Supplementary Information **for *ChemComm***

Effect of Elimination on Antifouling and pH-Responsive Properties of the Carboxybetaine Materials

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EXPERIMENTAL

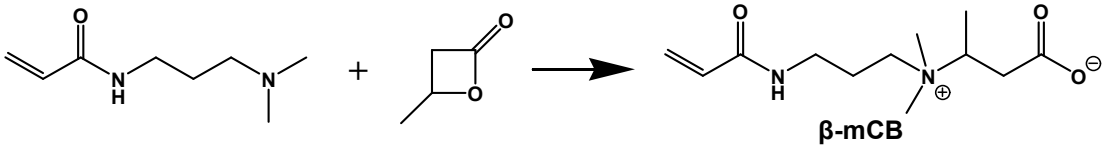
Materials

Bovine serum albumin (BSA), porcine stomach mucin, and chicken egg white lysozyme were purchased from MDBio Inc. Bovine plasma fibrinogen was purchased from Alfa Aesar. Live/dead® BacLight™ bacterial viability kit was purchased from Life Technologies. Dulbecco's Modified Eagle's Medium (DMEM) and fetal bovine serum (FBS) were purchased from Gibco. Luria-Bertani broth (LB broth) was purchased from BD. 3T3 Fibroblasts was purchased from Food Industry Research and

Development Institute (Taiwan). Bis[2-(2-bromoisobutyryloxy)undecyl] disulfide, β-butyrolactone, N,N-dimethyl-cysteamine, 2,2'-bipyridyl, copper(I) bromide, and 2-bromopropionic acid were purchased from Sigma-Aldrich. Acetonitrile (99.9%, Extra Dry) was purchased from ACROS Organics™. Tetrahydrofuran (THF) was purchased from Tedia Company. 3-bromoisobutyric acid, n-[3-(dimethylamino)propyl]acrylamide were purchased from Tokyo Chemical Industry. Sodium hydroxide was purchased from Fisher scientific. Copper(II) bromide was purchased from SHOWA Corporation. Methanol was purchased from AENCORE Chemical Company. Ethanol was purchased from ECHO Chemical Company.

Synthesis of β-substituted methyl carboxybetaine acrylamide (β-mCB)

Dissolve N-[3-(Dimethylamino)propyl]acrylamide (2.33 M) in anhydrous acetonitrile (10 mL) at 0°C under nitrogen protection. Dropwise add β-Butyrolactone (3.5 M) into the reaction solution at 0°C and allow mixing for 20 min on ice. The reaction solution was stirred at room temperature for 3 days. Afterwards, the white powder product was washed with pure acetone for three times and acetone was removed. And then, the product was dissolved in HPLC-graded ethanol, crystallized from HPLC-graded acetone and dried under vacuum. The white powder was obtained with a yield of 32.3%. The reaction formula is showed in Scheme S1.



Scheme S1. The synthesis of β-mCB.

Preparation for SI-ATRP initiator SAM

The β-mCB monomer was grafted onto gold-deposited substrates by SI-ATRP. Before the self-assembled monolayer (SAM) preparation, the substrates were washed with pure ethanol and acetone, and subsequently, cleaned with a UV cleaner for 10 min to remove organic contamination. The initiator SAMs were formed by soaking gold substrates in a pure ethanol solution containing 1 mM bis[2-(2-bromoisobutyryloxy)undecyl] disulfide at room temperature for 24 h. Before the polymerization, the substrates were rinsed with absolute ethanol and dried in a stream of nitrogen.

Preparation of SI-ATRP initiator silane SAM

The initiator silane SAMs were formed by soaking clean silicon substrates in a pure THF solution containing 40 mM 2-bromo-2-methyl-N-3-[(triethoxysilyl)propyl]propanamide (BrTMOS) at room temperature overnight. BrTMOS was synthesized as reported in the previous work.[1] Before the polymerization, the substrates were rinsed with THF and dried in a stream of nitrogen, followed by baking in the oven at 80^{o}C for 1 h.

SI-ATRP for preparation of poly(β-mCB)

Bpy, CuBr, and $CuBr_2$ were stored in a glove box filled with nitrogen before surface polymerization. A reaction solution was prepared by mixing bpy (0.93 mmol), β-mCB (6.6 mmol), CuBr (0.12 mmol), $CuBr_2$ (0.016 mmol) in deionized water and absolute methanol with a 2:3 volume ratio. The initiator-modified substrates were transferred to the reaction solution in a glove box. After 5 h, the substrates were removed and rinsed with methanol, and dried in a stream of nitrogen.

Potentiometric titration

β-mCB monomers at 0.1 M were dissolved in deionized water in a 100 mL flask. A pH meter after calibration was used. A 0.1 M HCl solution was added dropwise into the solution until the pH value = 1. Afterwards, 0.1 M NaOH solution was slowly added into the solution and the pH values were recorded as a function of the volume of the NaOH solution for plotting the titration curve.

Water Contact Angle

The water contact angle is one of a practical ways to measure the wettability of a surface. The contact angle goniometer (Phoenix mini, Surface Electro Optics) was used. A droplet with a volume of 5 μL from a micro syringe was placed at random positions on the substrate. The measurements were carried out for three times for each sample.

X-ray Photoelectron Spectroscopy

XPS spectra are obtained by irradiating a solid surface with a beam of X-ray while measuring the kinetic energy and electrons that are emitted from the top 1 to 10 nm of the material being analyzed. The XPS PHI 5000 VersaProbe system (ULVAC-PHI, Chigasaki, Japan) equipped with an Al Kα excitation source (25W, 100 μm) in a

vacuum of below 10^{-8} Pa. A dual beam charge neutralizer (7 V Ar^+ and flooding 1 V electron beam) was employed to compensate for the charge-up effect. The energy of emitted electrons is measured using an energy analyzer at a pass energy of 58.7 eV. All data were collected at 45° from the surface normal takeoff angle. The BE scale is referenced by setting the peak for Au_{4f} to 84 eV and 86 eV or for Si_{2p} to 103.5 eV. The ratio of peak intensity converted to atomic percentage using the sensitivity factors simulation was conducted with MULTIPAK software package.

Atomic Force Microscope

AFM was performed using a JSPM-5200 (JEOL). The measurements were operated in tapping mode with a soft tapping/NC probe (Nano World) under ambient conditions. The AFM was operated at a scan rate of 0.5 Hz and a set point of 0.9 V.

Bacterial Adhesion

Three bacterial species, *E. coli, S. epidermidis* and *P. aeruginosa*, were used in this work. *E. coli, S. epidermidis* and *P. aeruginosa* were cultured in 25 mL of a LB growth media at 37 °C for 16 h at a shaking rate of 200 rpm. The bacterial solutions were collected by centrifuging at 4000 rpm for 5 min. Afterwards, we diluted the bacterial solution with PBS to an optical density (O.D.) at 600 nm of 0.1, corresponding to ~8×10^7 cells/mL. The substrates were incubated into the bacterial solution at 37 °C for 3 h and 24 h. Afterwards, the substrates were washed with sterile PBS and shaken at 100 rpm for 5 min for three times to remove the loosely bound bacteria. The substrates were stained with LIVE/DEAD BacLight and covered with a cover slide for 15 min, followed by washing with sterile PBS. The adsorbed bacteria

were determined under a fluorescence microscope. The bacteria numbers were analyzed using ImageJ software.

Surface plasmon resonance (SPR) technology

The SPR sensor platform in this study is an SPR biosensor with six flow chambers from the Homola group.[2] The SPR sensor equipped with a self-referencing function enables sensitive detection to the refractive index unit (RIU) better than 10^{-6}, which is illustrated to the protein surface coverage as small as 0.2 pg/mm^2. The glass substrates were pre-coated with an adhesion layer (chromium, thickness ~2 nm), and deposited a gold layer with a thickness of 48 nm. A flow rate of 50 μL/min was controlled in all experiments, and experiments were performed at room temperature.

Synthesis of β-methyl Carboxybetaine disulfide

1 equivalent of N,N-dimethyl-cysteamine (0.7762 g, 3.7263 mmol) was dissolved with 5 mL of anhydrous acetonitrile in ice bath. 2.2 equivalent of beta-butyrolactone was added into the solution and stirred in an ice bath for 30 min. The mixture was protected in nitrogen and allowed to react at room temperature for 3 days. After 3 days, the mixture was concentrated using a rotavapor and 25 mL of diethyl ether was added. The mixture was stored in a refrigerator (-20˚C) for overnight before filtration. The obtained white powder was quickly separated using vacuum filtration (0.09 g, 6.92 % yield). ^{1}H NMR (200 MHz, D2O) δ(ppm): 3.6-3.8 (m, 2H), 3.52 (t, J=8.2 Hz, 4H), 2.7-3.0 (m,18H), 2.2 (d, J=13.6 Hz,2H), 1.28 (d, J=6 Hz, 6H)

Synthesis of α-methyl Carboxybetaine disulfide

5 mL of ethanol was added into 2 equivalent of 3-bromoisobutyric acid (1.397 g, 8.3706 mmol). In other flask, 1 equivalent of sodium carbonate (0.4435 g, 4.1853

mmol) was dissolved with 1 mL of water. The solution of sodium carbonate was then added into the solution of 3-bromoisobutyric acid and stirred for 1 h at room temperature. After 1 h of reaction, 1 equivalent of N,N-dimethyl-cysteamine (0.5812 g, 2.7902 mmol) was slowly added into the above reaction mixture. The resulting mixture was protected in nitrogen and allowed to react at 40°C for 3 days. After 3 days, the mixture was concentrated using a rotavapor and dissolved with HPLC-graded ethanol. Insoluble materials were discarded and soluble fraction was again concentrated then followed by crystallization with anhydrous acetone. The white solid was separated quickly using vacuum filtration (0.279 g, 26.36% yield). ^{1}H NMR (400 MHz, D2O) δ(ppm) : 3.6-3.8 (m, 8H), 3.1-3.3 (m, 18H), 1.23 (d, J=7.16 Hz, 6H)

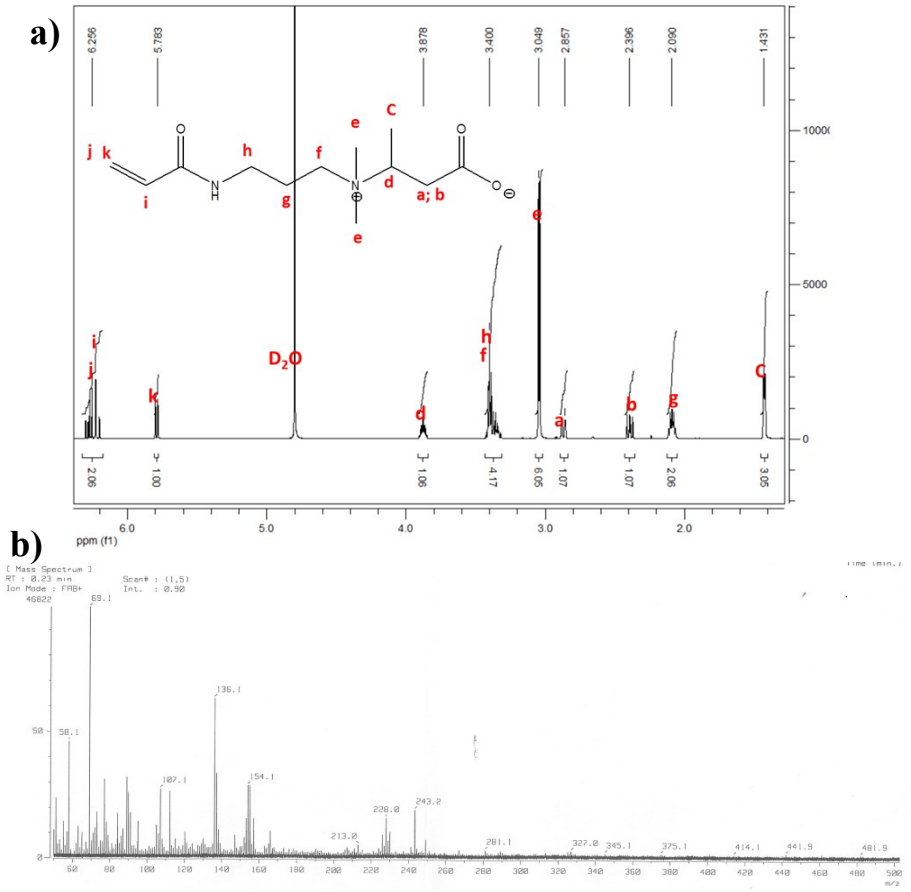


Figure S1. (a) ^{1}H NMR spectrum for β-mCB analysis. (b) High resolution mass spectrum for β-mCB.

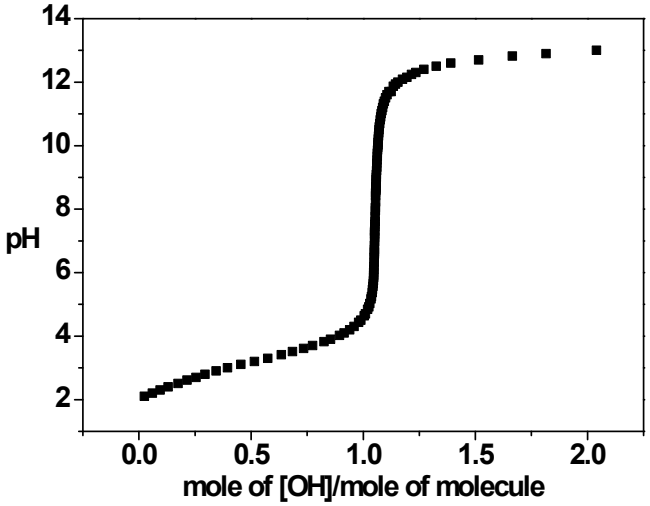


Figure S2. Potentiometric titration curve of β-mCB at a concentration of 0.1 M in deionized water.

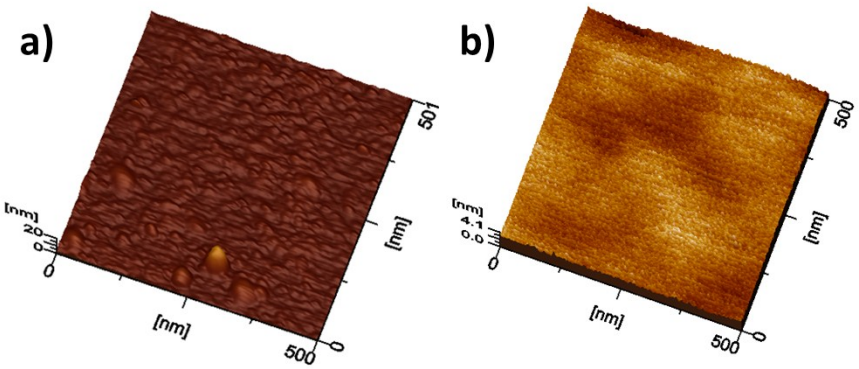


Figure S3. The taping-mode AFM images for (a) bare glass substrate and (b) poly(β-mCB) modified surface.

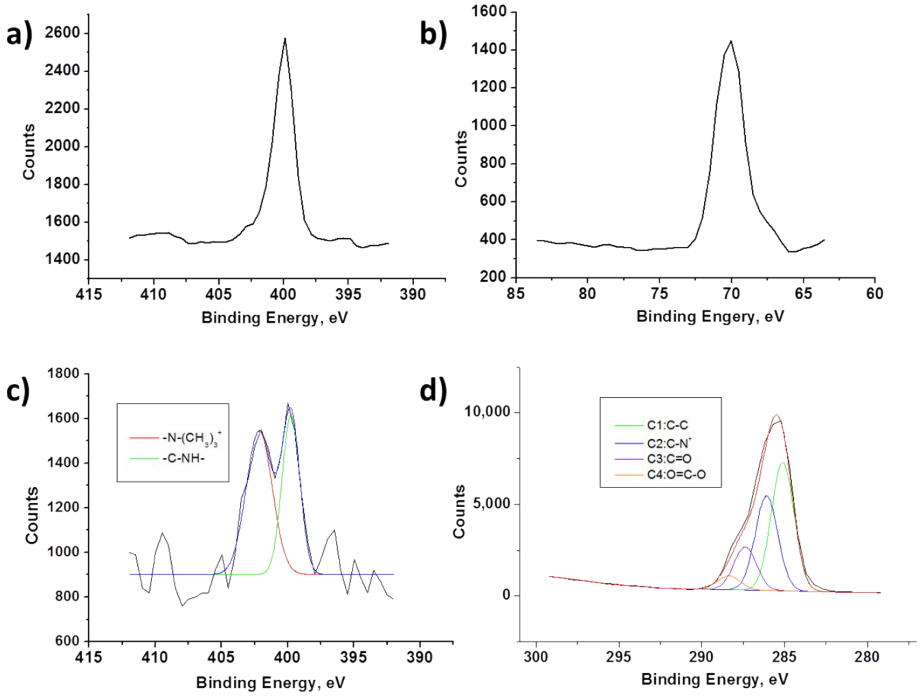


Figure S4. XPS spectra for ATRP initiator-modified and poly(β-mCB) grafted surfaces on SiO_2 substrate. The results of binding energy were acquired from (a) N1s and (b) Br3d for the ATRP initiator-modified surface; (c) N1s and (d) C1s for the poly(β-mCB) grafted surface

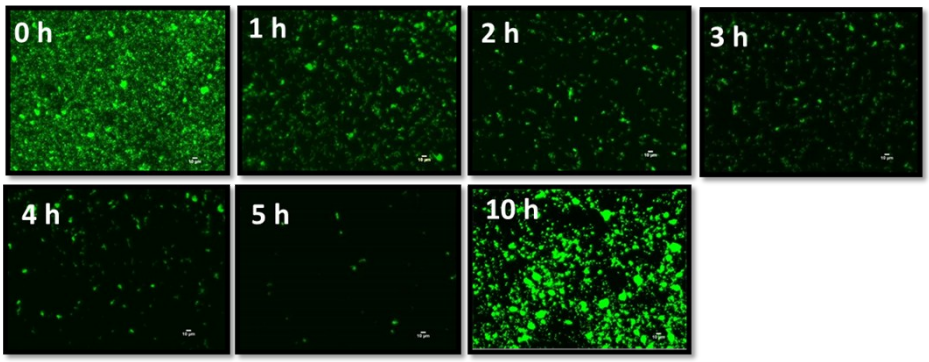


Figure S5. Fluorescence images for the bacterial adhesion test on the poly(β-mCB) films, prepared for different polymerization time.

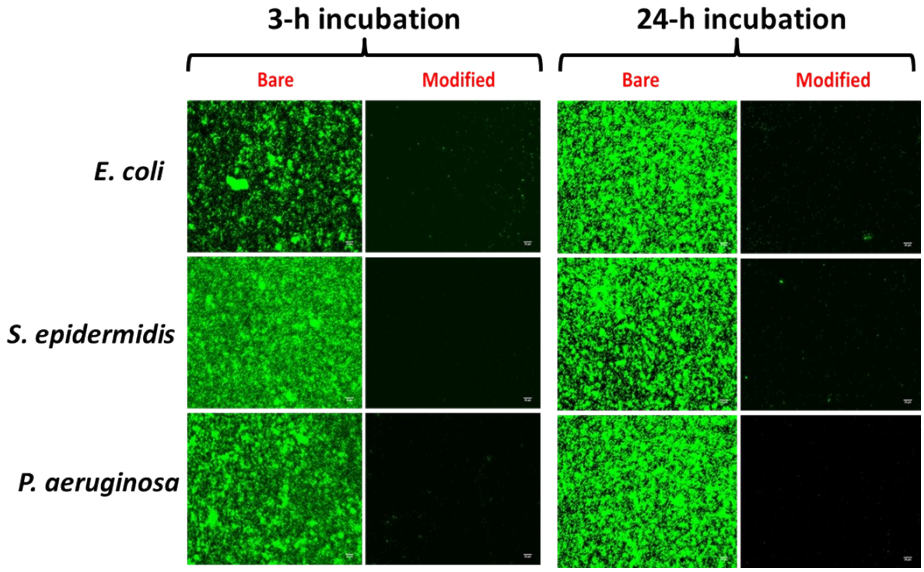


Figure S6. Fluorescence images of bacteria of *E. coli*, *S. epidermidis* and *P. aeruginosa* on bare glass and the poly(β-mCB) film with a polymerization time of 5 h.

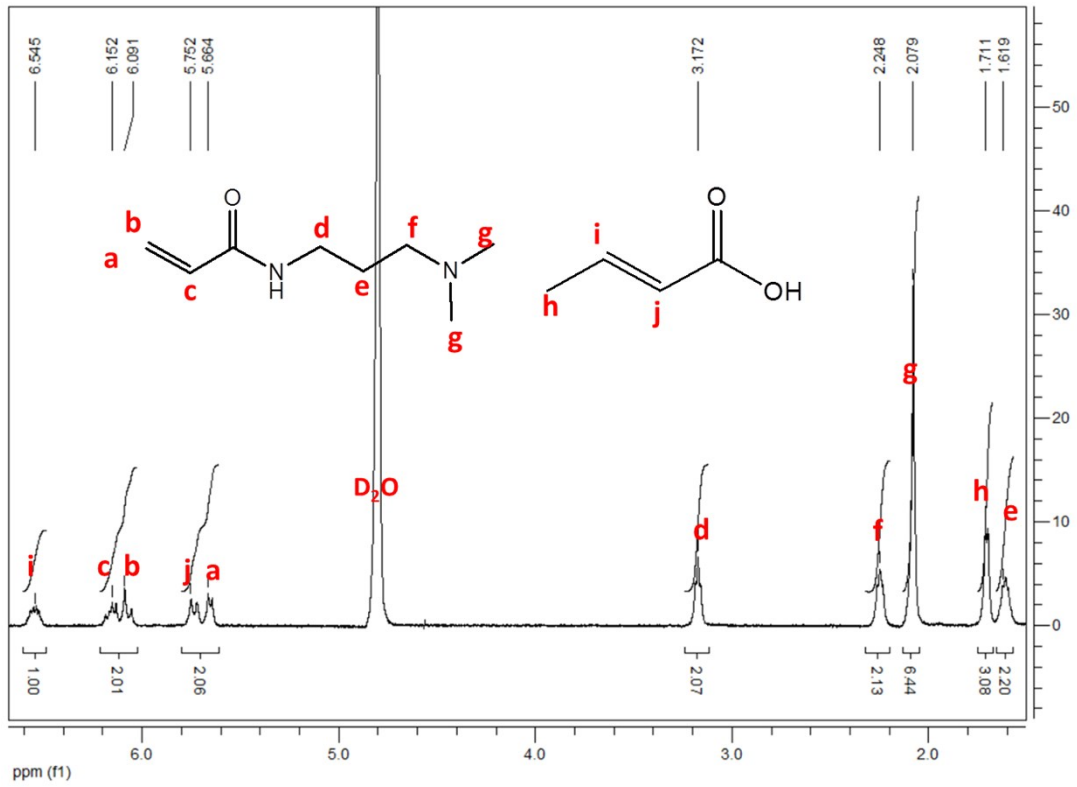


Figure S7. NMR spectrum for β-mCB incubated in 0.1 M NaOH solution for 35 h. The result shows that β-mCB degrades into N-[3-(dimethylamino)propyl]acrylamide and 2-butenoic acid.

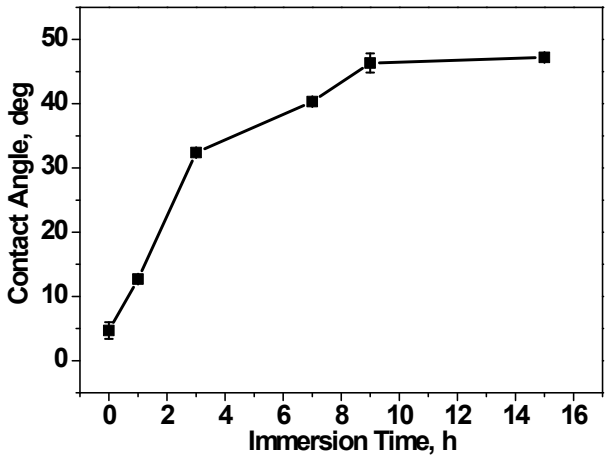


Figure S8. Contact angle measurements for poly(β-mCB) films after treatment with 0.1 M NaOH solutions for different immersion time.

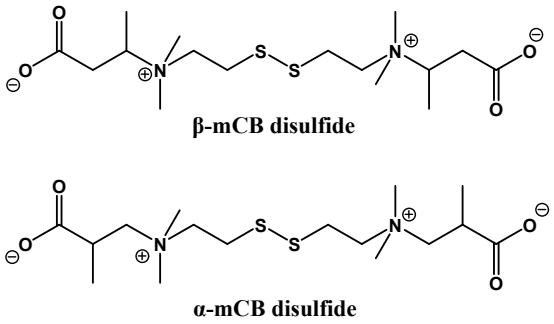


Figure S9. Chemical structures of α-mCB and β-mCB disulfide.

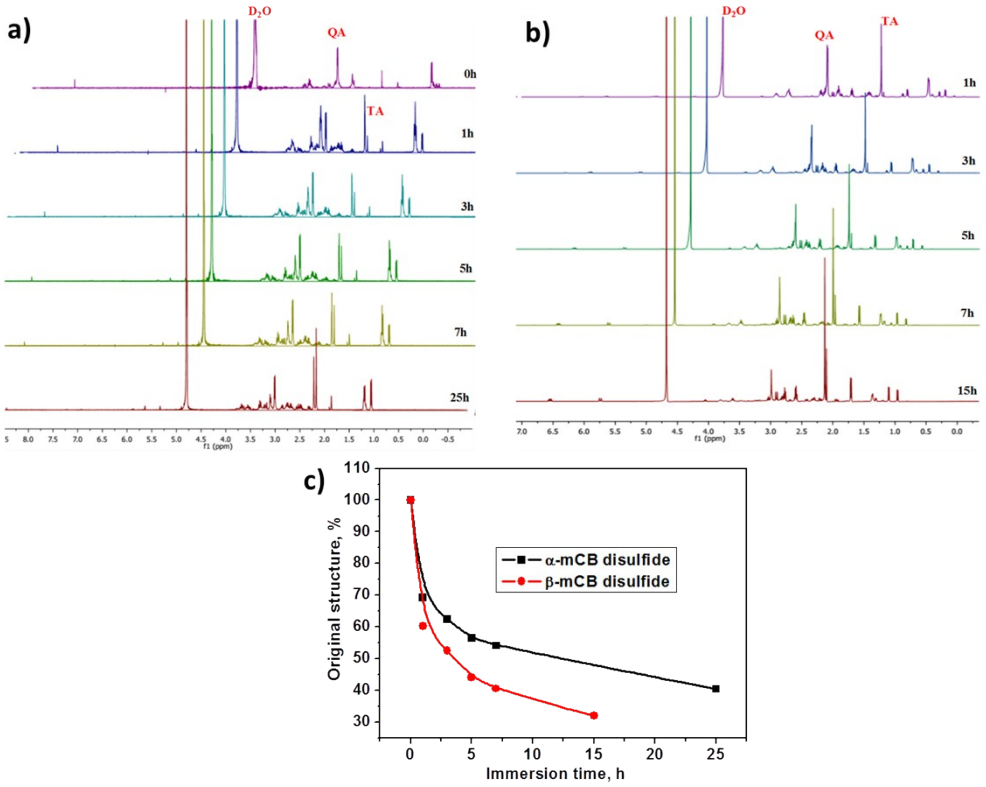


Figure S10. NMR tests for (a) α-mCB and (b) β-mCB disulfide in the presence of 0.1 M NaOH. (c) Plots of percentage of undegraded species versus time to show the conversion rate from the zwitterionic group to tertiary amine group for α-mCB and β-mCB disulfide.

References:

1 T. C. Zheng Zhang, Shengfu Chen, and Shaoyi Jiang, *Langmuir*, 2006, **22**, 10072.

2 M. Piliarik and J. Homola, *Sens. Actuator B-Chem.*, 2008, **134**, 353.