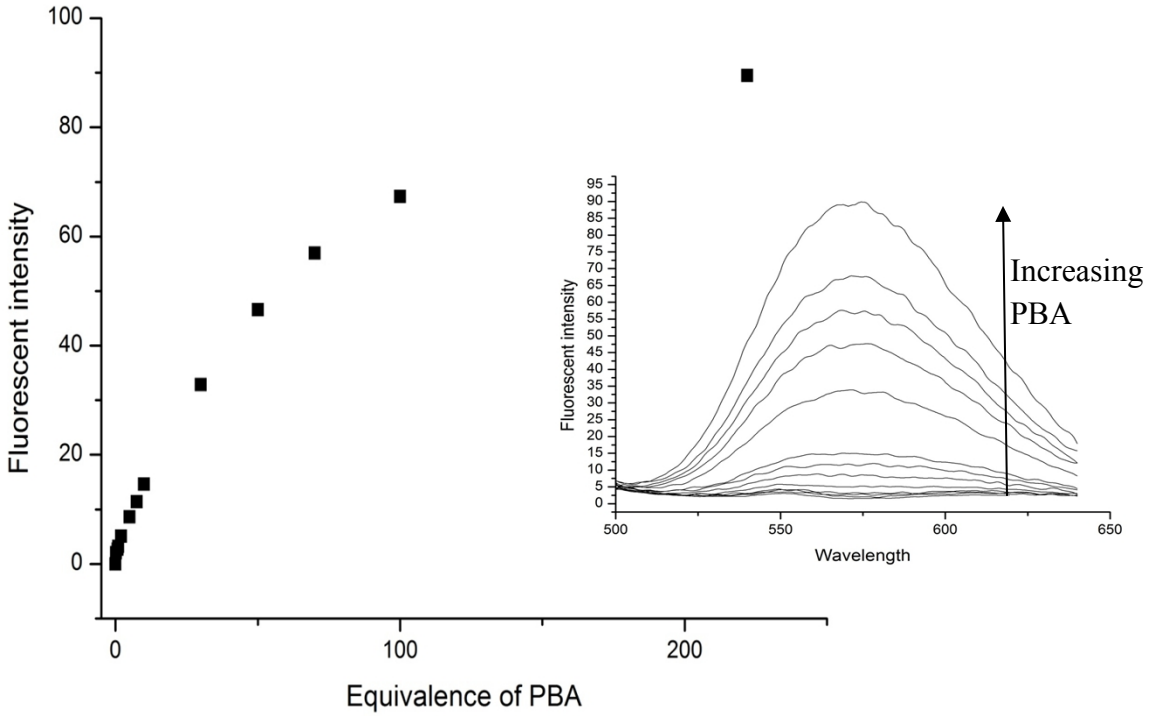


Figure S 4. Fluorescent intensity increases with increasing PBA in presence of ARS (9.0×10^{-6} M) Insert: Fluorescence emission spectra of ARS-PBA (0.25 – 222 equiv. PBA)

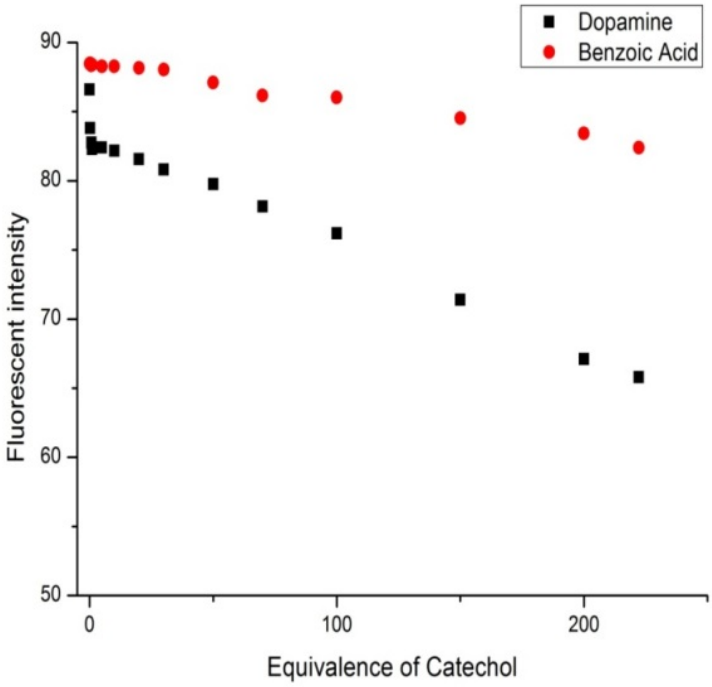


Figure S 5. Competitive binding of a phenylboronic acid with Alizarin Red S (9.0×10^{-6}M) and dopamine, two POEGMEMA polymers and the POEGMEMA-*b*-PMMA block copolymer micelle in phosphate buffer solution (pH 7.4 and pH5, 0.10 M).

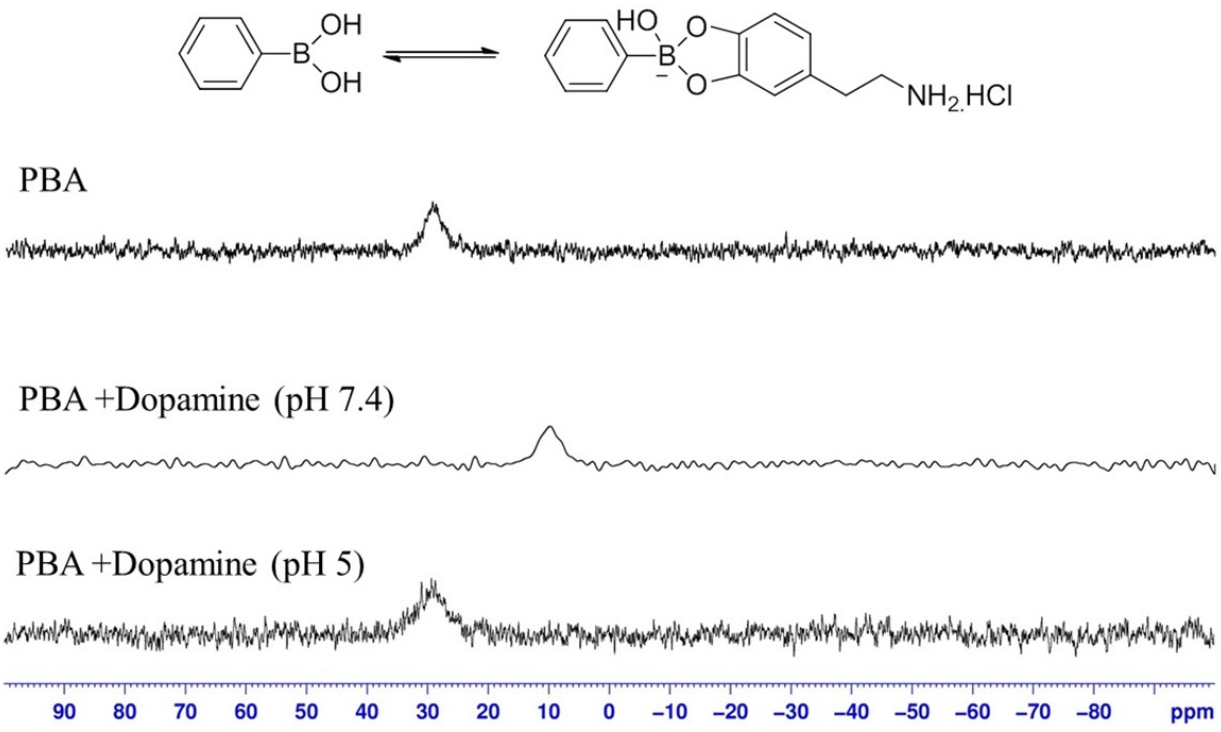


Figure S 6. ^{11}B-NMR spectra for top to bottom: PBA (32 mM) in pH 7.4 in D_2O solution, PBA (32 mM) and dopamine (740 mM) in pH 7.4 D_2O solution, PBA (32 mM) and dopamine (740 mM) in pH 5 D_2O solution.

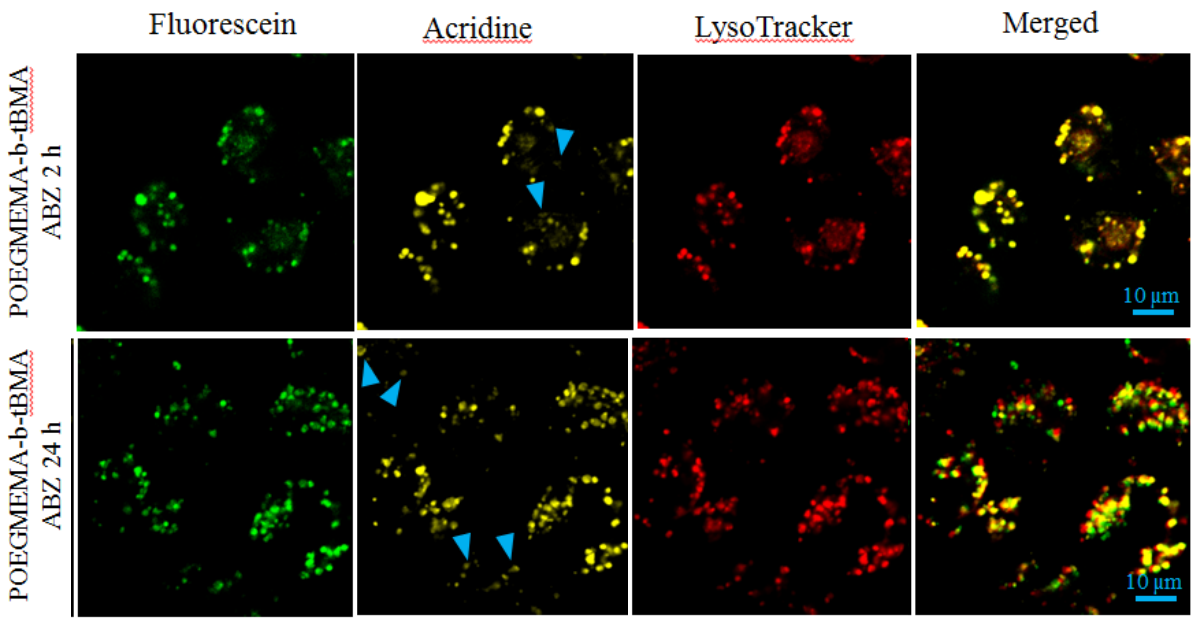


Figure S 7. Confocal microphotographs of Ovcar-3 cells after incubation with $POEGMEMA_{26}$-b-$tBMA_{175}$-ABZ micelles at 37 °C for 1 hr, 6 hrs and 24 hrs. , Polymers (Green) were labeled with fluorescein. Acridine (yellow) was added to the micelles. Lysosomes (Red) were stained with LysoTracker Red DND-99. Blue triangles indicate the intracellular parts with only acridine but without fluorescein-labeled polymers. Scale bar = 5 µm.

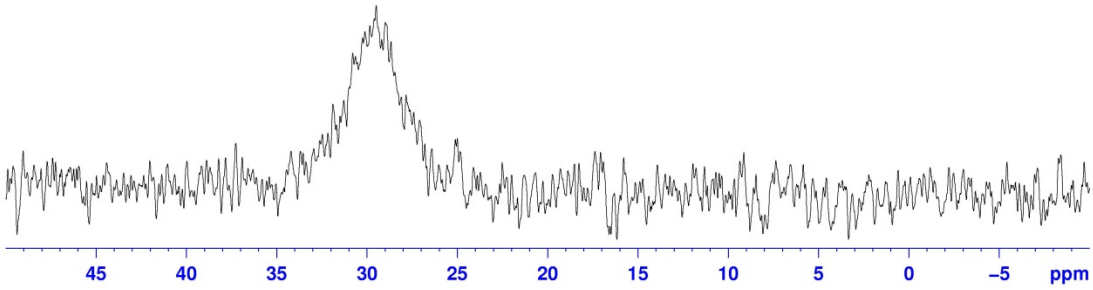


Figure S 8. ^{11}B-NMR spectra of hydrogen peroxide treated polymer comprised of dopamine terminated $POEGMEMA_{15}$ (50 mg) and PBA (4 mg) in PBS and D_2O solution.

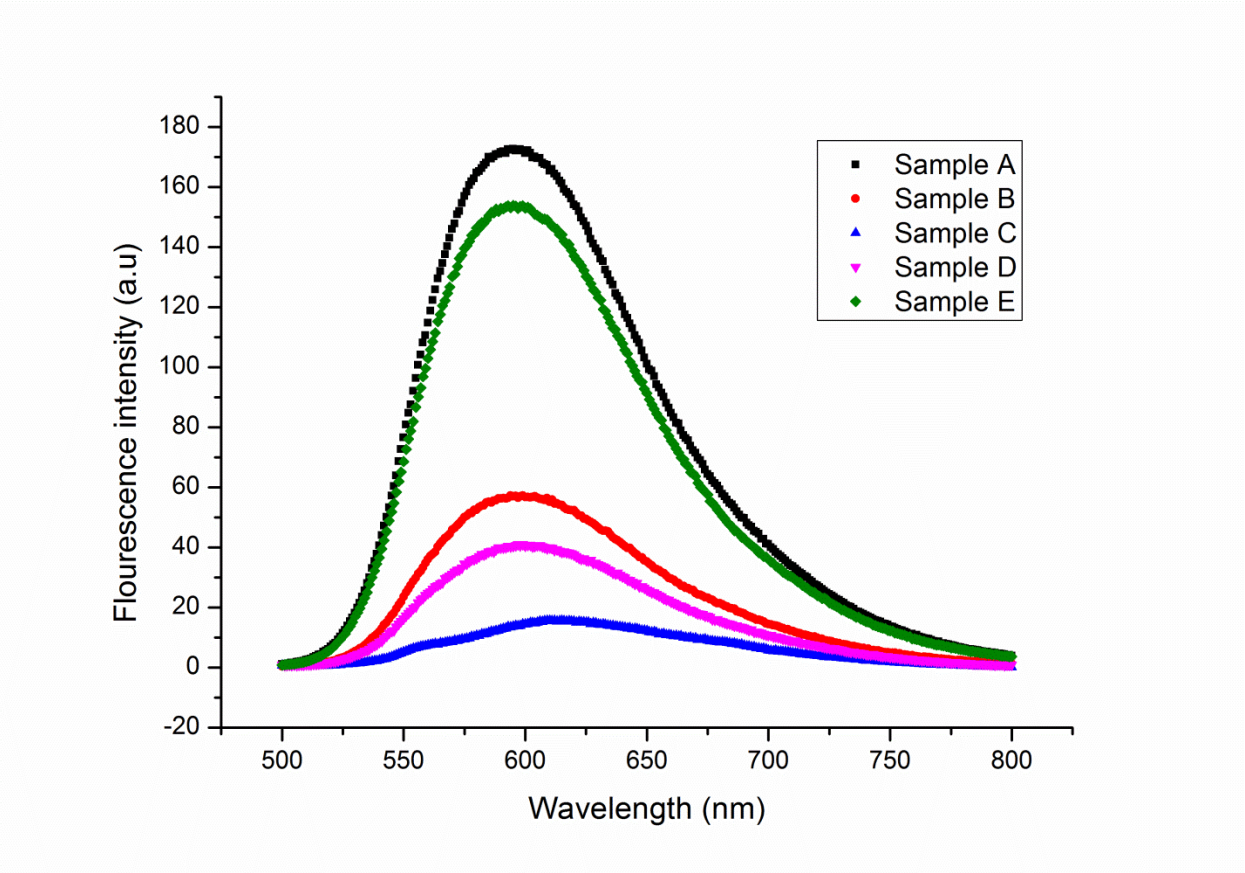


Figure S 9. Fluorescence spectra of ARS and PBA in DI water (A), ARS, PBA and dopamine in DI water (B), Ca^{2+}, ARS and PBA in DI water (C), Ca^{2+}, ARS, PBA and dopamine in DI water (D) and ARS and PBA in PBS buffer (E), see Table S1 for concentrations. Addition of Ca^{2+} to solution (A) leads to reduction of fluorescence caused binding between ARS and Ca^{2+} and therefore the depletion of the fluorescent ARS-PBA complex. Addition of dopamine to (C) led to competition of dopamine for Ca^{2+} with the ARS-Ca^{2+} complex. Some ARS has been liberated and is now available for binding with PBA, which cause the increase in fluorescence.

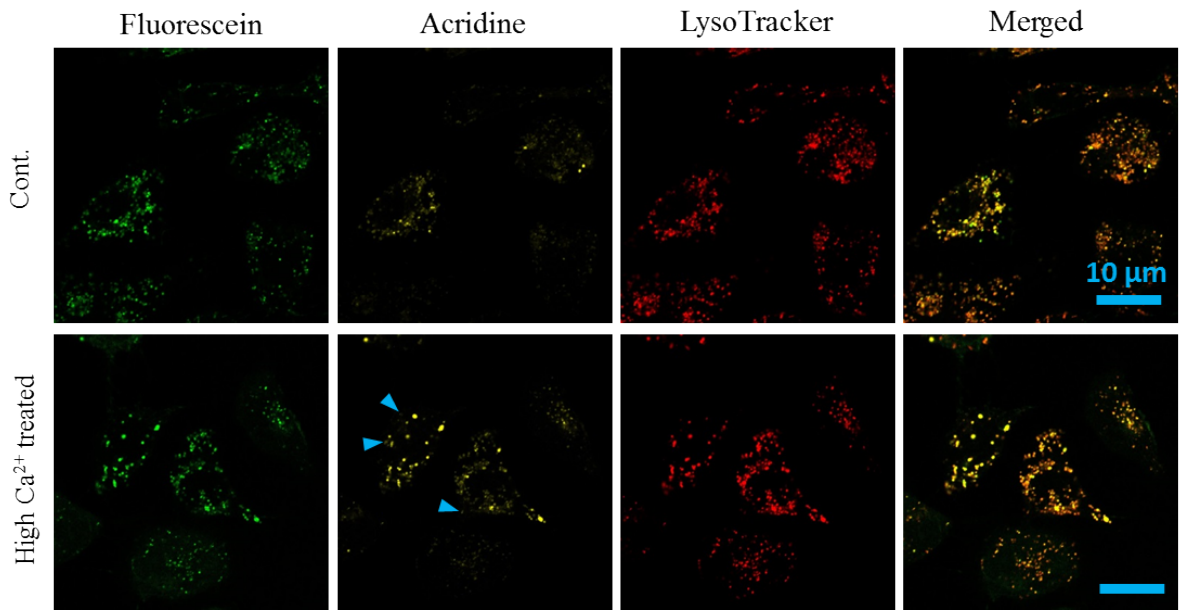


Fig S 10. Confocal microphotographs of Ovcar-3 cells incubated with micelles with and without extracellular Ca^{2+} overloading. Polymers (Green) were labelled with fluorescein. Acridine (yellow) was added to the micelles. Lysosomes (Red) were stained with LysoTracker Red DND-99. Blue triangles indicate the intracellular parts with only acridine but without fluorescein-labelled polymers. Scale bar = 10 µm.