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Electronic supplementary information for:

"Click-chemical" modification of Cellulose Acetate Nanofibers: A Versatile Platform for Biofunctionalization

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Experimental section

1. Materials

Cellulose acetate (CA), fluorescein isothiocyanate-labeled bovine serum albumin (FITC-BSA), N,N,N′,N′,N″-pentamethyldiethylenetriamine (PMDETA), N-(5-fluoresceinyl) maleimide and *but*-3-yn-1-ol were purchased from Sigma-Aldrich. Potassium hydrogen difluoride (KFHF), tert-butyldimethylchlorosilane, triazabicyclodecene (TBD), disperse Red 1 (DR1), poly(ethylene glycol) methyl ether (PEG-OH), mercaptoethan-1-ol, 2,2'-azoisobutyronitrile (AIBN), and 6-bromo-1-hexanol were purchased from TCI. Imidazole and methylimidazole were purchased from J&K Chemical. Dimethyl formamide (DMF), diethyl ether, methanol, ethanol and acetone were purchased from Sinopharm Chemical Reagent Co., Ltd and used as received. Azide-containing cresyl violet was synthesized as reported previously[1]. The preparation of tert-butyldimethylsilyl (TBDMS)-protected Disperse Red 1 and poly(ethylene glycol) methyl ether (referred to as TBDMS-DR1 and TBDMS-PEG, respectively) was as previously described[2]. Gram-negative *Escherichia coli* (*E. coli*) was provided by the China General Microbiological Culture Collection Center (Beijing, China).

2. Instruments and measurements

^{1}H nuclear magnetic resonance (NMR) spectra were recorded on an INOVA 400 MHz spectrometer (Agilent Technologies, USA). UV/Vis studies were performed using a microplate reader (Thermo Fisher Scientific, USA). A fluorescence microscope (BX51, Olympus，Japan) was used to observe the dyed nanofibers. The chemical compositions of fiber surfaces were determined using an ESCALAB MK I X-ray photoelectron spectrometer (Thermo Scientific, USA). The number-average molecular weights (Mn) and polydispersity (PDI) of the polymers were determined by gel permeation chromatography (GPC) using an Agilent PL-GPC 50 system equipped with a refractive index detector, a 5 μm Guard, a 5 μm MIXED-D column with PMMA standard samples, and 0.05 mol/L LiBr solution in DMF as the eluent at a flow rate of 1 mL/min operated at 50 °C.

3. Synthesis

3.1 Synthesis of poly(3-(fluorosulfonyl)propyl methacrylate) (PFPM)

3-(fluorosulfonyl)propyl methacrylate (FPM) was synthesized as reported previously[3]. PFPM was synthesized by free radical polymerization. Briefly, FPM(1.0 g, 4.76 mmol) and 7.45 mg AIBN(0.045 mmol) were added to 4 mL DMF and reacted at 70°C overnight under nitrogen after bubbling for 30 min. The resulting solution was then added dropwise to 100 mL ice-cold ether, and filtered to obtain the product as a white precipitate. The precipitate was dried overnight (Mn=2.9×10^4 g/mol, PDI=2.25 (Figure S2)).

The chemical structures of FPM and PFPM were confirmed by ^{1}H NMR spectrometry (Figure 1Sa and b). FPM: ^{1}H NMR (400MHz, $CDCl_3$), δ ppm: 2.01(s, 3H, C***H***$_3$-), 2.38(m, 2H, -C***H***$_2$-), 3.56(m, 2H, SO_2FC***H***$_2$ -), 4.36(t, 2H, -C***H***$_2$-O-), 5.66(s, 1H, C***H***$_2$=), 6.18(s, 1H, C***H***$_2$=) (Figure S1a).

PFPM: ^{1}H NMR (400MHz, $CDCl_3$), δ ppm: 0.93(m, C***H***$_3$-), 1.82(m, -C***H***$_2$-), 2.17(m, -C***H***$_2$-), 3.90-4.49 (m, SO_2FC***H***$_2$ – and -C***H***$_2$-O-) (Figure S1b).

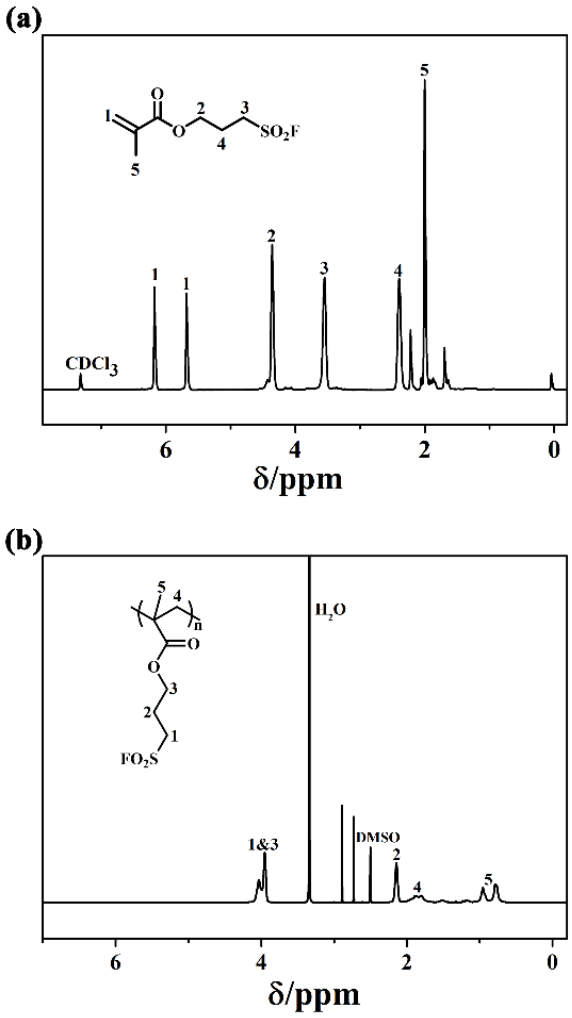

Figure S1. ^{1}H NMR of (a) FPM; (b) PFPM.

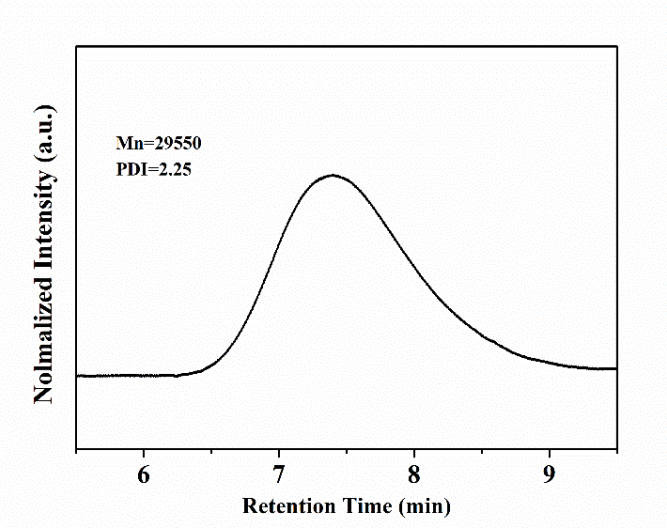

Figure S2. GPC trace of PFPM.

3.2 Synthesis of 1-(6-((tert-butyldimethylsilyl)oxy)hexyl)-3-methyl-1H-imidazol-3-ium bromide (TBDMS-IL)

First, 3.0 g (0.02 mol) 6-bromo-1-hexanol, 3.0 g (0.02 mol) tert-butyldimethylchlorosilane and 3.36 g (0.05 mol) imidazole were dissolved in 20 mL dichloromethane, and stirred overnight. The resulting solution was washed with saturated sodium bicarbonate and saturated brine, and dried with anhydrous magnesium sulfate. The crude products were purified by column chromatography using ethyl acetate and hexane (1:4, v/v) as eluting agents to obtain a transparent liquid ((6-bromohexyl)oxy)(tert-butyl)dimethylsilane. Then, 3.0 g (0.01 mol) ((6-bromohexyl)oxy)(tert-butyl)dimethylsilane) and 0.9 g (0.01 mol) methylimidazole were stirred overnight under nitrogen. The product was obtained by precipitation several times in diethyl ether as a transparent viscous liquid. ^{1}H NMR (400MHz, $CDCl_3$), δ ppm: 0.01(s, 6H, $((CH_3)_3C)(C\boldsymbol{H}_3)_2$-Si-), 0.85(s, 9H, $(C\boldsymbol{H}_3)_3$-C-), 1.36–1.92 (m, 8H, –$C\boldsymbol{H}_2C\boldsymbol{H}_2C\boldsymbol{H}_2C\boldsymbol{H}_2$–), 3.56(t, 2H, –$C\boldsymbol{H}_2$O), 4.10(s, 3H, N–$C\boldsymbol{H}_3$), 4.30(t, 2H, –$C\boldsymbol{H}_2$–N), 7.40-7.29 (s, 2H, –N–C***H***=C***H***–N–), 10.37 (s, 1H, –N=C***H***–) (Figure S3).

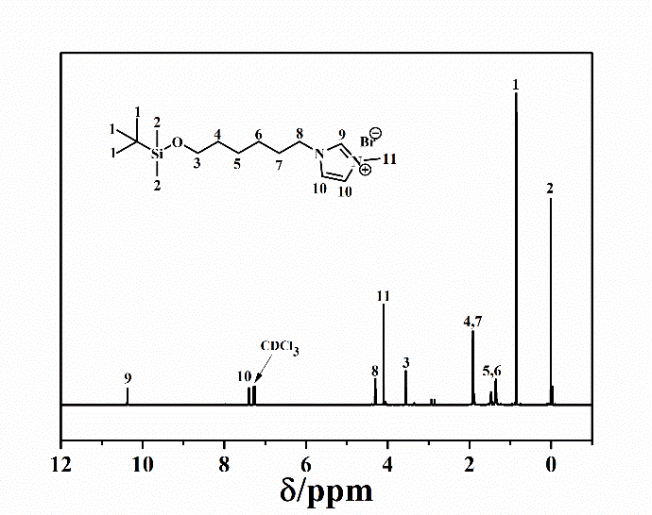

Figure S3. ^{1}H NMR of TBDMS-IL.

3.3 Synthesis of 2-((tert-butyldimethylsilyl)oxy)ethane-1-thiol and (but-3-yn-1-yloxy)(tert butyl)dimethylsilane (TBDMS-Thio and TBDMS-Yne, respectively)

TBDMS-Thio: 1.0 g (12.80 mmol) of 2-mercaptoethan-1-ol, 1.45 g (13.06 mmol) of imidazole, and 1.56 g (26.88 mmol) of TBDMS-Cl were dissolved in 7 mL of dichloromethane, and the solution was stirred overnight at room temperature. The reaction solution was washed with water and the dichloromethane removed by evaporation under reduced pressure to yield a clear oil. ^{1}H NMR (400MHz, $CDCl_3$), δ ppm: 0.05(s, 6H, $(C\boldsymbol{H}_3)_2$-Si-), 0.89(s, 9H, $(C\boldsymbol{H}_3)_3$-C-), 2.62(m, 2H, SHC$\boldsymbol{H}_2$-), 3.71(m, 2H, C$\boldsymbol{H}_2$-O-), 5.28(s, 1H, S***H***-) (Figure S4).

TBDMS-Yne: 0.9 g (20 mmol) of 3-Butyn-1-ol, 1.45 g (13.06 mmol) of imidazole, and 1.56 g (26.88 mmol) of TBDMS-Cl were dissolved in 7 mL of dichloromethane, and the solution was stirred overnight at room temperature. The reaction solution was washed with water and the dichloromethane removed by evaporation under reduced pressure to yield a clear oil. ^{1}H NMR (400MHz, DMSO-d6), δ ppm: 0.07(s, 6H, $(C\boldsymbol{H}_3)_2$-Si-), 0.90(S, 9H, $(C\boldsymbol{H}_3)_3$-C-), 2.37(m, 2H, CH≡CC$\boldsymbol{H}_2$-), 2.80(s, 1H, C***H***≡C-), 3.71(m, 2H, C$\boldsymbol{H}_2$-O-) (Figure S5).

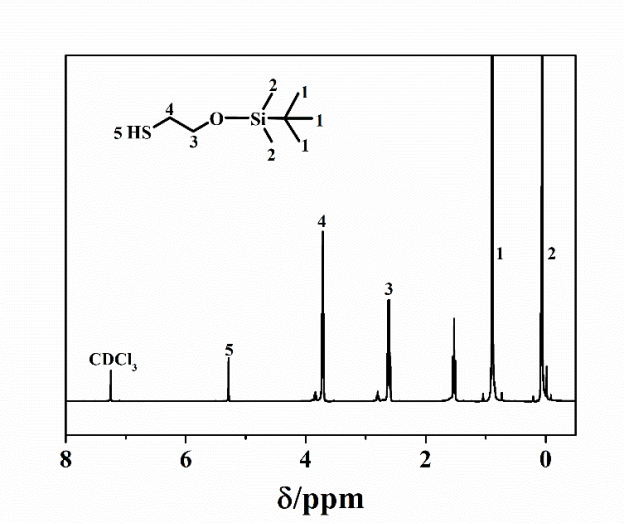

Figure S4. ^{1}H NMR of TBDMS-Thio.

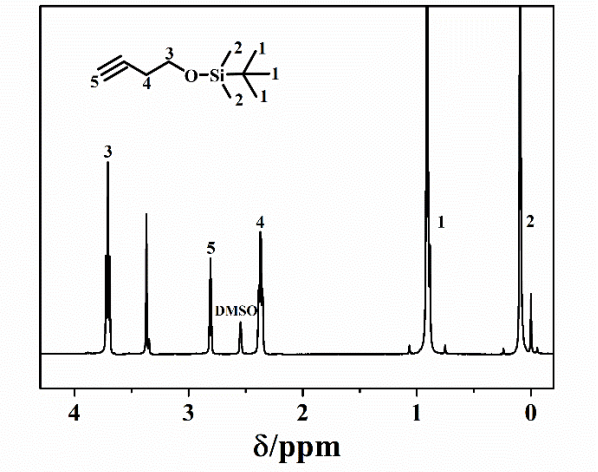

Figure S5. ^{1}H NMR of TBDMS-Yne.

4. Fabrication of CA nanofibers via electrospinning

The CA nanofibers were fabricated by electrospinning (SS-2535H, Beijing Ucalery Technology Development Co., Ltd, China). PFPM and cellulose acetate were blended in different ratios (0, 10 and 20 wt% of PFPM) in DMF-acetone (2:3, v/v) solution. Electrospinning was performed at an applied voltage of 16 kV, a collection distance of ~20 cm, and a solution flow rate of 0.75 mL/h at 40% relative humidity and room temperature. The obtained CA nanofiber mats were carefully removed from the collector and dried under vacuum. The mats were then cut into discs of 6 mm diameter.

Table S1. XPS analysis of CA-0, CA-10 and CA-20

Sample	XPS atom concentration (%)			
	[C]	[O]	[S]	[F]
CA-0	56.6	43.4	—	—
CA-10	39.2	58.3	1.9	0.6
CA-20	37.9	57.7	2.4	2.0

5. CA nanofiber functionalization

5.1 Conjugation of Disperse Red 1 dye to CA nanofibers

20.0 mg TBDMS-DR1, and a catalytic amount of TBD were dissolved in ethanol, and placed in a 10 mL flask with several pieces of nanofiber mat. After 2 min reaction, the samples were cleaned with ethanol and dried under a flow of argon.

5.2 PEG-biofunctionalized CA nanofibers

1.2 g TBDMS-PEG, and a catalytic amount of TBD were dissolved in water, and placed in a 10 mL flask with several pieces of CA nanofiber mat. After 5 min reaction, the samples were cleaned with water and dried under a flow of argon.

5.3 Ionic liquid derivative-biofunctionalized CA nanofibers

0.6 g TBDMS-IL was dissolved in 3 mL methanol, and placed in a 10 mL flask with several pieces of CA nanofiber mat. The reaction was catalyzed by TBD. After 5 min reaction, the samples were cleaned with methanol and dried under a flow of argon.

5.4 Thiol and alkynyl-functionalized CA nanofibers

0.6 g TBDMS-Thio or TBDMS-Yne was dissolved in 3 mL toluene, and placed in a 10 mL flask with several pieces of CA nanofiber mat. The reaction was catalyzed by TBD. After 5 min reaction, the samples were cleaned with toluene and dried under a flow of argon.

6. Protein adsorption on PEG-functionalized CA nanofiber mats

Modified and unmodified CA nanofiber mats were immersed in 1 mg/mL FITC-BSA solution for 30 min. After washing 3 times with water, the samples were immersed in 250 µL 2% SDS solution for 1 h. 200 µL of the resulting solution was placed a 96-well plate, and the fluorescence intensity was measured (emission and excitation wavelengths 485 and 518 nm respectively, Figure S6).

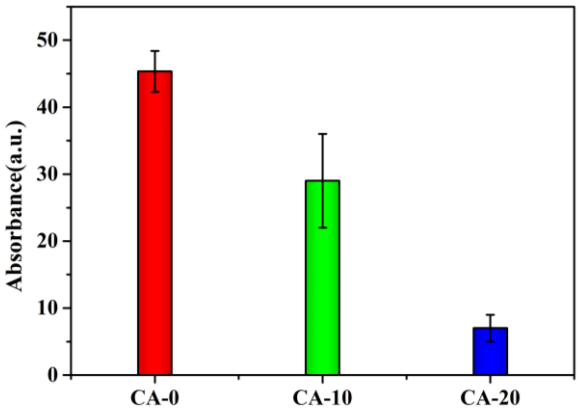

Figure S6. The absorbance of BSA-FITC on CA-0, CA-10 and CA-20 surfaces. Error bars represent the standard deviation (n = 3).

7. Bacterial adhesion on PEG-functionalized CA nanofibers

Escherichia coli (*E. coli*, ATCC-700926) were used as model bacteria. The bacteria were incubated in Luria-Bertani broth medium (LB, Sigma-Aldrich) and nutrient bouillon medium (NB, Sigma-Aldrich), grown overnight with shaking at 37°C. The bacteria were harvested during the exponential growth phase via centrifugation. The supernatant was discarded, and the cell pellet was re-suspended in phosphate-buffered saline (PBS, pH = 7.4) at a final concentration of approximately 1×10^7 cells·mL^{-1}.

The modified and unmodified mats were incubated in 500 µL *E. coli* suspension at 37°C for 2 h to attach the bacteria. The mats were then washed with sterile water to remove loosely bound bacteria, placed in centrifuge tubes containing 500 µL deionized water and centrifuged at 8.0×10^3 rpm for 5 min to release the attached cells. The released cells were appropriately diluted, placed on gelatinous Luria agar plates and incubated at 37°C for 18 h. The number of viable cells was then determined in colony-forming units (CFU).

8. Bactericidal activity of Ionic liquid derivative-biofunctionalized CA nanofibers

Mats were sterilized with 75% ethanol, and dried before bacterial attachment. After bacterial attachment for 2 h, the mats were placed face down in centrifuge tubes filled with PBS (pH = 7.4), and centrifuged at 8.0×10^3 rpm for 5 min to release the attached cells. The released cells were appropriately diluted with PBS (pH = 7.4), placed on gelatinous Luria agar plates and incubated at 37°C for 18 h. The number of viable cells was then determined in colony-forming units (CFU).

9. Thio-Michael addition reaction on thiol-functionalized CA nanofiber mats

Thiol-functionalized CA nanofiber mats were immersed in N-(5-fluoresceinyl) maleimide aqueous solution, and reacted overnight at room temperature. The resulting materials were cleaned with deionized water and dried under argon. The mats were then imaged in a fluorescence microscope with 40 × objective.

10. CuAAC on alkynyl-functionalized CA nanofibers

Alkynyl-functionalized CA nanofiber mats were immersed in azide-containing cresyl violet methanol solution for 30 min. PMDETA (55.0 mg, 0.32 mmol) and CuBr (23.0 mg, 0.16 mmol) were added. The solution was deoxygenated by bubbling argon gas for 30 min and allowed to react for 3 h. The resulting nanofiber mats were cleaned with methanol and dried under argon. The mats were then imaged in a fluorescence microscope with 40 × objective.

References

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2. P. Wang, Y. Dong, X. Lu, Z. Wu and H. Chen, *Macromol. Rapid Commun.*, 2018, **39**, 1700523.
3. J. Yatvin, K. Brooks and J. Locklin, *Angewandte Chemie International Edition*, 2015, **54**, 13370-13373.