Supporting Information

Poly(oligoethylene glycol methacrylate) dip-coating: turning cellulose paper into a protein-repellent platform for biosensors

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1. Materials

Oligo (ethylene glycol) methyl ether methacrylate with an average number-average molecular weight of 475 g/mol ($OEGMA_{475}$, Sigma Aldrich, 95%) and (diethylene glycol) methyl ether methacrylate ($M(EO)_2MA$, Sigma Aldrich, 98%) were purified using a column of basic aluminum oxide (Sigma Aldrich, type CG-20) to remove the monomethyl ether hydroquinone (MEHQ) and butylated hydroxytoluene (BHT) inhibitors. Acrylic acid (AA, Sigma Aldrich, 99%), thioglycolic acid (TGA, Sigma Aldrich, 98%), 2,2-azobisisobutryic acid dimethyl ester (AIBMe, Wako Chemicals, 98.5%), adipic acid dihydrazyde (ADH, Alfa Aesar, 98%), N'-ethyl-N-(3-dimethylaminopropyl)-carbodiimide (EDC, Carbosynth, Compton CA, commercial grade), bovine serum albumin (BSA, Sigma Aldrich, 97%), fibrinogen from human plasma (Sigma Aldrich), IgG from rabbit serum (Sigma Aldrich, 95%), β-galactosidase from *Escherichia coli* (Grade VI, Sigma Aldrich), goat anti-rabbit IgG (whole molecule, Sigma), normal rabbit IgG-HRP (Santa Cruz Biotechnology), rhodamine 123 (Sigma Aldrich, 85%), fluorescein 5-isothiocyanate (5-FITC, Sigma Aldrich, 90%), and fluorescein-5-thiosemicarbazide (5-FTSC, Sigma Aldrich, 80%) were all used as received. TMB peroxidase substrate solution and peroxidase substrate solution B were purchased from KPL, Kirkegaard & Perry Laboratories, Inc. Hydrochloric acid (1M) was received from LabChem Inc. (Pittsburgh, PA). For all experiments, Milli-Q grade distilled deionized water (DIW) was used. Phosphate buffered saline (PBS) was diluted from a 10X liquid concentrate (Bioshop Canada Inc.). N-(2,2-dimethoxyethyl)methacrylamide (DMEMAm) monomer was synthesized according to a previously reported method.[1]

2. Polymer synthesis and characterization

Polymer characterization: Size exclusion chromatography (SEC) was performed using a Waters 2695 separations module equipped with a Waters 2996 photodiode array detector, a Waters 2414 refractive index detector, a Waters 2475 multi λ fluorescence detector and four Polymer Labs PLgel individual pore size columns maintained at 40 °C, with 5 μm bead size and pore sizes of 100, 500, 103 and 105 Å. THF was used as the eluent at a flow rate of 1.0 ml min^{-1}, and poly(methyl methacrylate) standards were used for calibration. ^{1}H-NMR and

^{13}C-NMR were performed on a Bruker AVANCE 600 MHz spectrometer using deuterated chloroform as the solvent (see section 20 of Supporting Information for full spectra)

Synthesis of aldehyde-functionalized poly(oligoethylene glycol methacrylate) (POA): POA precursors were prepared by adding AIBMe (38 mg, 0.16 mmol), $OEGMA_{475}$ (0.90 g, 1.9 mmol), $M(EO)_2MA$ (3.1g, 16 mmol), DMEMAm (0.60 g, 3.5 mmol) and TGA (7.5 µL, 0.15 mmol) to a 50 mL Schlenk flask. 1,4-Dioxane (20 mL) was added and the solution was purged with nitrogen for at least 30 minutes. Subsequently, the flask was sealed and submerged in a pre-heated oil bath at 75°C for 4 hours under magnetic stirring. After polymerization, the solvent was removed and the poly($OEGMA_{475}$-co-$M(EO)_2MA$-co-DMEMAm) polymer was purified by dialysis against DIW over 6 cycles (6+ hours each time) and lyophilized to dryness. The acetal groups of POA precursors were subsequently converted to aldehydes by dissolving 4.0 g of the copolymer prepared above in 75 mL DIW and 25 mL 1.0 M HCl in a 250 mL round-bottom flask. The solution was left to stir for 24 hours, dialyzed for a minimum of 6 (6+ hour) cycles, and lyophilized to dryness. The polymers were dissolved in PBS and stored as 20 w/w% solutions at 4°C. The number-average molecular weight was determined to be $14 \cdot 10^3$ g/mol (Đ = 2.03) from size exclusion chromatography using THF as a solvent. The aldehyde content was determined to be 24 mol% (equivalent to 19 reactive aldehyde groups per chain) using ^{1}H-NMR based on the proton signals of the methoxy (O-CH_3, 3H, δ = 3.3 ppm) and aldehyde (CHO, 1H, δ = 9.2 ppm) groups.

Synthesis of the hydrazide-functionalized poly(oligoethylene glycol methacrylate) (POH): POH precursors were prepared by adding AIBMe (38 mg, 0.16 mmol), $OEGMA_{475}$ (0.95 g, 2.0 mmol), $M(EO)_2MA$ (3.1g, 16 mmol), AA (0.55 g, 7.6mmol), and TGA (7.5 µL, 0.15 mmol) to a 50 mL Schlenk flask. 1,4-Dioxane (20 mL) was added, and the solution was purged with nitrogen for at least 30 minutes. Subsequently, the flask was sealed and submerged in a pre-heated oil bath at 75°C for 4 hours under magnetic stirring. After the solvent was removed, the resulting poly($OEGMA_{475}$-co-$M(EO)_2MA$-co-AA) polymer was purified by dialysis against DIW over 6 cycles (6+ hours per cycle) and lyophilized to dryness. The carboxylic acid groups of POH precursor were subsequently converted to hydrazide groups via a

carbodiimide-mediated conjugation of a large excess of adipic acid dihydrazide. The polymer (4.0 g) was dissolved in 100 mL DIW and added to a 250 mL round-bottom flask. ADH (4.33 g, 24.8 mmol, 8.16 mol eq.) was added and the pH of the solution was adjusted to pH = 4.75 using 0.1 M HCl. Subsequently, EDC (1.93 g, 12.4 mmol, 3.80 mol eq.) was added and the pH was maintained at pH = 4.75 by the dropwise addition of 0.1 M HCl over 4 hours. The solution was left to stir overnight, dialyzed against DIW over 6 cycles (6+ hours per cycle) and lyophilized to dryness. The polymers were dissolved in PBS and stored as 20 w/w% solutions at 4°C. The number-average molecular weight was determined to be $17 \cdot 10^3$ g/mol (Đ = 2.08) from size exclusion chromatography using THF as a solvent. The degree of hydrazide functionalization was determined to be 22 mol% (equivalent to 22 reactive hydrazide groups per chain) by conductometric base-into-acid titration by comparing the carboxylic acid content before and after ADH conjugation (0.1 M NaOH titrant, 50 mg polymer in 50 mg of 1 mM NaCl titration solution, ManTech automatic titrator).

Note that a 10mol% OEGMA475 (n = 7-8 ethylene oxide repeat units on the side chain) and 90 mol% $M(EO)_2MA$ (n = 2 ethylene oxide repeat units on the side chain) copolymer was chosen for both the hydrazide and aldehyde-functionalized copolymers. This particular copolymer, in the absence of hydrazide and aldehyde functionalization, has a thermal phase transition temperature just above room temperature (~32-33°C) and as such can more easily adsorb on to the cellulose paper in the first POA dipping step relative to a polymer prepared with 100% OEGMA475. However, the hydrogel chosen still effectively repels proteins with near-equal efficacy to the 100% OEGMA475 polymer (see reference 13b in the main manuscript for data on bulk hydrogels) due to the still significant presence of the longer side chain comonomer, and dip coating of 100% OEGMA475 polymers onto the filter paper actually facilitated a lower protein repellency than the 10mol% OEGMA475 polymer used (~ 1.7-fold higher IgG adsorption) owing to the reduced surface coverage that can be achieved. Thus, the composition chosen represents an optimized chemistry for both promoting effective coverage of the paper and low non-specific protein adsorption.

3. Quartz crystal microbalance with dissipation monitoring (QCM-D)

The adsorption of POA and POH on cellulose and the ability of the resulting POEGMA interface to repel proteins were investigated using quartz crystal microbalance with dissipation (QCM-D, Q-Sense E4, Gothenburg, Sweden) equipped with QSX 334 cellulose sensors. The resonance frequency and dissipation shifts of the oscillating crystals were simultaneously monitored at the fundamental frequency (5 MHz) and its seven overtones (15, 25, 35, 45, 55, 65 and 75 MHz) at 25°C under constant fluid flow (0.1 mL/min). The cellulose chip surface was allowed to equilibrate for 3 hours in PBS buffer prior to measurements. All polymers (4%, w/v) and proteins (100 μg/mL) were dissolved in the same PBS solution and flowed over the pre-equilibrated chip until the measured frequency (and thus the adsorption of the polymer or protein added) reached a plateau characterized by a frequency change of less than 1 Hz/10 min. The adsorbed mass (Δm) was determined using Sauerbrey's equation under the assumptions that the adsorbed layer is rigid, uniformly distributed on the surface, and small compared to the crystal's mass (Equation 1)[2]:

$$\Delta m = -\frac{C \cdot \Delta f}{n} \qquad \text{(Equation 1)}$$

In this equation, C is the sensitivity constant (17.7 $ngHz^{-1}cm^{-2}$ for a 5 MHz crystal, as provided by the manufacturer), Δf is the measured change in frequency, and n is an overtone number. Only the changes in the normalized frequencies and dissipations of the fifth overtone are reported; analogous results were achieved with other overtones.

Figure 2a (main manuscript) shows the measured frequency changes upon sequential addition of POA and POH to the cellulose-coated QCM chip. Following rinsing to remove unbound polymer after each adsorption step, the bound masses of POA and POH (including bound water, assuming three moles of water is bound per mole of ethylene glycol repeat units in the POEGMA side chain[3]) were calculated to be 390 and 550 ng/cm^2, respectively. In addition, the significant increase in dissipation following treatment of the surface with POH suggests an increase in the modulus of the surface consistent with covalent cross-linking of POA and POH to form a hydrogel.

The bound masses of the four proteins assayed (BSA, fibrinogen, rabbit IgG, and β-galactosidase, see Table S1 for representative properties) to the resulting POEGMA-coated surface defined in Figure 2a are shown in the main text (Figure 2c), with data calculated according to the raw normalized frequency changes measured in Figure S1.

Table S1. The molecular weight and isoelectric point of proteins with varying properties obtained from the NCBI protein database and ExPASy SIB Bioinformatics Resource Portal

Protein	Molecular Weight (kDa)	Isoelectric point
Albumin (BSA)	69.3	4.7 - 5.82
Fibrinogen	340	5.5
IgG1	150	8.6 ± 0.4
β-galactosidase	464	4.61

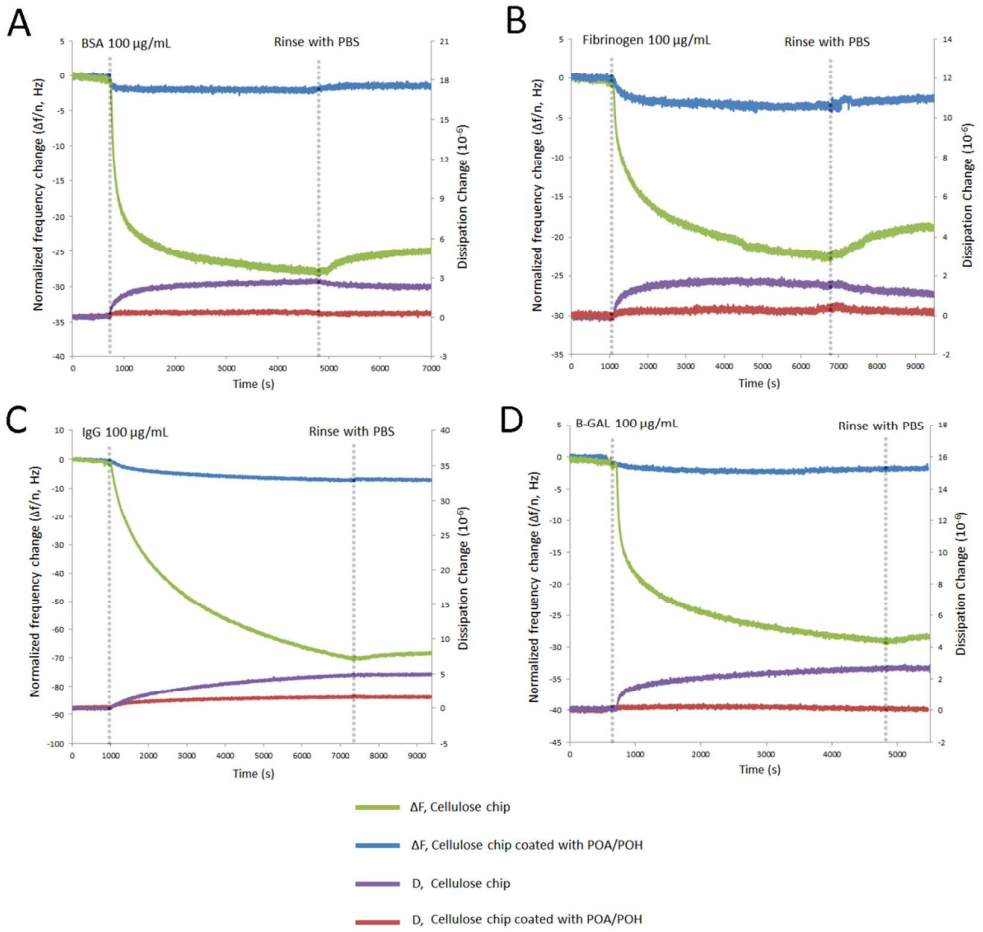


Figure S1. QCM-D results of the measured frequency change (ΔF) and dissipation (D) following adsorption of 100 µg/mL solutions of BSA (A), fibrinogen (B), rabbit IgG (C) and β-GAL (D) on a pre-equilibrated cellulose sensor with or without a POA/POH coating (as defined in Figure 2a)

4. Contact angle and droplet penetration assay

Water contact angle measurements were conducted using a Model 100-00-115 NRL contact angle goniometer (Ramé-Hart, Succasunna, NJ) equipped with a Sanyo VC8−3512T camera. Contact angles were measured by applying 5 μL droplets of distilled deionized water on the surface of both cellulose-coated QCM chips and Whatman 40# filter paper samples. For the QCM chips, the measurement of contact angle was conducted following rinsing with PBS until a stable frequency plateau was achieved (see Fig. 2a) and subsequent drying of the chip under moderate nitrogen flow. For paper samples, the droplet evolution was tracked by video, and screen shots were taken 1 second before and 1, 3, 5, 7 seconds after the droplet was applied to the paper to track both the initial contact as well as the kinetics of the penetration of the droplet into the paper network.

5. Paper dipping method

The POEGMA polymers were dissolved in 4% (w/v) phosphate buffered saline (PBS) solutions. Samples of Whatman 40# ashless filter paper were cut into small pieces (1 cm × 2 cm) or strips (0.8 cm × 8 cm) and then dipped in the polymer solutions by completely submerging the paper in the solution. POA was used in the first dipping step for all reported experiments since initiating the sequential dipping with POA instead of POH was demonstrated in preliminary work to facilitate improved protein repellency; we attribute this result to the enhanced affinity between aldehyde-functionalized POA and paper, which can more effectively anchor the POEGMA polymer to the fiber network. After 4 h of gentle shaking (~30 rpm) at room temperature, the paper samples were removed from the solution and washed twice with PBS. Afterwards, all the samples were dried overnight at ambient conditions (~23°C and ~30% relative humidity). Subsequently, the dried paper was dipped in the 4% (w/v) POH solution for another 4 hours and then washed and dried using the same procedure outlined above. It should be noted that preliminary work was performed to investigate the utility of multiple dipping cycles on the ability of the dip-modified paper to suppress non-specific protein adsorption. While step-by-step mass gain (i.e. POEGMA adsorption or grafting) was achieved on sequential dipping cycles, no significant

improvement in protein repellency was observed; as such, a single dipping cycle was used for all experiments discussed in this manuscript.

6. Surface topography

The filter paper samples were subjected to optical profilometry and scanning electron microscopy (SEM) imaging before and after dipping in the POA and POH solutions. Optical profilometry images were obtained using a WYKO Model NT 1100 optical profilometer operating under the VSI measurement mode at a magnification of 20x. For SEM analysis, samples were sputter-coated with platinum (layer thickness = 15 Å) to avoid charging effects and were imaged using a JEOL Model JSM-7000F SEM (Tokyo, Japan) operating at 2.8 kV and a probe distance of 5.9 mm.

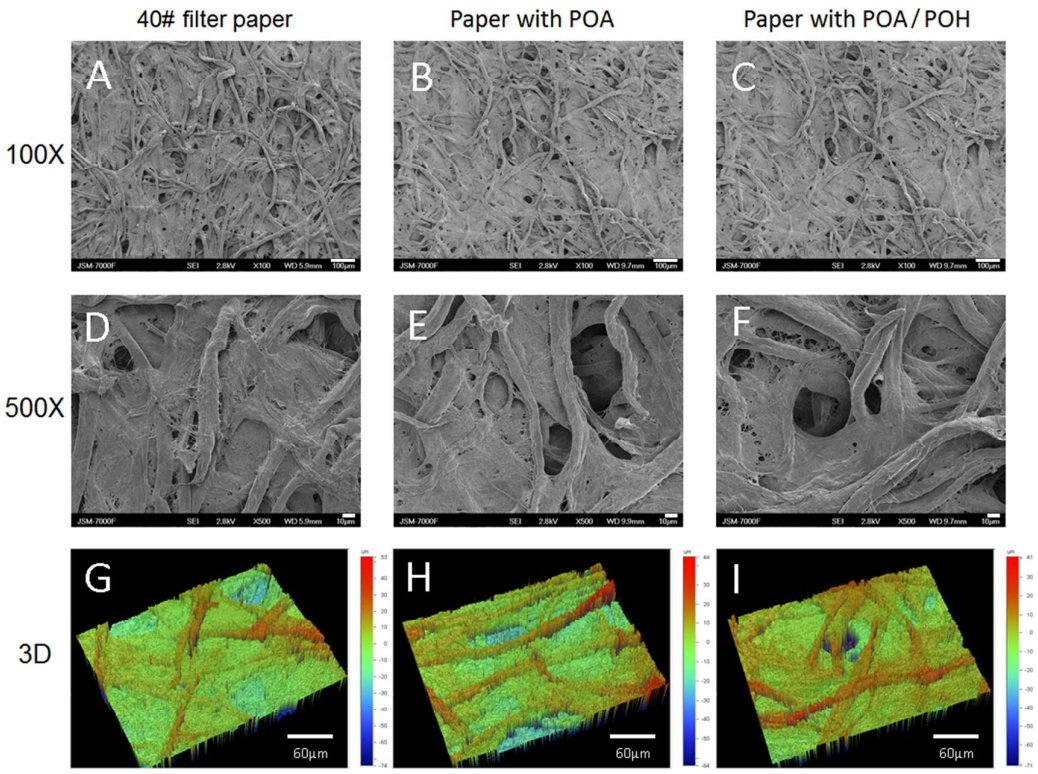


Figure S2. Scanning electron microscopy (SEM, A-F) and optical profilometry (G-I) images of 40# filter paper (A,D,G) and filter paper coated with POA (B,E,H) or with POA/POH (C,F,I) at 100x (A-C), 500x (D-F) and 400x (G-I) magnification.

As noted in the manuscript, no significant change in the morphology or roughness of the

paper fiber network was observed upon POA or POA/POH dipping, as evidenced by the lack of change in both the SEM images as well as the optical profilometry results before and after both POA and POA/POH dipping.

7. Paper mechanics

The tensile strength of paper before and after dip modification was measured to assess the effect of dip coating on the paper mechanics. Prior to the test, paper samples were equilibrated overnight at 23°C and 50% relative humidity. Testing was conducted following TAPPI T494 (Tensile properties of paper and paperboard, using constant rate of elongation apparatus) using a 50 N load cell and operating at a deformation rate of 10 mm/min along the machine direction of paper. The rectangular sample width was 8.0 mm and the grip distance was 20.0 mm. The thickness of all paper samples tested (including dipped papers) was 0.201 ± 0.006 mm (TMI digital micrometer), such that differences in modulus are not attributable to differences in thickness. Tensile strength, modulus, and elongation to break were calculated using Series IX standalone software (Version 1.1). Ten samples were tested in each group, with results expressed in terms of the mean ± standard deviation. Table S1 shows the tensile properties of unmodified filter paper, filter paper treated with POA, and filter paper treated with sequential dipping of POA/POH.

Table S2. Mechanical properties of 40# filter paper and paper coated with POA and POH obtained by tensile force measurements.

	Displacement at Peak (mm)	**Load at Peak (N)**	**Break Displacement (mm)**
40#	0.70±0.11	20.3±0.8	0.91±0.29
POA	0.94±0.06**	21.0±1.2	1.19±0.22*
POA/POH	0.94±0.08**	21.3±1.0*	1.18±0.25*

	0.2% Yield Strength (MPa)	**Modulus (MPa)**	**Energy at Break (mJ)**
40#	8.9±0.4	970±120	12.2±2.5
POA	7.3±0.7##	850±90#	16.8±2.0**
POA/POH	7.6±0.4##	910±70	17.3±2.5**

N=10; *p<0.05,**p<0.001, significantly higher than 40# filter paper; #p<0.05, ##p<0.001, significantly lower than 40# filter paper.

Following initial dipping of POA, the displacement at peak, break displacement, and energy at break all significantly increased while the load at peak and the modulus were not affected. Overall, this result suggests that the paper becomes more difficult to break as a result of POA adsorption. Subsequent dipping (and covalent bond formation) with POH maintains these same properties, although gel formation does not induce significant further strengthening of the paper. This demonstrated enhancement (or at least maintenance) of key paper mechanical properties is a point of significant contrast between this dipping method and other methods of paper modification (i.e. chemical grafting or paper oxidation), which typically significantly decrease the tensile strength of the paper. Note that the "0.2% Yield Strength" reported is an offset yield point often reported in the literature in cases in which the true yield point is not easily defined based on the shape of the stress-strain curve and refers to the stress measured at 0.2% strain.[4]

8. FTIR of polymers

The infrared spectra of the reactive pre-gel polymers and the gel-coated paper samples were collected using a Nexus 6700 Fourier-transform infrared (FTIR) spectrometer (Thermo Fisher Scientific Inc.) equipped with an infrared microscope (Nicolet Continuμm). An attenuated total reflection (ATR) attachment was used for the measurement. The lyophilized samples of POA and POH were measured to obtain their individual spectra as shown in Figure S3.

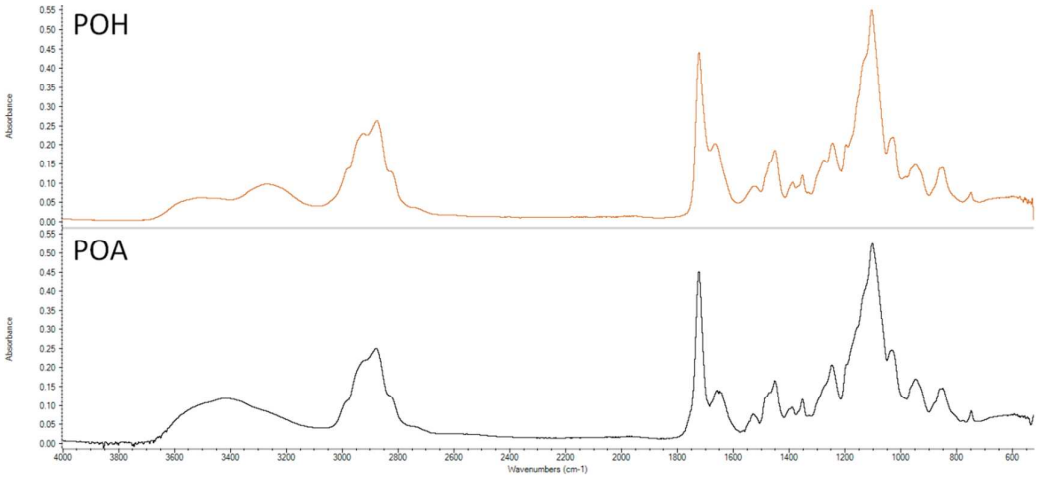


Figure S3. FTIR spectra of hydrazide-functionalized POEGMA (POH) and aldehyde-functionalized POEGMA (POA).

ATR-FTIR for POA (cm^{-1}): 3412m, 2877s, 1723s, 1657w, 1649w, 1529w, 1451m, 1389w, 1352w, 1246m, 1103s, 1033w, 947w, 850w, 748w.

ATR-FTIR for POH (cm^{-1}): 3265m, 2876s, 1722s, 1665w, 1524w, 1451m, 1388w, 1353w, 1245m, 1104s, 1029w, 947w, 850w, 749w.

The IR spectra of POA and POH are largely the same, with the strong peak at 1723 cm^{-1} and a medium intensity peak at ~1245 cm^{-1} representing stretching vibrations indicative of the C=O ester groups that connect the backbone of the POEGMA polymers to the oligo(ethylene glycol) side chains and a very strong peak at 1103 cm^{-1} representing the antisymmetric stretching vibration of C-O-C in the oligo(ethylene glycol) in the side chain. Hydrazide and aldehyde groups both typically appear in the range of the ester signals and are thus convoluted with the higher intensity ester peaks.

9. FTIR of paper surface

The diffuse reflectance infrared Fourier transform spectroscopy (DRIFTS) method was employed to characterize the polymer-treated paper surface. DRIFTS was used in this case since it is well-documented to be useful for analyzing polymers (including foams, powders, fibers and composites) on rough and heterogeneous surfaces (like paper). The spectra of nascent 40# filter paper and the paper samples dipped with POA and sequential dipping of POA/POH are shown in Figure S4. The presence of the peaks at 1727-1735 cm^{-1} (corresponding to the ester groups in POEGMA, indicated by the dashed rectangle in each spectrum) confirms the deposition of POEGMA on to the paper surface as a result of the dipping procedure.

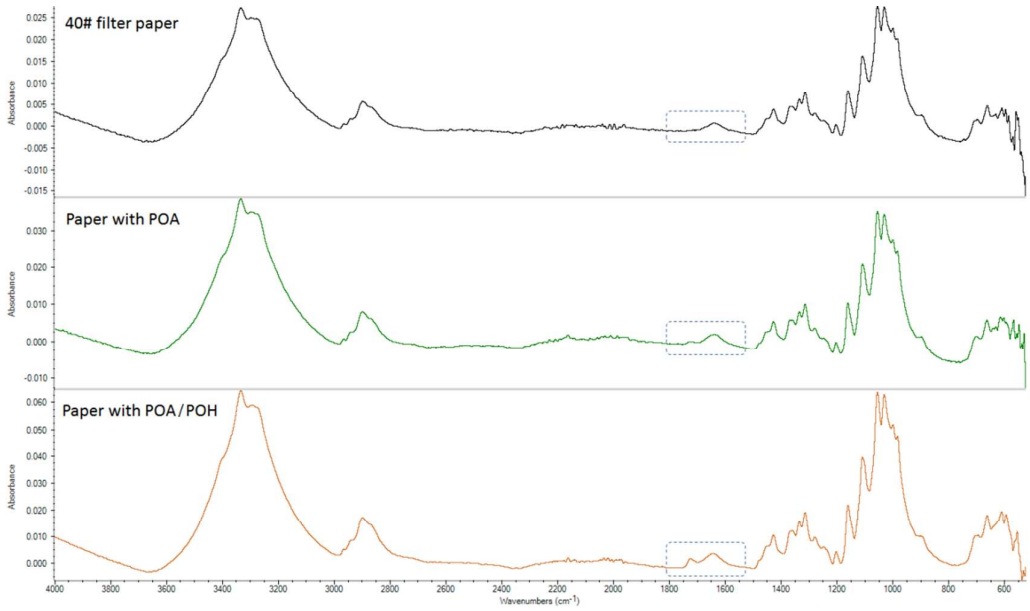


Figure S4. FTIR spectra of 40# filter paper, paper dipped with POA, and paper dipped with POA/POH.

10. Dry and wet weight of paper samples

Round pieces of unmodified and modified paper with diameters of ~6.6 mm were submerged in 250 µL of PBS for 24 hours inside a 96-well plate at room temperature. The dry and wet weight of the paper samples were measured before and after dipping. For dry weight measurements, the samples were dried in a 60°C oven and then equilibrated at 23°C and 50% relative humidity overnight prior to measurement to ensure consistency between samples. For wet weight measurements, the excess water on the surface of sample was removed by a rapid press (using 2.4 kg hand roller) between two Kimwipes to ensure that only absorbed/adsorbed water is considered in the swollen mass measurements. Results are shown in Figure S5.

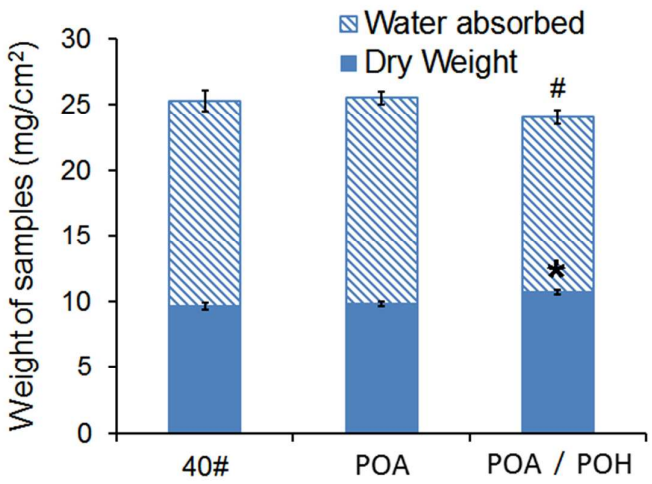


Figure S5. Dry and fully wetted weight of paper samples (n=12, *p<0.05 (dry), #p<0.05 (wet) relative to 40# filter paper).

As discussed in the manuscript, POEGMA dipping results in an increase in paper dry weight (associated with the immobilization of a POEGMA gel on the fiber surface) but a slight albeit significant decrease in wet weight. We hypothesize this result is related to the clogging of small pores in the fibers and/or between fibers by POEGMA gel formation.

11. Polymer distribution on paper

To confirm that the dipping method successfully modified the fiber surface, the sequential dipping procedure was repeated using rhodamine 123-labeled POA and fluorescein-labeled POH. For this purpose, POH was tethered with fluorescein isothiocyanate (FITC) via direct reaction between the isothiocyanate and hydrazide groups. Typically, 1 g of POH was reacted with 5 mg of FITC overnight (at least 12 h) under gentile mechanical agitation at pH 8 and room temperature, targeting modification of only a small (~2 mol%) fraction of the available hydrazide groups so as not to disrupt the cross-linking capacity of the polymer. Analogously, POA (1 g) was labeled with rhodamine 123 (5 mg) via reductive amination between the amino group in rhodamine 123 and a small (~2 mol%) fraction of aldehyde groups in POA, with the resulting Schiff base reduced using sodium cyanoborohydride (8.25 mg, 10 fold molar excess to the rhodamine) to create a stable conjugate. All reactions were performed in a foil-covered reaction flask to prevent photobleaching during synthesis. The resulting labeled

polymers were dialyzed exhaustively against deionized water (MWCO 3500 Da) for 6 cycles (6+ hours per cycle, all in the dark) to remove unbound FITC, lyophilized to dryness, dissolved in PBS as 4% w/v solution, and stored at -4°C in the dark. Fluorescently-labeled POA and POH solutions were subsequently used in place of non-labeled POA and POH in the dipping method outlined in section 4 to prepare a labeled POEGMA-coated paper. Following modification, the dried paper sample was exposed to blue (488 nm) and green (543 nm) light using a Zeiss 510 inverted confocal microscope to collect fluorescence images defining the localization of fluorescein-labeled (POH) and rhodamine-labeled (POA) polymers respectively on the paper surface. The images are shown in Figures 3a and S6, with the two figures presented representing images collected at two different z-planes within the sample. Each figure shows similar co-localization of POA and POH on the fiber surface, indicating that the bulk of the paper was successfully surface modified with POEGMA by dipping.

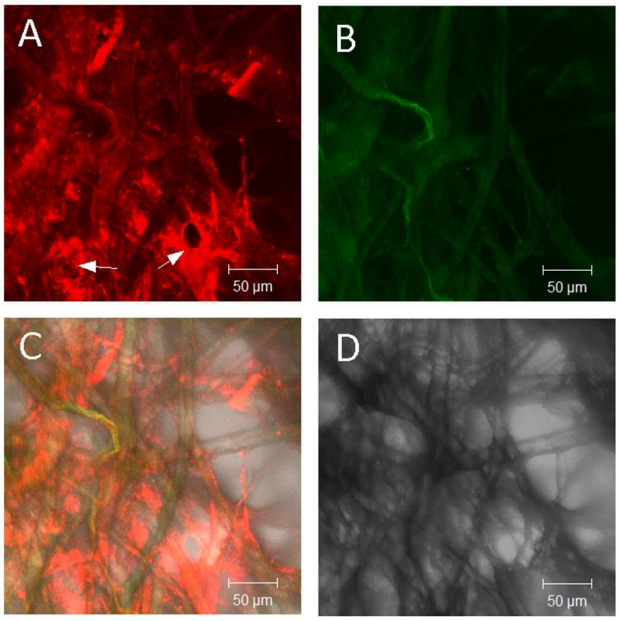


Figure S6. Cellulose fiber networks treated by fluorescently-labeled POA and POH as viewed by confocal laser scanning microscopy (CLSM) at a different z-plane from Figure 3a. A: rhodamine 123-labeled POA; B: fluorescein-labeled POH; C: merged CLSM image; D bright field image. Arrowheads in A indicate the concentration of POA at the edge and junction points between fibers.

12. Mercury intrusion porosimetry

Mercury intrusion porosimetry measurements were performed using a Quantachrome Poremaster GT mercury intrusion porosimeter (Boca Raton, USA) using high pressure mode and a fixed speed pressure gradient from 1.4 to 4195 kg·cm^{-1} (10.6 µm –3.57 nm pore diameter range). The average pore diameter, total porosity, and pore size distribution were determined as a function of intruded volume using the Poremaster (Version 5.00) software (Quantachrome). Figure S7 shows the number distribution of pores and the total pore volume of the paper before and after POEGMA dip coating treatment.

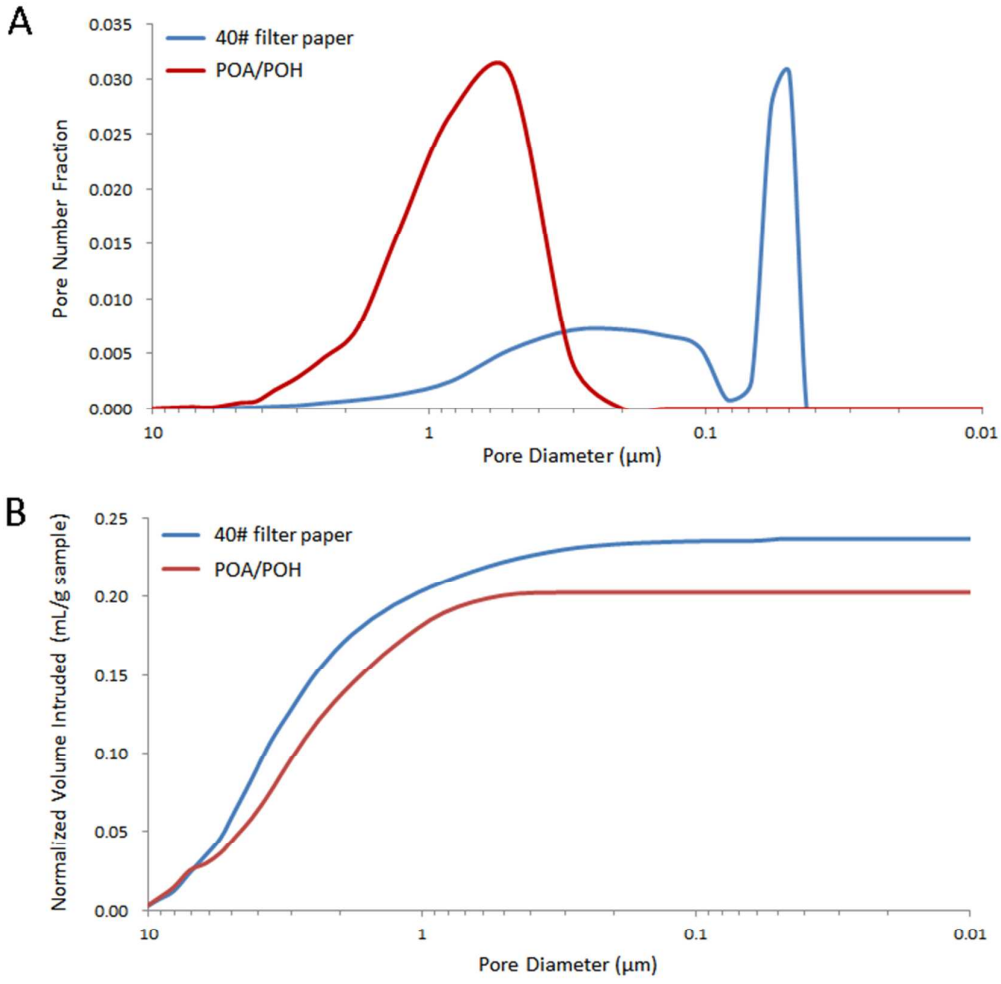


Figure S7. Number distribution of pore diameter (A) and normalized volume of mercury intrusion (B) of 40# filter paper and filter paper coated with POA/POH operated at high pressure model.

This data indicates that the porosity measured by high pressure mercury porosimetry slightly decreased after coating with polymers, with the decrease in porosity attributable almost exclusively to the elimination of smaller pores (< 0.05 μm) while larger pores (~ 0.5 μm average size) remain relatively unaffected. This result further supports our hypothesis that POEGMA dipping results in the filling of nanopores/micropores within individual fibers but has little impact on the pores between fibers (i.e. the mesh size of the paper) relevant for defining transport properties. It should be noted that the high pressure operation of the porosimeter effectively collapses the macropore network of the paper, requiring the addition of the interparticle porosity data obtained under low pressure (~61% for both treated and untreated paper) to accurately estimate the total pore fraction inside the paper at ambient pressure. However, the high pressure data can more directly probe the fate of the smaller pores as a result of POEGMA treatment, of direct interest here in interpreting the role of POEGMA in regulating paper interfacial properties.

13. Residual aldehyde and hydrazide groups on paper surface

Fluorescent labeling was used to assess the presence of unreacted (free) aldehyde and hydrazide groups on POA and POA/POH treated paper. Following POEGMA modification of the paper surface using the dipping method described in section 4 (using non-fluorescent polymers), the dried POA and POA/POH dipped paper samples were soaked in solutions of 0.05 g/L fluorescein-5-thiosemicarbazide (5-FTSC, aldehyde-reactive) or 5-fluorescein isothiocyanate (5-FITC, hydrazide-reactive) overnight at pH 8 in a 96-well plate to detect the number of residual functional aldehyde or hydrazide functional groups respectively. Non-reacted probes were subsequently removed from the paper by soaking and rinsing the paper in pH 8 PBS buffer (15 wash cycles) to ensure that only covalently-tethered probe remained. The relative fluorescence intensity of the surface (directly correlated to the number of free aldehyde or hydrazide groups on the paper, depending on the probe used) was measured by a VICTOR 3 multi-label microplate reader using an excitation wavelength of 488 nm and an emission wavelength of 535 nm (Figure S8).

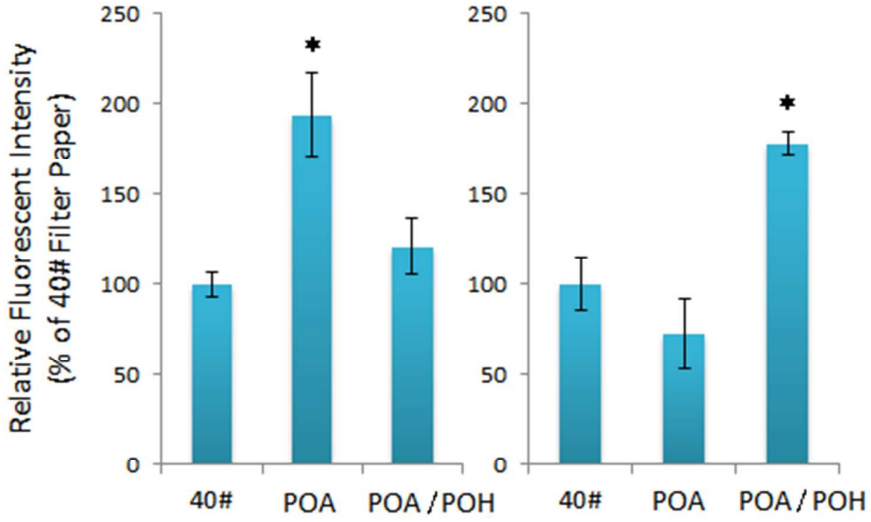


Figure S8. Relative density of aldehyde and hydrazide groups throughout the paper surface with or without POA and POH coating. The aldehyde and hydrazide groups were labeled by fluorescein-5-thiosemicarbazide (5-FTSC) and 5-fluorescein isothiocyanate (5-FITC) respectively. The fluorescence intensity of the paper samples was measured by fluorescent plate reader at the excitation wavelength of 488 nm and emission wavelength of 535 nm and normalized to the results obtained with unmodified 40# filter paper.

Addition of POA in the first dipping step results in a significant increase in the number of free aldehyde groups but no change in the number of hydrazide (nucleophilic) groups, as anticipated for POA adsorption to the paper. In the second dipping step, those excess aldehyde groups are consumed (returning to the concentration of aldehydes observed in unmodified 40# filter paper) while the number of free hydrazide (nucleophilic) groups significantly increases, indicating both the successful immobilization of POH and the occurrence of covalent cross-link formation between aldehyde groups in POA and hydrazide groups in POH. Thus, these results confirm that a thin interfacial hydrogel layer is formed on the fiber surface as a result of dipping.

14. Layer-by-layer dipping

Given the results above that suggest a large excess of hydrazide groups at the interface following a single POA/POH dipping cycle, we investigated the production of hydrogel films by sequential POA/POH dipping cycles in a process analogous to polyelectrolyte

layer-by-layer assembly methods. Figure S9(a) shows the mass change measured over each cycle of POA/POH sequential dipping, while Figure S9(b) shows the resulting protein adsorption to these multi-layer dipped filter papers.

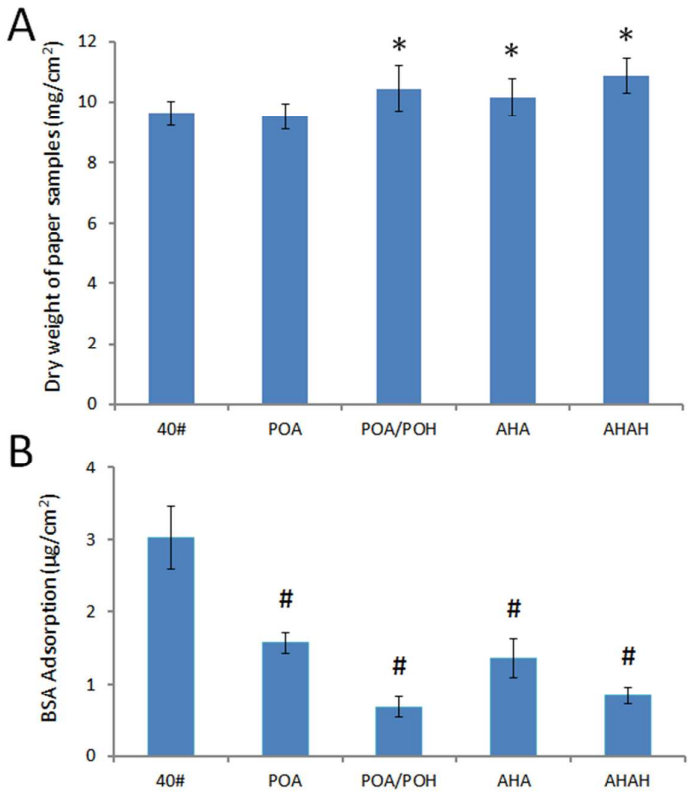


Figure S9. Dry mass change (n=6) (a) and the adsorption of BSA (100 µg/mL protein concentrations, n=6) (b) to layer-by-layer POA/POH dipped papers prepared by sequential dipping cycles. AHA and AHAH) indicate three or four dipping steps respectively using POA/POH sequentially. *p<0.05 higher, #p<0.05 lower relative to 40# filter paper

A significant mass increase is observed upon each POA/POH dipping cycle (Fig. S9(a)), indicating the effectiveness of a layer-by-layer approach to build a hydrogel interface on the filter paper. However, no significant decrease in protein adsorption is observed as a result of this sequential hydrogel fabrication process; a single POA/POH dipping cycle is as effective as multiple cycles in terms of reducing non-specific adsorption ($p > 0.05$, Fig. S9(b)). Based on this result, coupled with the increased work associated with layer-by-layer approaches and the increased risk of macropore blockage as the hydrogel becomes thicker, a single POA/POH

dipping cycle was used to produce all blocked filter papers discussed in the main manuscript. Note that regardless of the number of dipping cycles used, paper surfaces in which POA is used as the terminal dipping polymer (i.e. the surface has excess aldehydes) consistently underperform paper surfaces in which POH is used as the terminal dipping polymer (i.e. the surface has excess hydrazides). We hypothesize this result is attributable to the lack of reactivity of hydrazide groups to proteins relative to aldehyde groups, which may form Schiff bases with terminal or pendant amines on proteins.

15. Capillary rise

Capillary rise experiments were performed on filter paper strips of width 0.8 cm, height 8 cm and thickness 0.2 mm prepared both with and without POEGMA coatings. One end of the strip was vertically submerged into a PBS bath at a depth of 1 cm, and the time required for the rising edge of the water to travel up the test strip was recorded at each centimeter of capillary rise to a total height of 5 cm from the point of submergence. At least three experiments were performed for each group of sample, with the reported capillary rise rates representing the mean ± standard deviation of these three experiments.

16. Protein adsorption

Protein adsorption to unmodified and dipped paper samples was assessed by submerging two paper samples (1cm × 2cm) in PBS within a single well of a 12 well plate and allowing the paper samples to equilibrate over 24 hours. Afterwards, unabsorbed PBS was removed and 500 μL of 100 μg/mL protein solution (bovine serum albumin, fibrinogen, rabbit IgG, or β-galactosidase) was added to each well. The samples were incubated for 2 hours at 25°C under gentle shaking. After 2 hours, the paper samples were removed and the residual protein concentration in the solution was measured using a Bradford protein assay (1:1 sample to reagent ratio) according to the manufacturer's protocol.[5] Results were quantified by measuring the absorption of the residual (unadsorbed) protein solution at 595 nm using a Nanodrop 2000c spectrophotometer (Thermo Scientific). Each experiment (POA/POH coated paper as well as the controls) was done in triplicate, with reported errors representing the

standard deviation of the replicates.

17. Benchmarking protein adsorption performance of POEGMA-blocked paper to BSA-blocked paper

To illustrate the performance of the POEGMA dip-coated paper relative to the current standard of BSA blocking in terms of resisting non-specific protein adsorption, IgG adsorption experiments were conducted on both a model cellulose surface using QCM and on Whatman 40# filter paper using a fluorescence technique.

QCM on Cellulose-Modified Surface: BSA-blocked surfaces were first prepared by flowing BSA solution (1%, w/v in PBS) over cellulose-modified QCM chips until the QCM signal reached a plateau (i.e. adsorption equilibrium was achieved). The chips were subsequently washed with PBS until a plateau dissipation was observed (i.e. no further BSA desorption occurred). Next, rabbit IgG solution (25, 50, 100, 200, 500, or 1000 μg/mL in PBS) was flowed over the chip until steady state was achieved, after which the chip was subjected to a PBS wash (again until equilibrium was achieved). The adsorption of IgG was calculated by Sauerbrey equation, as described in section 3 of Supporting Information.

Figure S10 shows the Sauerbrey masses of rabbit IgG adsorbed to the QCM chip as a function of IgG concentration. Both POA/POH dip-coated paper as well as BSA-blocked paper performed similarly, with protein adsorption to either interface less than $1/10^{th}$ of that observed for the bare cellulose chip.

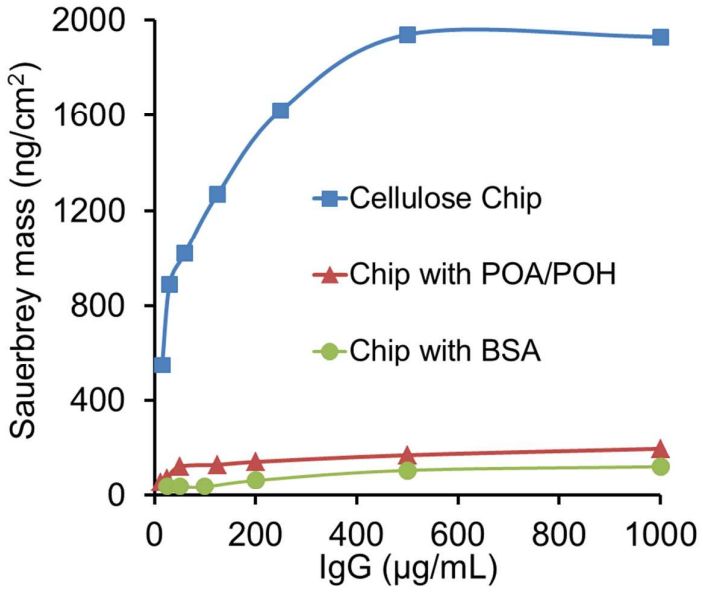


Figure S10. Sauerbrey mass (from QCM) as a function of time for rabbit IgG adsorption to a bare cellulose chip and cellulose QCM chips pre-treated with POA/POH and BSA

Filter paper: To assess the protein adsorption performance of Whatman 40# filter paper blocked with POA/POH relative to paper blocked with BSA, IgG was labeled with FITC according to the Thermo Scientific product protocol (# 53027). Briefly, 50 µg FITC (15- to 20-fold molar excess) was reacted with 1 mg IgG (500 µL, 2 mg/mL) at pH 8.5. Labeled protein was separated from free label via centrifugal filtration (10000 RPM, 10 min) using a membrane with a molecular-weight cutoff (MWCO) of 10 kDa (IgG=150 kDa, FITC=389 Da) instead of using resin in the protocol.

The filter paper was cut into a 1cm×2cm strip, submerged in BSA (1%, w/v) for 30 min, and then washed with PBS. The BSA-blocked paper (two pieces per well) was next submerged in the FITC-labeled IgG solution (100 µg/mL, 500 µL) for 2 hours and removed after a gentle shaking of the container. The fluorescent intensity of the solution before and after paper soaking (including the PBS rinse volumes) was measured using a VICTOR 3 multi-label microplate reader (excitation = 488 nm, emission = 535 nm). Fluorescence intensity values were converted to protein concentrations based on a calibration curve (R^2 = 0.997), with the amount of protein adsorbed to the paper calculated based on the difference between total

protein content of the solution before and after paper exposure. Figure S11 shows the comparative adsorption result between unmodified (control), BSA-blocked, and POA/POH-blocked filter paper.

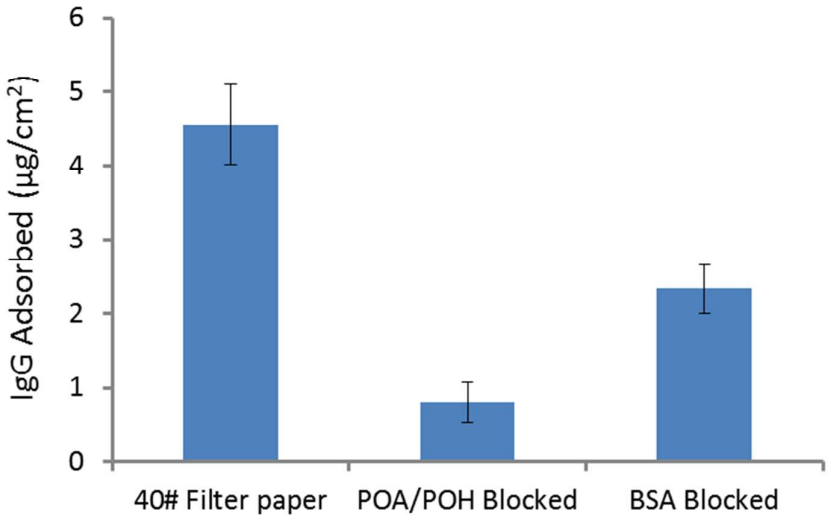


Figure S11. Amount of rabbit IgG adsorbed per unit cross-sectional area on BSA-blocked, POA/POH-blocked, and control (unmodified) paper.

BSA-blocked paper is significantly less effective at preventing protein adsorption relative to POA/POH-blocked filter paper, with approximately 3-fold less IgG adsorbed to the POA/POH-blocked paper. This result can be attributed to two factors: (1) POA/POH polymers are significantly smaller (~20 kDa compared to ~66 kDa) and significantly more flexible than BSA and thus can effectively block smaller pores in the paper that BSA cannot and (2) POA/POH forms a covalently cross-linked thin layer at the fiber interface that is fixed in place, while BSA can exchange with IgG dynamically via non-covalent interactions.

18. Construction of β-galactosidase (β-GAL) test strips

To assess the capacity of POEGMA-coated paper for selective lateral flow assays, a paper-based test strip was designed that utilized chlorophenol red β-galactopyranoside (CPRG) as a colorimetric sensor to confirm the mobility of β-galactosidase (β-GAL) within the paper

matrix. CPRG (yellow color) is enzymatically converted by β-GAL into chlorophenol red (red-magenta color); capture of the chlorophenol red product by poly-L-arginine hydrochloride results in the red color changing to purple, allowing for site-specific colorimetric detection of the presence of β-GAL.

Test strips were constructed by dip-coating paper with POA/POH as described in section 4 and subsequently depositing CPRG (5μL, 3 mM) and poly-L-arginine hydrochloride (5 μL, 2% w/v) at a specific location on a 0.8 cm x 8 cm paper strip by pipetting to form a localized detection band (see Figure 4a, strips 1-3). The strips were air dried prior to use for testing. Subsequently, the mobility of β-GAL was assayed by placing the test strips into the β-GAL solution (10U/mL in PBS, pH=6.8) at a depth of 1 cm and allowing the liquid to move up the strip until it crossed the poly-arginine band.

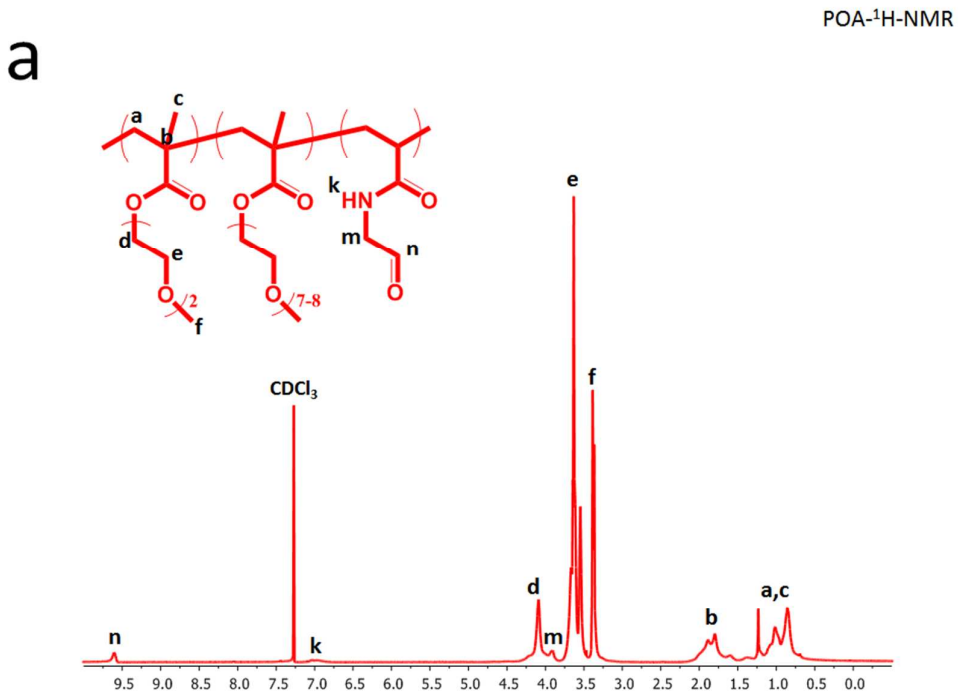
19. Indirect paper-based ELISA

Prior to the experiment, a paper microzone plate was fabricated using a wax printer (Xerox Phaser 6580). The patterned black wax on the 40# filter paper was melted in an oven at 120 °C for 2 minutes to form the hydrophobic barriers. Goat anti-rabbit IgG (the antigen) was subsequently transferred to the patterned paper at various concentrations in PBS solutions in 3 μL aliquots into different test zones on the microzone plate and allowed to dry for 10 minutes under ambient conditions (~23^{o}C, ~30% relative humidity). Each test zone was subsequently blocked by adding 3 μL total volume of various blocking agents: (1) POA and POH (both 4% (w/v) with 0.05% (v/v) Tween-20 in PBS), added in sequence and dried for 15 minutes each; (2) BSA (consisting of 1% (w/v) BSA and 0.05% (v/v) Tween-20 in PBS), dried for 30 minutes – "BSA-blocked paper" in the manuscript; (3) skimmed milk (5% in PBS), dried for 30 minutes – "skimmed milk-blocked paper" in the manuscript. After blocking, a 3 μL solution containing horseradish peroxidase (HRP)-conjugated rabbit IgG (the antibody) in an incubation buffer (0.05% (v/v) Tween-20 in PBS) was added to each zone and allowed to incubate for 75 seconds. The whole paper was then washed three times by submerging into a PBS buffer with gentle shaking (3 x 1 minute cycles). Finally, a 3 μL

solution of the colorimetric substrate for horseradish peroxidase (1:1 TMB peroxidase substrate solution and peroxidase substrate solution B, KPL) was added to each test zone immediately after the washing step and allowed to react for 5 minutes under ambient conditions. The paper microzone plate was scanned by a desktop scanner (CanoScan LiDE 700F, Canon), and the integrated intensity and mean gray value of the color were measured using ImageJ software. The detection limit was defined as three times the standard deviation of the control group.[6]

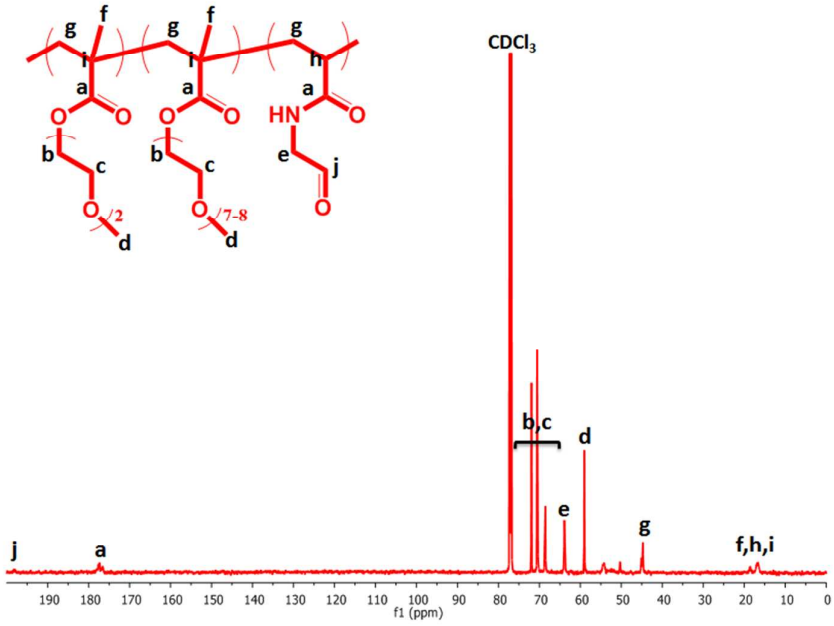
20. ^{1}H NMR and ^{13}C NMR spectra of POA and POH

^{1}H-NMR and ^{13}C-NMR were performed on a Bruker AVANCE 600 MHz spectrometer using deuterated chloroform as the solvent. Chemical shifts are reported relative to residual deuterated solvent peaks. Peak assignments are given on each spectrum based on the anticipated chemical structure of each polymer.



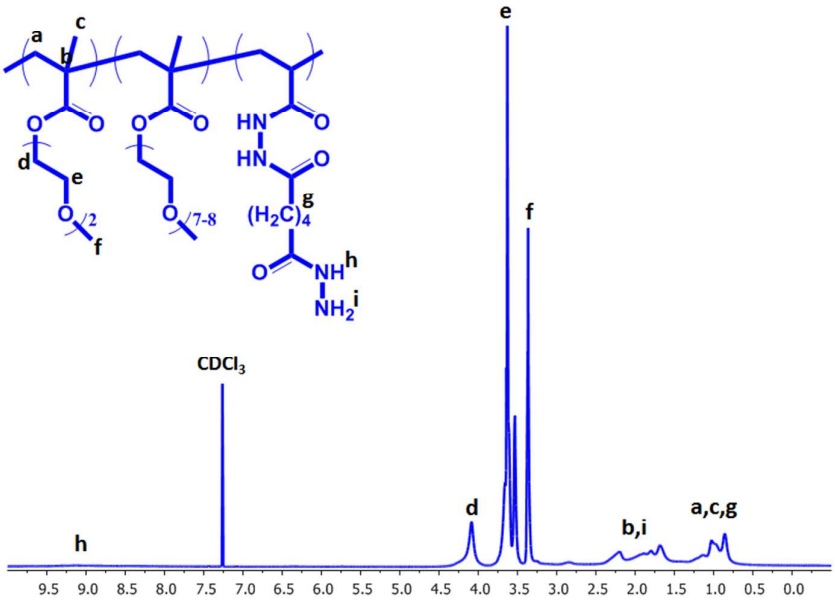
b

POA-^{13}C-NMR



c

POH-^{1}H-NMR



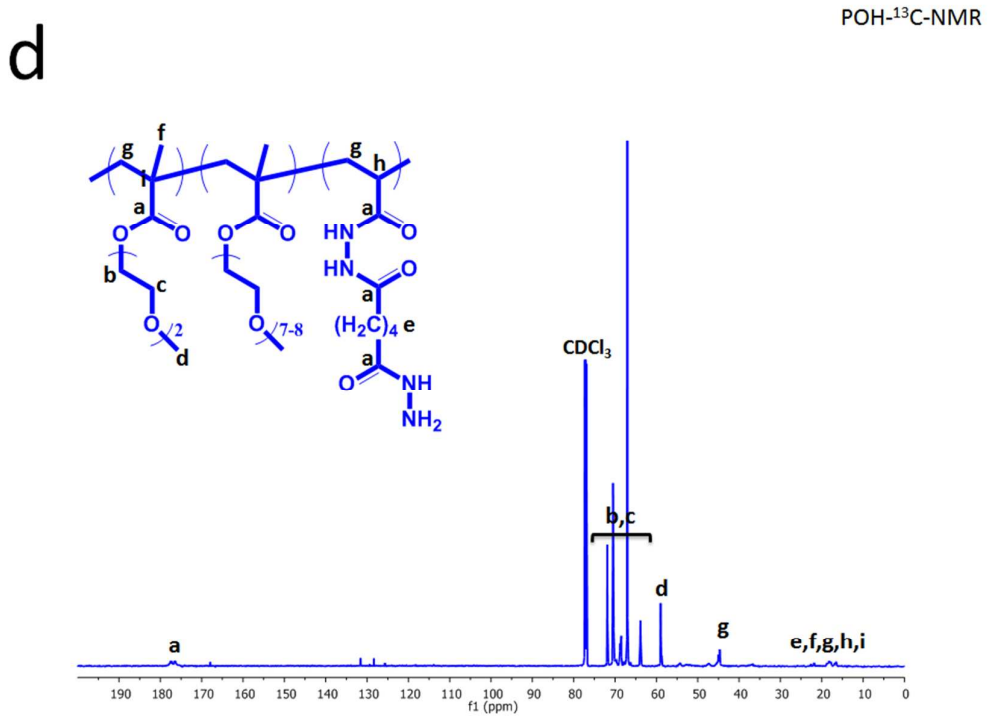


Figure S12. (a) ^{1}H NMR and (b) ^{13}C NMR spectra of POA; (c) ^{1}H NMR and (d) ^{13}C NMR spectra of POH.

21. Thermal analysis of POA and POH

Thermogravimetric analysis: Thermogravimetric analysis (TGA) was performed using a TA-Q50 instrument under an argon atmosphere. Samples were heated at 10 °C/min to 800 °C.

Figure S13 shows the TGA curves of POA and POH. Both polymers were thermally stable up to temperatures of >200 °C, facilitating storage stability. POH is more hydrophilic and hygroscopic than POA so absorbs water once removed from the drying oven, resulting in a small but significant water fraction that is removed at ~100 °C in the POH data.

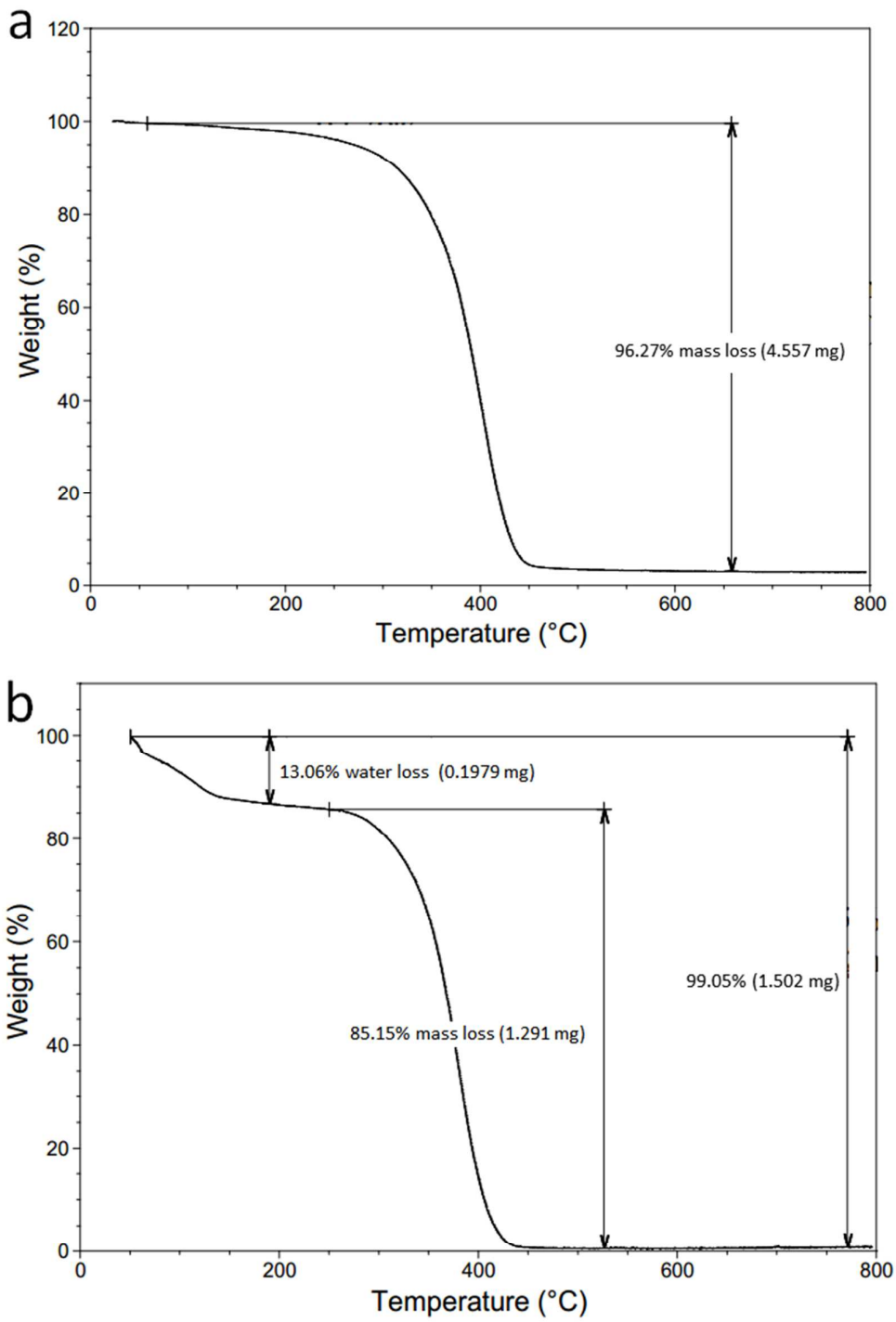
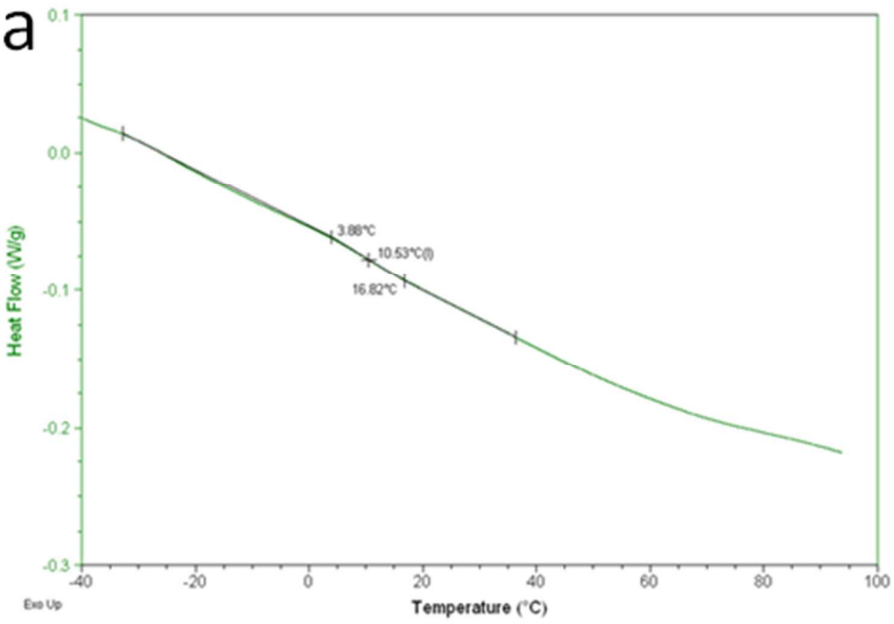


Figure S13. The weight loss of POA (a) and POH (b) as a function of temperature measured at a heating rate of 10 °C/min. using TGA

Differential Scanning Calorimetry: Differential scanning calorimetry (DSC) was performed using a TA Instruments Q200 DSC. 15 mg of a dried polymer sample was placed in a Tzero

hermetic aluminum sample pan. An empty reference pan was used during testing. A modulated temperature ramp from -50°C to 100°C was performed on each sample using a 60 second modulation at an amplitude of ±1°C and a 3°C/min. heating rate. The glass transition temperature within this range was determined using the TA Instruments Analysis software.

Figure S14 shows the DSC results for POA and POH. Glass transitions were observed for both polymers, with both polymers exhibiting relatively low T_g values of ~11°C for POA and ~-14°C for POH. Note that the relevance of this result to the performance of the hydrogel in the present application is somewhat unclear, although the fact that the polymer is in the rubbery state at room temperature and dry should at least assist with maintaining paper flexibility even at very high polymer concentrations.



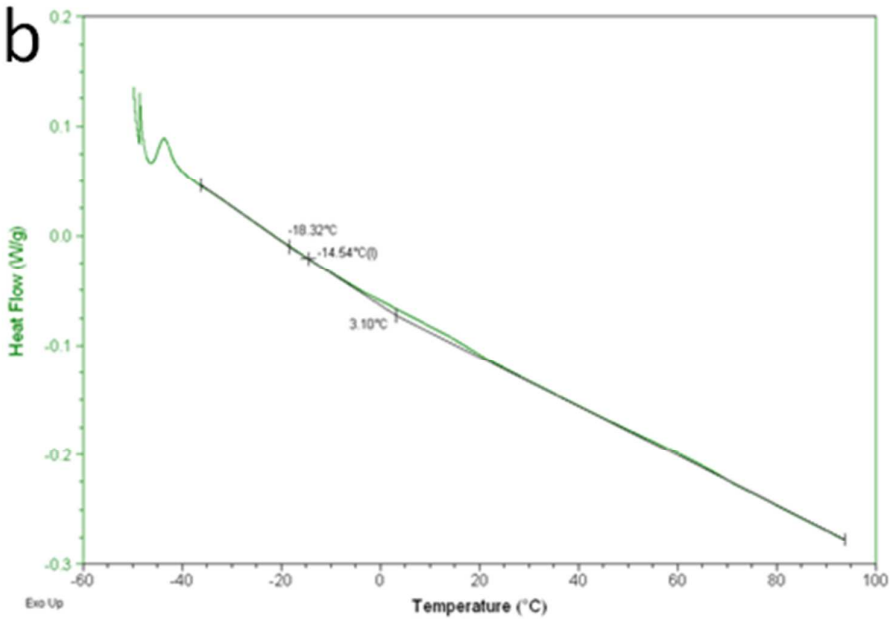


Figure S14. Differential scanning calorimetry results for POA (a) and POH (b).

22. Supplementary references

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