Electronic Supplementary Material (ESI) for Chemical Communications
This journal is © The Royal Society of Chemistry 2013

Supplementary Information

ATRP Synthesis of Poly(2-(Methacryloyloxy)ethyl Choline Phosphate): a Multivalent Universal Biomembrane Adhesive

Xifei Yu[1,2], Xiaoqiang Yang[1,2], Sonja Horte[1,2], Jayachandran N Kizhakkedathu[1, 2, 3] and Donald E. Brooks[1, 2, 3, *]

[*] Corresponding Author: E-mail: don.brooks@ubc.ca;

[1] Centre for Blood Research, University of British Columbia, 2350 Health Sciences Mall, UBC, Vancouver, V6T 1Z3, Canada, Tel: 604 822 7081, Fax: 604 822 7742;

[2] Department of Pathology and Laboratory Medicine, UBC;

[3] Department of Chemistry, UBC

This journal is © The Royal Society of Chemistry 2013

Materials and Methods.

An ATRP initiator of 3-azidopropyl 2-bromo-2-methylpropanoate was synthesized according to previous report [1]. 2-chloro-2-oxo-1,3,2-dioxaphospholane was synthesized as described in the literature [2]. All chemicals were purchased from Sigma-Aldrich (ON, Canada) and used without further purification unless otherwise mentioned. Proton NMR spectra were recorded on a Bruker AV400 spectrometers using solvent as the internal standard. Proton decoupled ^{31}P-NMR spectra were recorded on Bruker AV400 spectrometers using D_2O or Methanol-d_4 as the solvent and deuterated phosphoric acid as the internal standard.

The molecular weights and polydispersities of copolymers were determined by gel permeation chromatography (GPC) on a Waters 2690 Separation module fitted with a DAWN EOS multi-angle laser light scattering (MALLS) detector from Wyatt Technology Corp (Santa Barbara, CA) with 18 detectors placed at different angles (laser wavelength λ = 690 nm) and a refractive index detector from Wyatt Technology Corp. operated at λ = 620 nm. Sodium nitrate (0.1 M) aqueous solution was used as an eluent at a flow rate of 0.8 mL/min.

Synthesis of 2-(Methacryloyloxy)ethyl Choline Phosphate, MCP

A scheme for the synthesis of 2-(Methacryloyloxy)ethyl Choline Phosphate (MCP) is given in Scheme S1. The synthesis of choline phosphate is very sensitive to moisture. Based on previous classical synthetic methods of phosphorylcholine (PC) [3], a reaction

This journal is © The Royal Society of Chemistry 2013

intermediate of 2-methoxy-2-oxo- 1,3,2-dioxaphospholane (MDP) is implied. However, the MDP is very easily hydrolyzed via endocyclic cleavage between pH 8~15, generating many side products [4, 5]. To reduce this side hydrolysis reaction, we developed the present synthetic strategy where 2-(dimethylamino)ethyl methacrylate is used, acting as both catalyst and reactant instead of the classic two steps reaction [3].

It should be noted that this reaction should be performed under absolutely anhydrous conditions to minimize the side products. Since the side products have similar properties, avoidance of side reactions is also critical for purification of the products. Therefore, all the glassware was flame dried and protected by dried argon at each step. Acetonitrile was dried by distillation against CaH_2, and distilled freshly before using. 2-(dimethylamino) ethyl methacrylate (98%) was dried by CaH_2, stirred at 0 °C for 72 h, and then distilled under reduced pressure before using. Anhydrous methanol was prepared by the distillation of sodium methoxide (25%) methanol solution.

Methanol (1.92 g, 0.06 mol), 2-(dimethylamino)ethyl methacrylate (18.87 g, 0.12 mol), acetonitrile (50 mL) and monomethyl ether hydroquinone (200 mg, as an inhibitor) were added to a 100 mL schlenk flask, and cooled to -78 ºC. To this cooled mixture, 2-chloro-2-oxo-1,3,2-dioxaphospholane (7.12 g, 0.05 mol) was added dropwise over 2 h. The reaction was continued 2 h at -78 ºC, and was allowed to warm to room temperature (22 °C) for another 4 h. The reaction mixture then was cooled to -20 °C, filtered off the precipitate using an air-free funnel, and the resulting mixture was

directly filtered into a schlenk flask under argon atmosphere. The resulting solution was stirred overnight at 65-70 ºC. The reaction mixture was cooled to room temperature and precipitated into tetrahydrofuran. The precipitate was washed with THF until the supernatant was clear and colorless. The product was dried and collected as a yellow waxy solid, 2-(Methacryloyloxy) ethyl Choline Phosphate; yield was 5.4 g. The typical yield of the reaction is between 20 to 40% with careful control of the reaction conditions. The product was stored as a solution containing 1000 ppm monomethyl ether hydroquinone in methanol (50%) in the dark at -80 ºC.

^{1}H-NMR (δ: ppm, 400 MHz, methanol-d_4): 6.16, 5.72 (C=C$\boldsymbol{H}_2$, 2s, 2H), 4.65-4.64 (COOC$\boldsymbol{H}_2$, t, 2H), 4.28-4.26 (POC$\boldsymbol{H}_2$, t, 2H), 3.88-3.85 (C$\boldsymbol{H}_2$N$(CH_3)_2CH_2CH_2OOC$, t, J = 4.80 Hz, 2H); 3.76-3.72 (CH_2N$(CH_3)_2$C$\boldsymbol{H}_2CH_2OOC$, t, J = 4.57 Hz, 2H), 3.62, 3.58 (OC$\boldsymbol{H}_3$, 2s, 3H), 3.28 (N(C$\boldsymbol{H}_3)_2$, s, 6H), 1.97 (-C$\boldsymbol{H}_3$, s, 3H) and ^{31}P-NMR (δ: ppm, 400 MHz, methanol-d_4): 0.58 (s) are shown in Fig S1.

TOF-MS ES+, m/z: 296.13 (M + 1H), see Fig S2.

ATRP Synthesis of poly(2-(Methacryloyloxy)ethyl Choline Phosphate), PMCP

The inhibitor of monomethyl ether hydroquinone in MCP was been removed using the Inhibitor Removers (Sigma-Aldrich, catalog number: 306312) before the polymerization of MCP.

An ATRP initiator of 3-azidopropyl 2-bromo-2-methylpropanoate (25 mg, 0.1 mmol), 4,4'-dinonyl-2,2'-dipyridyl (dNPy, 81.6 mg, 0.2 mmol), MCP (1.5 g), and

methanol (5 mL) were added into a clean, dry 25 mL schlenk flask, which was then put through three cycles of freeze-pump-thaw with argon prior to the addition of CuBr (14.3 mg, 0.1 mmol). The flask was further freeze-pump-thaw treated with argon three times after the addition of CuBr. The mixture was maintained at 25 ºC for 4 hours. The polymerization was stopped by exposing the contents to air. The reaction mixture was filtered and precipitated into hexane. Finally, dialysis was used to remove the excessive MCP and the copper catalyst with MWCO 1000 membrane. After freeze-drying, PMCP was collected with $M_{n,\,NMR}$ = 12K, $M_{n,\,GPC}$ = 16.5K, M_w/M_n = 1.14. Yield is 1.3 g (87%).

^{1}H-NMR (δ: ppm, 400 MHz, methanol-d_4): 4.68-4.42 (COOC***H***$_2$), 4.28-4.05 (POC***H***$_2$), 3.89-3.75 (C***H***$_2$N(CH$_3$)$_2$C***H***$_2$), 3.75-3.55 (OC***H***$_3$), 3.30-3.25 (N(C***H***$_3$)$_2$), 2.45-2.45 (C***H***$_2$), 1.6-1.3 (C***H***$_3$); ^{31}P-NMR (δ: ppm, 400 MHz, methanol-d_4): 0.52 (s), shown in Fig S3.

Care should be taken in storing the polymers; dry or in methanolic solutions are preferred. Under aqueous conditions we have seen a slow degradation.

Synthesis of fluorescently labeled PMCP

PMCP (M_n = 12K, PDI = 1.14, 100 mg), prop-2-yn-1-amine (1 mg), copper sulfate pentahydrate (5 mg), sodium ascorbate (10 mg) and methanol (5 mL) were added and mixed into a 20 mL one-necked flask. The reaction was continued for 24 h at room temperature, and then Alexa Fluor-488 carboxylic acid, succinimidyl ester (1 mg, Invitrogen, USA) added to the reaction mixture and the stirring was continued for

This journal is © The Royal Society of Chemistry 2013

another 12 h. An Alexa Fluor 488 labeled PMCP (AF488-PMCP) was obtained after dialyzing the polymer for 24 h against water using MWCO 1000 membrane, yield was 53 mg.

Confocal Laser Scanning Microscopy (CLSM) analysis:

Preparation of red blood cells: EDTA-anticoagulated whole human blood (6 ml) was collected from healthy donors after obtaining consent. The protocol was approved by the University of British Columbia clinical ethical committee. The whole blood was centrifuged at 2300 rpm for 5 min. The plasma and buffy coat layers (platelets and white cells) were discarded. The RBC pellet was then washed with 40 ml of phosphate buffered saline (PBS) and was repeated three times.

Adsorption of AF488-PMCP to RBCs: AF488-PMCP (1 mL, 1 mg/mL in PBS solution, pH 7.4) was mixed with washed RBCs (50 μL) and equilibrated for 5 min room temperature. The RBCs were then washed with the phosphate buffered saline (PBS) to remove the free labeled polymer, and viewed using a confocal Microscope (Olympus Fluoview FV1000, Japan).

Adsorption of AF488-PMCP to CHO cells: The CHO cells (ATCC, USA) were seeded on glass bottom dishes (Matteck Corporation, Ashland, USA) and cultured in Dulbecco's Modified Eagle's Medium (DMEM, Invitrogen, USA) supplemented with 10% FBS (Invitrogen, USA) overnight. The medium was discarded and replaced with DMEM containing 1 mg/ml of AF488-PMCP. After incubation at the temperature and

This journal is © The Royal Society of Chemistry 2013

for the time indicated the medium was discarded and the cells were washed twice with PBS. The cells then were fixed with 4 % formaldehyde for 15 min at 37°C and washed three times with PBS. Fixed cells were stained with a sufficient amount of wheat germ agglutinin (WGA)-Alexa Fluor 633 conjugate solution (7 μg/ml in PBS, Invitrogen, USA) for 10 min at room temperature and washed twice with PBS. Finally, the cells were mounted in ProLong Gold antifade reagent wit DAPI (Invitrogen, USA) and viewed by confocal microscopy.

Adsorption of PMCP to human pancreatic carcinoma cells (Hs766T): The Hs766T cells (ATCC, USA) were seeded on glass bottom dishes and cultured in DMEM-F12 (Invitrogen, USA) supplemented with 10% FBS medium two days. The medium was discarded and replaced with DMEM-F12 containing 1 mg/ml of AF488-PMCP. After incubation at the temperature and for the time indicated the medium was discarded and the cells were washed twice with PBS. The cells then were fixed with 4 % formaldehyde for 15 min at 37°C and washed three times with PBS. Fixed cells were stained with a sufficient amount of wheat germ agglutinin (WGA)-Alexa Fluor 633 conjugate solution (7 μg/ml in PBS) for 10 min at room temperature and washed twice with PBS. Finally, the cells were mounted in ProLong Gold antifade reagent with DAPI and viewed by confocal microscopy.

Scanning Electron Microscopy (SEM) Analysis:

RBC morphology after incubation with different polymers was assessed using

This journal is © The Royal Society of Chemistry 2013

SEM analysis. RBC pellets were equilibrated with PMCP in PBS (0.1 mg/mL, 1 mg/mL, 10 mg/mL) for 10 min at room temperature. After equilibration, the RBCs were washed with saline (pH 7.4) to remove unbound polymers at room temperature and the RBCs were fixed overnight at 4 °C using 2.5 % glutaraldehyde in PBS. Afterwards, the RBC suspensions were centrifuged at 500 rpm for 10 min, the supernatant discarded, the fixed RBCs washed once with saline and then were dropped on a silicon wafer. The RBCs were dehydrated by immersing the wafer in 70, 85, 95, 100 % (v/v) ethanol for 10 min, respectively. Finally, the RBCs were dried overnight at room temperature. The RBCs were coated with gold (5 nm) and examined by SEM (Hitachi S4700, Japan).

References:

[1] P. D. Topham, N. Sandon, E. S. Read, J. Madsen, A. J. Ryan and S. P. Armes, *Macromolecules*, **2008**, *41*, 9542–9547

[2] N. T. Thuong and P. Chabrier, *Bull. Soc. Chim. Fr.* **1974**, *32*, 6521.

[3] S. K.Bhatia and J. Hajdu, *J. Org. Chem.* **1988**, *53*, 5034-5039.

[4] K. Taira, T. Fanni, and D. G. Gorenstein, *J. Org. Chem.* **1984**, *49*, 4531-4536.

[5] R. Kluger, F. Covitz, E. Dennis, and L. D. Williams, *J. Am. Chem. Soc.* **1969**, *91*, 6066-6072.

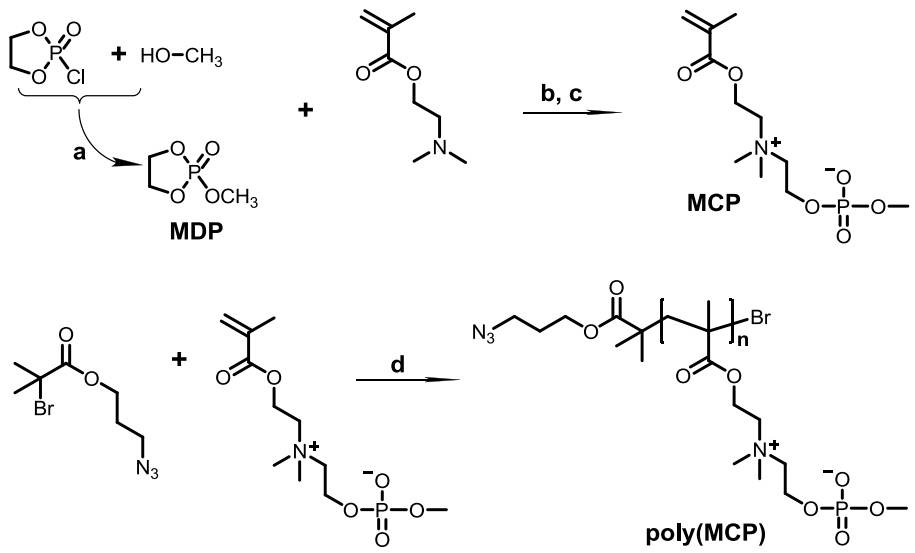
This journal is © The Royal Society of Chemistry 2013

Table S1. Summary of reaction conditions, molecular weight data, and monomer conversions for the homo-polymerization of MCP Using ABMP[a] as initiator in protic media.

monomer	solvent	catalyst[a]	Time h	target D_p	M_n[b] g mol^{-1}	Mw/Mn[b]
MCP	Methanol	CuBr	3	20	7500	1.12
MCP	4:1 methanol/water	CuBr	3	20	7900	1.21
MCP	2:1 methanol/water	CuBr	3	20	7200	1.28
MCP	1:1 methanol/water	CuBr	3	20	8100	1.30
MCP	1:4 methanol/water	CuBr	3	20	7400	1.35
MCP	Water	CuBr	3	20	8400	1.41
MCP	Methanol	CuBr	4	50	16500	1.14
MCP	4:1 methanol/water	CuBr	4	50	16000	1.18
MCP	2:1 methanol/water	CuBr	4	50	17300	1.25
MCP	1:1 methanol/water	CuBr	4	50	16600	1.36
MCP	1:4 methanol/water	CuBr	4	50	17100	1.37
MCP	Water	CuBr	4	50	16900	1.44

[a] 3-azidopropyl 2-bromo-2-methylpropanoate (ABMP) was used as ATRP initiator, and 4,4'-dinonyl-2,2'-dipyridyl (dNPy) was used as the ligand.

[b] The molecular weights (M_n) and polydispersities (M_w/M_n) of PMCPs were determined by gel permeation chromatography (GPC)/multi-angle light scattering.



Scheme S1. The synthetic strategies for 2-(Methacryloyloxy)ethyl Choline Phosphate and Poly(2-(Methacryloyloxy)ethyl Choline Phosphate).

Reagents and conditions: (a) Triethylamine and methylene chloride at -20 ºC. (b) Methanol, 2-(dimethylamino)ethyl methacrylate, 2-chloro-2-oxo-1,3,2-dioxaphospholane and acetonitrile at -78 ºC. (c) Raise the reaction temperature of step (b) to 65 ºC. (d) 4,4'-dinonyl-2,2'-dipyridyl (dNPy), CuBr and methanol at RT. All materials and glassware (a-c) were absolutely anhydrous. All the synthetic methods are described in detail above.

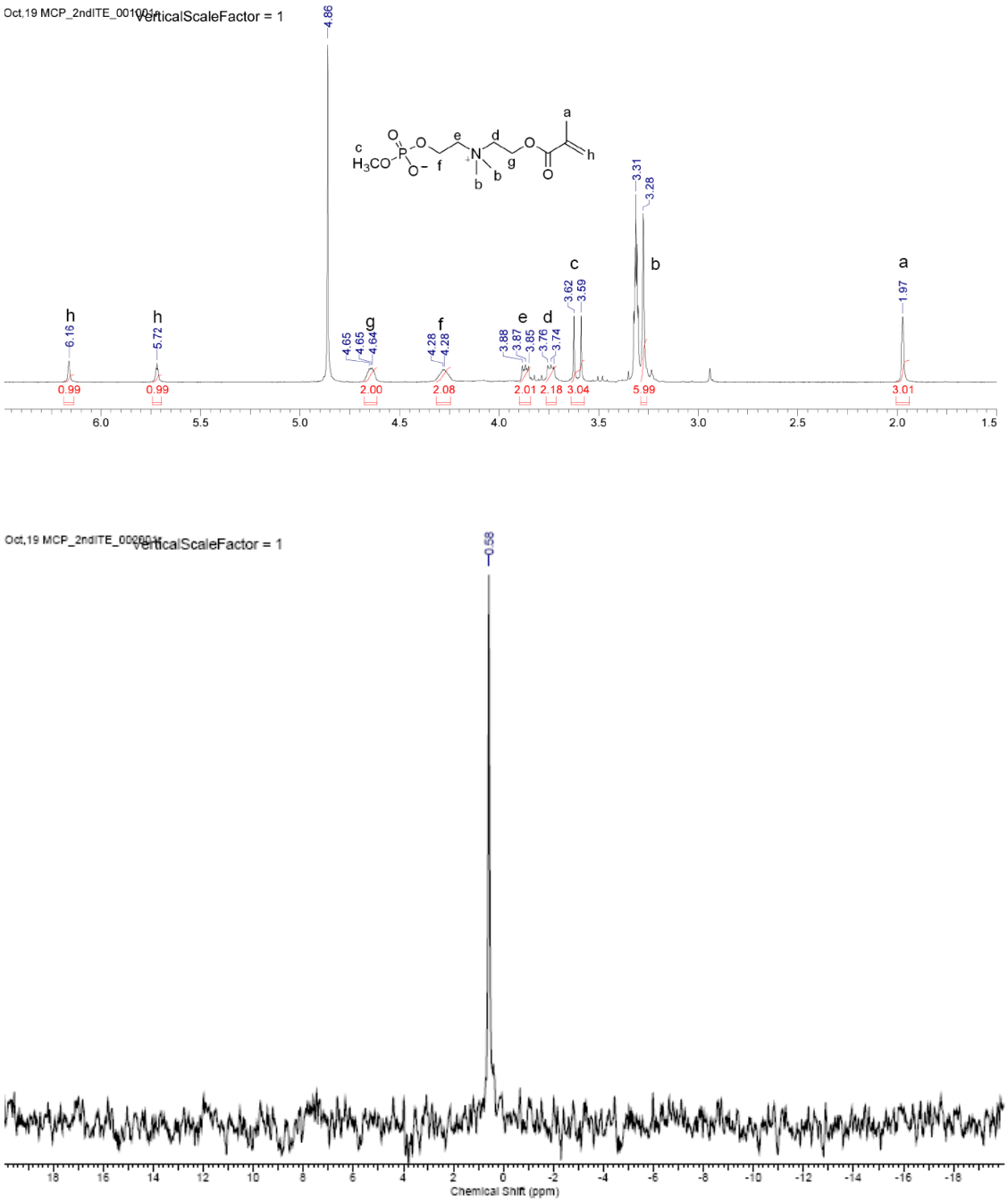


Figure S1 ^{1}H-NMR and ^{31}P-NMR spectrum of 2-(Methacryloyloxy)ethyl Choline Phosphate (MCP).

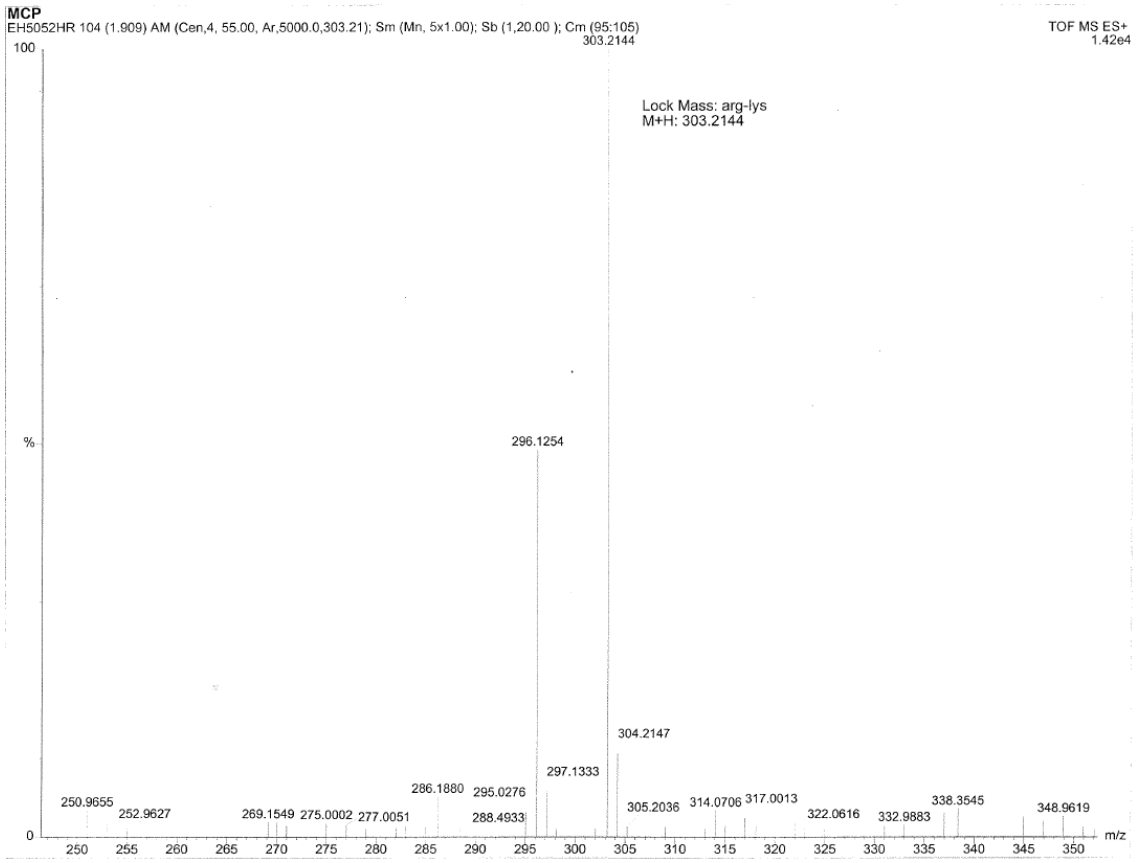


Figure S2 TOF MS(ESI$^+$) spectrum of 2-(Methacryloyloxy)ethyl Choline Phosphate.

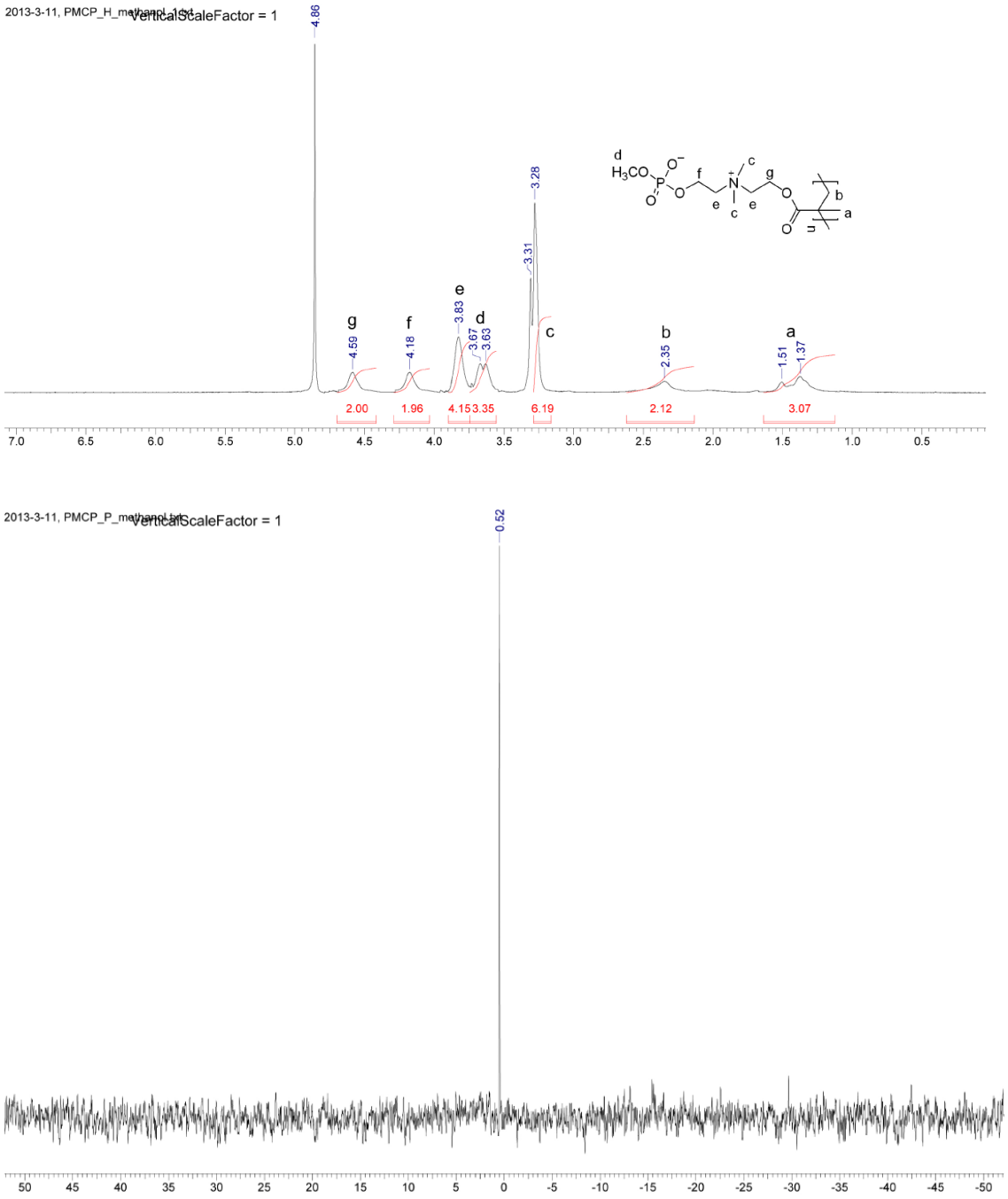


Figure S3 ^{1}H-NMR and ^{31}P-NMR spectrum of poly(2-(Methacryloyloxy)ethyl Choline Phosphate), PMCP.

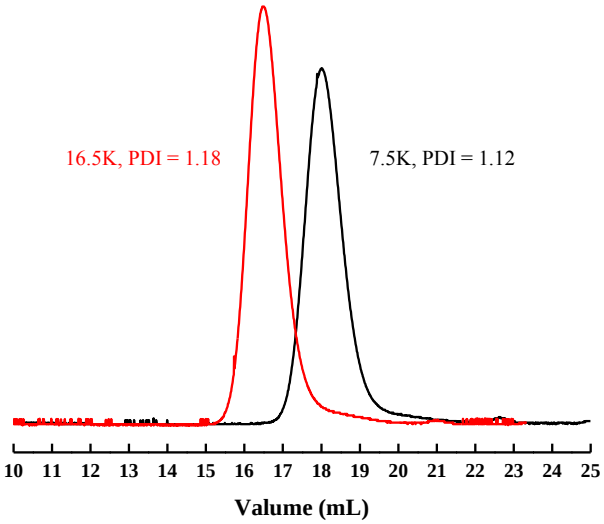


Figure S4. GPC traces of poly(2-(Methacryloyloxy)ethyl Choline Phosphate). Sodium nitrate (0.1 M) aqueous solution was used as an eluent at a flow rate of 0.8 mL/min.

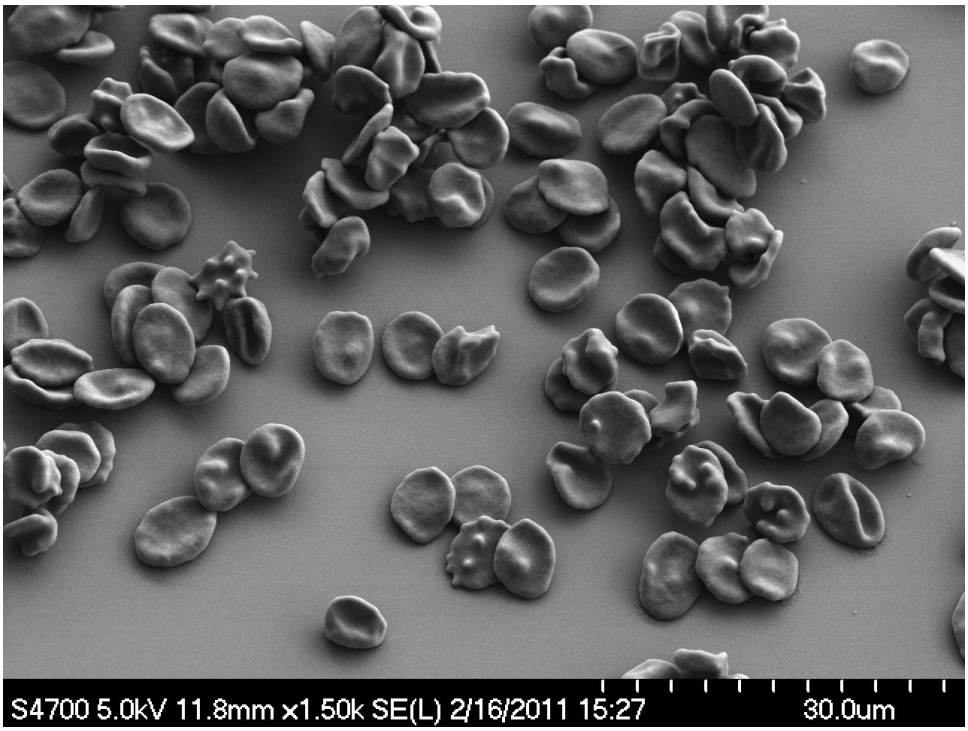


Figure S5 SEM morphologies (×1.5K) of red cell incubation in 0.1 mg/mL PMCP (M_n = 12K, PDI = 1.14) in isotonic saline solution.

This journal is © The Royal Society of Chemistry 2013

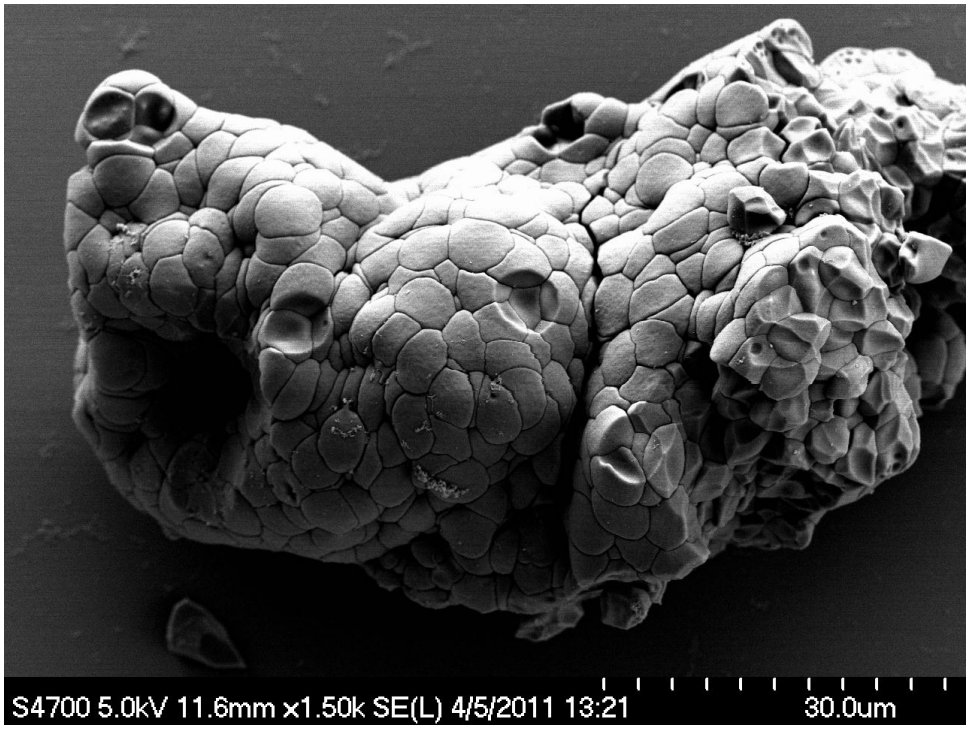


Figure S6 SEM morphologies (×1.5K) of red cell incubation in 1 mg/mL PMCP (M_n = 12K, PDI = 1.14) in isotonic saline solution.

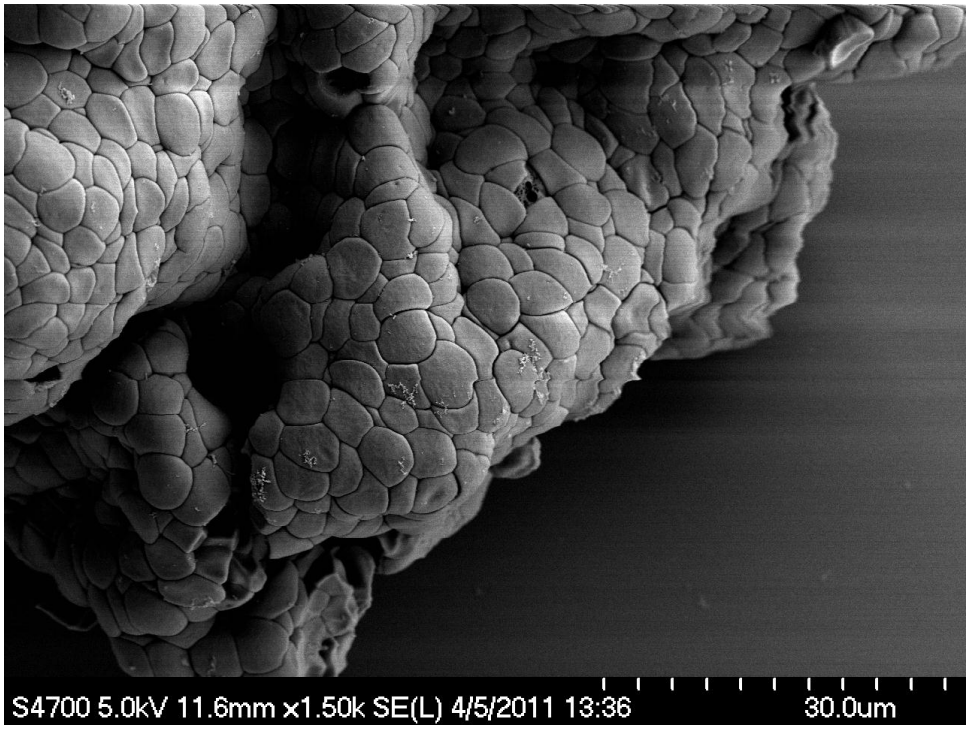


Figure S7 SEM morphologies (×1.5K) of red cell incubation in 10 mg/mL PMCP (M_n = 12K, PDI = 1.14) in isotonic saline solution.

This journal is © The Royal Society of Chemistry 2013

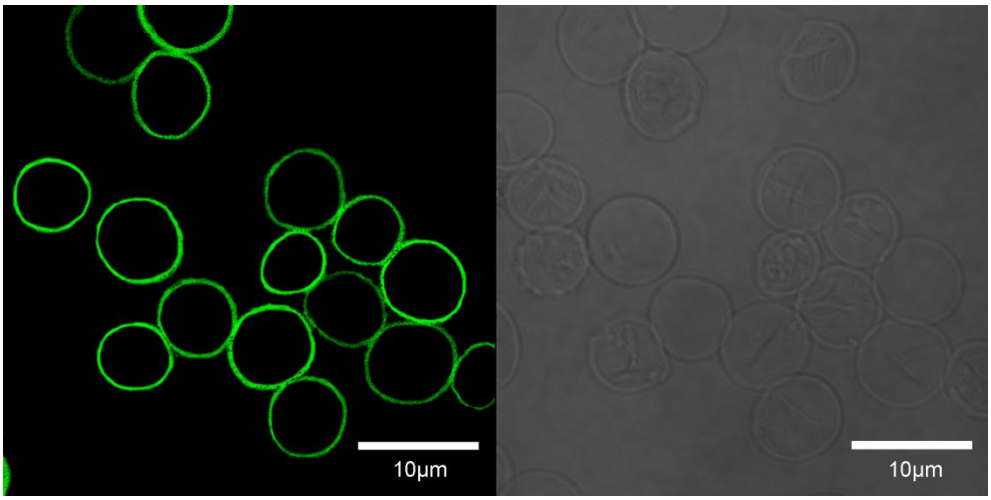


Figure S8. Confocal Laser Scanning Micrograph images of human RBCs binding with AF488-PMCP (1 mg/mL phosphate buffered saline solution).

This journal is © The Royal Society of Chemistry 2013

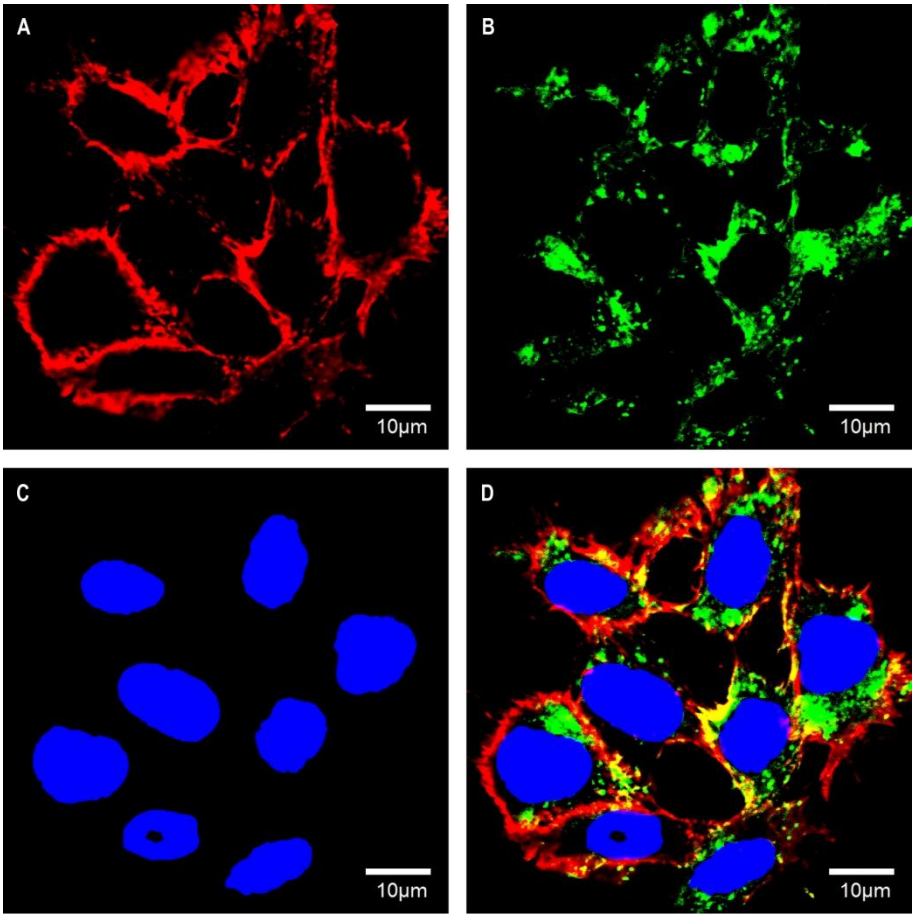


Figure S9. Confocal image analysis of PMCP adsorption and internalization by human pancreatic carcinoma cells

Confocal images of human pancreatic carcinoma cells (Hs766T) incubated with 1 mg/mL AF488-PMCP in Dulbecco's Modified Eagle's Medium solution at 37 ºC for 30 min: **A)** cell membrane stained with wheat-germ agglutinin Alexa Fluor 633; **B)** AF488-PMCP; **C)** nuclei stained with DAPI; **D)** overlay of A), B) and C).