Electronic Supplementary Material (ESI) for Chemical Communications.

Supporting Information

SuFEx-Based Strategies for the Preparation of Functional Particles and Cation Exchange Resins

Andrew J. Kassick,[a,b] Li Chen,[c] Marina Kovaliov,[a,b] Robert Mathers,[d] Jason Locklin,[c] Saadyah Averick [a,b*]

[a] Neuroscience Disruptive Research Lab, Allegheny Health Network Research Institute, Allegheny General Hospital, Pittsburgh, PA 15212, USA.
[b] Neuroscience Institute, Allegheny Health Network, Allegheny General Hospital, Pittsburgh, PA 15212, USA.
[c] Department of Chemistry and College of Engineering, University of Georgia, Athens, GA 30602, USA.
[d] Department of Chemistry, The Pennsylvania State University, New Kensington, PA 15068, USA

*Corresponding author: saadyah.averick@ahn.org

MATERIALS AND METHODS

Materials. Styrene and divinylbenzene were purchased from Sigma-Aldrich (St. Louis, MO) and were passed through basic alumina prior to use. Sodium dodecyl sulfate was obtained from Invitrogen (Carlsbad, CA). 1,8-Diazabicyclo[5.4.0]undec-7-ene, triazabicylcodecene, and ammonium persulfate were purchased from Sigma-Aldrich (St. Louis, MO). *N, N*-Dimethylformamide was purchased from ACROS Organics (Morris Plains, NJ). PVA, phenol, *tert*-butyldimethylchlorosilane, and imidazole were obtained from Sigma-Aldrich (St. Louis, MO). Methanol and 2-propanol were purchased from Pharmco-AAPER (Shelbyville, KY). Water was purified via a Millipore Synergy water purification system. 4-Vinylphenyl sulfofluoridate (VPSF) was prepared from 4-acetoxystryrene, sourced from ACROS Organics (Morris Plains, NJ) according to the literature procedure.[1] The enzymes used in this work were *Thermomyces Lanuginosus* lipase (lipozyme® TL) or green fluorescent protein (GFP) as a control. All other reagents and solvents were used as received unless otherwise noted.

Emulsion polymerization of VPSF and 10 wt% DVB. A 25 mL Schlenk flask equipped with a stir bar was charged with SDS (89 mg) and 6 mL of H_2O. The resulting clear, colorless solution was treated with $(NH_4)_2S_2O_8$ (53 mg). A mixture of VPSF monomer **5** (1.58 g) and DVB (0.175 g) was then added slowly dropwise via pipette to the solution under vigorous stirring. The now milky white reaction mixture was thoroughly degassed with N_2 for 30 min then heated at 50 °C for 18 h with constant stirring. Saturated aqueous NaCl (30 mL) was added to the resulting thick, white suspension. The mixture was triturated and filtered. The collected solid was washed with H_2O (2 × 20 mL) and MeOH (2 × 20 mL) then suspended in 20 mL of DMF. The suspension was added dropwise to a solution of 1:1 MeOH/H_2O (300 mL). The mixture was filtered and the solid was washed with H_2O (3 × 20 mL) and MeOH (3 × 20 mL). The white solid was dried on a Buchner funnel under vacuum to provide 1.35 g of the desired polymeric material.

Emulsion polymerization of styrene/VPSF (90:10) and 10 wt% DVB. A 25 mL Schlenk flask equipped with a stir bar was charged with SDS (89 mg) and 6 mL of H_2O. The resulting clear, colorless solution was treated with $(NH_4)_2S_2O_8$ (55 mg). A 90:10 mixture of styrene (1.42 g) and VPSF monomer **5** (0.158 g) containing 10 wt% DVB (0.175 g) was then added slowly dropwise via pipette to the solution under vigorous stirring. The milky white reaction mixture was thoroughly degassed with N_2 for 30 min then heated at 50 °C for 18 h with constant stirring. Saturated aqueous NaCl (30 mL) was added to the resulting thick, white suspension. The mixture was triturated and filtered. The collected solid was washed with H_2O (2 × 20 mL) and MeOH (2 × 20 mL) then suspended in 20 mL of DMF. The suspension was added dropwise to a solution of 1:1 MeOH/H_2O (300 mL). The mixture was filtered and the solid was washed with H_2O (3 × 20 mL) and MeOH (3 × 20 mL). The white solid was dried on a Buchner funnel under vacuum to provide 1.40 g of the desired polymeric material.

Emulsion polymerization of styrene/VPSF (70:30) and 10 wt% DVB. A 25 mL Schlenk flask equipped with a stir bar was charged with SDS (89 mg) and 6 mL of H_2O. The resulting clear, colorless solution was treated with $(NH_4)_2S_2O_8$ (55 mg). A 70:30 mixture of styrene (1.11 g) and VPSF monomer **5** (0.474 g) containing 10 wt% DVB (0.176 g) was then added slowly dropwise via pipette to the solution under vigorous stirring. The milky white reaction mixture was thoroughly degassed with N_2 for 30 min then heated at 50 °C for 18 h with constant stirring. The resulting white suspension was cooled to ambient temperature and quenched with saturated aqueous NaCl (30 mL). The mixture was triturated and filtered. The collected solid was washed with H_2O (2 × 20 mL) and MeOH (2 × 20 mL) then dissolved in 20 mL of DMF (with sonication). The solution was added dropwise to a solution of 1:1 MeOH/H_2O (300 mL). The resulting precipitate was filtered and washed with H_2O (3 × 20 mL) and MeOH (3 × 20 mL). The collected material was dried on a Buchner funnel under vacuum to provide 1.45 g of a white solid.

Suspension polymerization of styrene/VPSF (90:10) and 10 wt% DVB. A 250 mL round bottomed flask was charged with 100 mg PVA and 70 mL of H_2O. The mixture was heated at 65 °C to dissolve the PVA. The resulting clear, colorless solution was cooled to ambient temperature whereupon a solution of styrene (8.1 g), SuFEx monomer (0.9 g), 10 wt% DVB (1.0 g), and benzoyl peroxide (100 mg) was added slowly dropwise under vigorous stirring (900 rpm). The resulting suspension was heated at 90 °C for 18 h with constant stirring. The reaction was cooled to ambient temperature then added to ~400 mL of ^{i}PrOH with stirring. The precipitate was filtered, washed several times with ^{i}PrOH (3 x 50 mL), and dried under reduced pressure to obtain 7.5 g of a white solid.

Suspension polymerization of styrene/VPSF (70:30) and 10 wt% DVB. A 250 mL round bottomed flask was charged with 100 mg PVA and 70 mL H2O. The mixture was heated at 65 °C to dissolve the PVA. The resulting clear, colorless solution was cooled to ambient temperature whereupon a solution of styrene (6.3 g), SuFEx monomer (2.7 g), 10 wt% DVB (1 g), and benzoyl peroxide (100 mg) was added dropwise under vigorous stirring (900 rpm). The resulting suspension was heated at 90 °C for 18 h with constant stirring. The reaction was cooled to ambient temperature then added to ~400 mL of ^{i}PrOH with stirring. The precipitate was filtered, washed several times with ^{i}PrOH (3 x 50 mL), and dried under reduced pressure to obtain 2.3 g of a white solid.

Suspension polymerization of VPSF and 10 wt% DVB. A 250 mL round bottomed flask was charged with 70 mg PVA and 50 mL H2O. The mixture was heated at 65 °C to dissolve the PVA. After cooling to ambient temperature, a solution of SuFEx monomer (6.3 g), 10 wt% DVB (0.7 g), and benzoyl peroxide (70 mg) was added slowly dropwise under vigorous stirring (800 rpm). The resulting suspension was heated at 90 °C for 45 h with constant stirring. The reaction was cooled to ambient temperature then added to ~350 mL of ^{i}PrOH with stirring. The precipitate was filtered, washed several times with ^{i}PrOH (4 x 50 mL), and dried to obtain 6.54 g of a white solid.

Deprotection of DVB-crosslinked styrene/VPSF beads. *KOH hydrolysis*: Fluorosulfonated polymer beads (100 mg) were suspended in 5 mL of aqueous 2M KOH in a scintillation vial equipped with a magnetic stir bar, and the reaction was stirred vigorously at 85 °C for 2 d. The mixture was treated with several drops of 2M HCl aqueous solution, and then centrifuged to obtain white powders, which were further washed with water and ethanol. Products were subsequently dried in a vacuum oven at 80 °C. *TBD-catalyzed hydrolysis*: Fluorosulfonated polymer beads (100 mg) were dispersed in 5 mL of anhydrous acetonitrile, and then TBS-benzyl alcohol (10 eq to VPSF in beads) and TBD catalyst (20 mol% with respect to TBS alcohol) were added to the dispersion. The mixture was stirred at 70 °C overnight, followed by centrifuge and washed with ethanol. Products were subsequently dried in a vacuum oven.

Synthesis of TBS-Phenol. Phenol (2.00 g, 21.25 mmol), *tert*-butyldimethylchlorosilane (3.36, 22.31 mmol), and imidazole (3.18 g, 46.75 mmol) were dissolved in 25 mL of anhydrous dichloromethane (DCM), and the reaction was maintained overnight at ambient temperature. The mixture was extracted with water twice and brine once and dried with $MgSO_4$. The solvent was removed under reduced pressure. ^{1}H NMR (300MHZ, $CDCl_3$) δ: 7.23 (m, 2H), 6.98 (t, 1H), 6.88 (d, 2H), 1.01 (s, 9H), 0.22 (s, 6H). ^{13}C NMR δ: 155.63, 129.35, 121.25, 120.11, 25.69, 18.20, -4.42.

Methylene Blue dye staining. Methylene blue (10.0 mg) was dissolved in 10 mL deionized water, which was then added to protected and deprotected beads and sonicated for 1 min. The beads were separated from methylene blue solution through vacuum filtration, and then thoroughly washed with water and methanol.

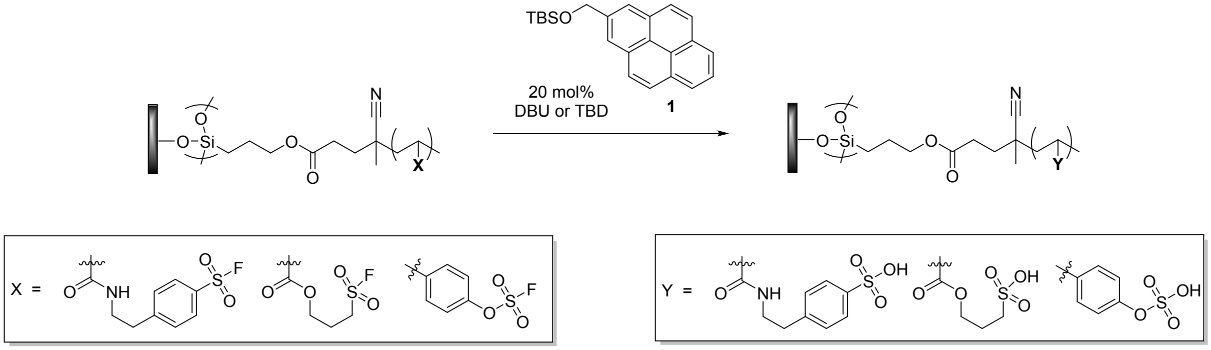
Ion exchange capacity (IEC) determination. IEC was determined by standard titration method.[2] The beads **10a-c** were loaded on the column and saturated NaCl solution was passed through, followed by deionized water. The effluent was titrated to neutral with 0.1 M NaOH.

TBS-Phenol and fluorosulfonated polymer beads click reaction. SuFEx beads with different compositions (50 mg) were suspended in 5 mL of anhydrous dimethylformamide (DMF). TBS-Phenol (10 equiv) and 20 mol% diazabicycloundec-7-ene (DBU) were then added to the solution, and the reaction was maintained at 60 °C for 24 h. Several drops of 2M HCl aqueous solution was added to the mixture, and then centrifuged to obtain white product. After washing with ethanol twice, the powders were dried in vacuum oven overnight.

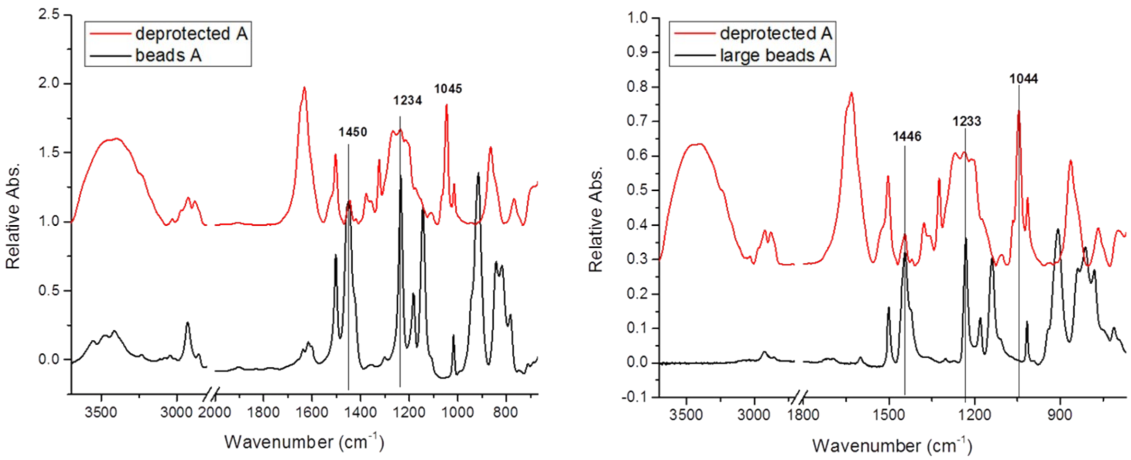
Characterization. Infrared spectra were taken using a Thermo-Nicolet model 6700 spectrometer equipped with a variable angle grazing angle attenuated total reflection (GATR-ATR) accessory (Harrick Scientific) at 64 scans with 4 cm^{-1} resolution. Beads morphology images were taken on a FEI Inspec F FEG scanning electron microscope at 20 kV. Measurements of beads size and zeta potential were performed using a Zetasizer Nano Series (Malvern) with dynamic light scattering (DLS). Elemental analysis for C, H, S, and F was performed on 20 mg of original (**7a-c**) and KOH or TBD/TBS-benzyl alcohol treated beads (**10a-c**) by Atlantic Microlab, Inc. (Norcross, GA). C, H and S were analyzed by combustion using automatic analyzers, while F was analyzed by flask combustion followed by ion chromatography.

Immobilization of Lipase/GFP. Immobilization was performed using 33 mg of protein per gram of wet support. A suspension of 70:30 sty/VPSF (**10 wt% DVB**) (24 mg) in 4 mL of PBS buffer was treated with 40 μL of lipase (20 mg/mL) or 20 μL of GFP (40 mg/mL). The suspension was gently mixed with a Barnstead/Thermolyne LABQUAKE shaker for 24 hours at ambient temperature. After immobilization the suspension was filtered and the supported lipase was washed several times with distilled water.

Enzyme activity was determined by measuring the increase in absorbance at 405 nm produced by the released *p*-nitrophenol in the hydrolysis of *p*-nitrophenyl palmitate (p-NPP). *p*-NPP stock solution (1 mM) was prepared, first *p*-NPP was dissolved in 3 mL isopropanol and 50 mL of 0.01 M potassium phosphate buffer (pH 7.4) (3% Triton X-100) was added. A volume of 500 μL of buffered *p*-NPP solution was added to 50 μL protein/beads solution (0.002 mg/mL enzyme), and 450 μL of 0.01 M potassium phosphate buffer was added. The mixture was incubated for 20 min at 37 °C. The samples were filtered and the *p*-nitrophenol (*p*-NP) release was monitored at 405 nm in 96-well Nunc clear polystyrene plate on a BioTek Cytation 3 plate reader. A blank sample was always used containing distilled water instead of enzyme solution.

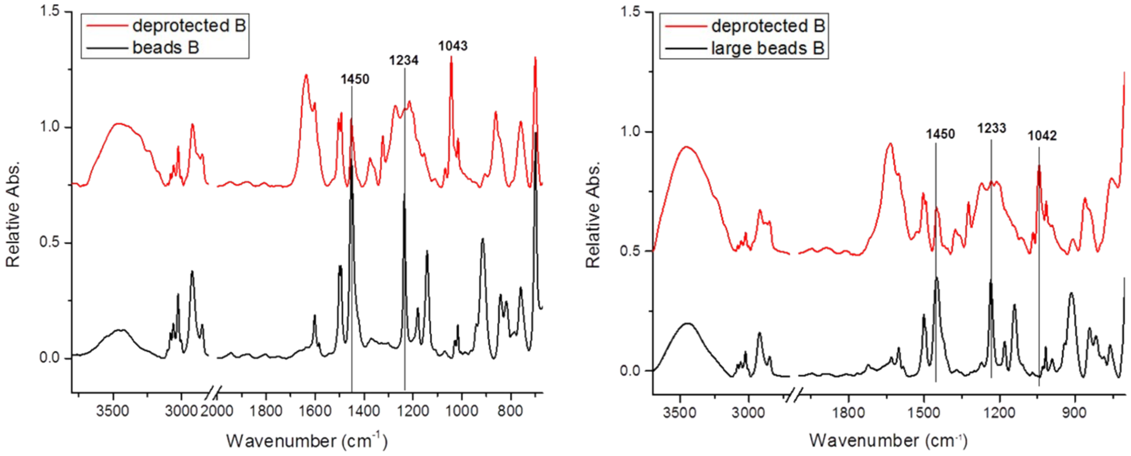


Scheme S1. Unexpected SuFEx-based hydrolysis of sulfonyl fluorides and fluorosulfonates with benzyl silyl ethers



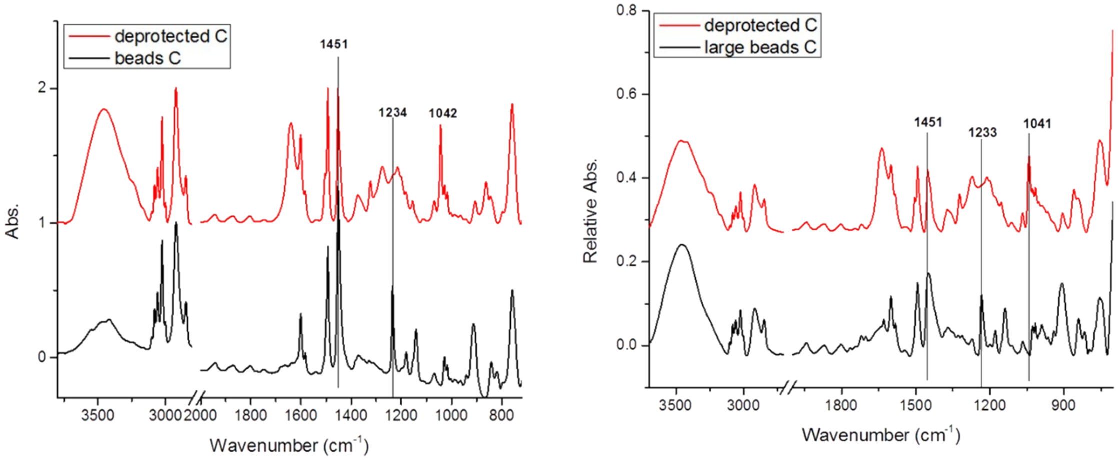
A **B**

Figure S1. IR analysis of TBD-catalyzed hydrolysis reactions. (A) Small fluorosulfonated polymer beads **6a** (B) Large fluorosulfonated polymer beads **7a**.



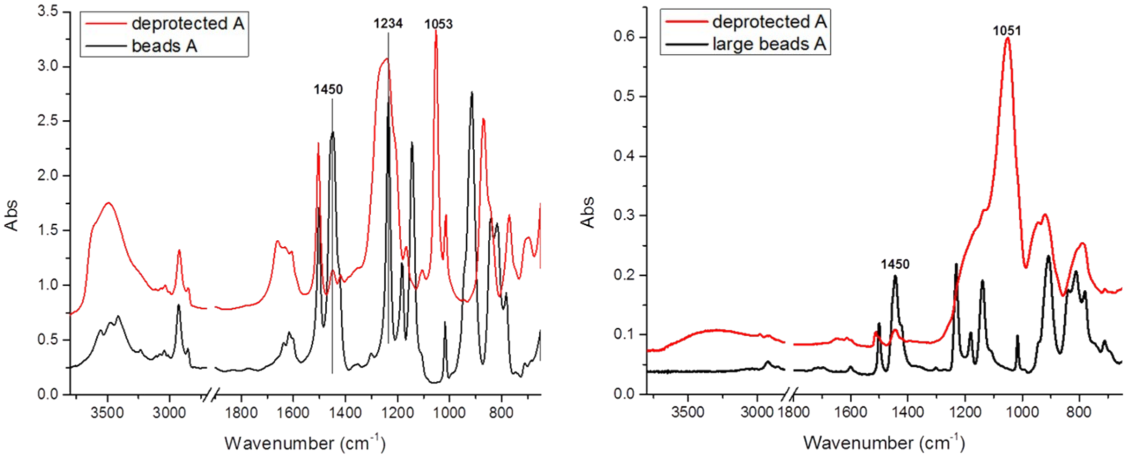
A **B**

Figure S2. IR analysis of TBD-catalyzed hydrolysis reactions. (A) Small fluorosulfonated polymer beads **6b** (B) Large fluorosulfonated polymer beads **7b**.



A B

Figure S3. IR analysis of TBD-catalyzed hydrolysis reactions. (A) Small fluorosulfonated polymer beads **6c** (B) Large fluorosulfonated polymer beads **7c**.



A B

Figure S4. IR analysis of KOH-mediated hydrolysis reactions. (A) Small fluorosulfonated polymer beads **6a** and (B) Large fluorosulfonated polymer beads **7a**.

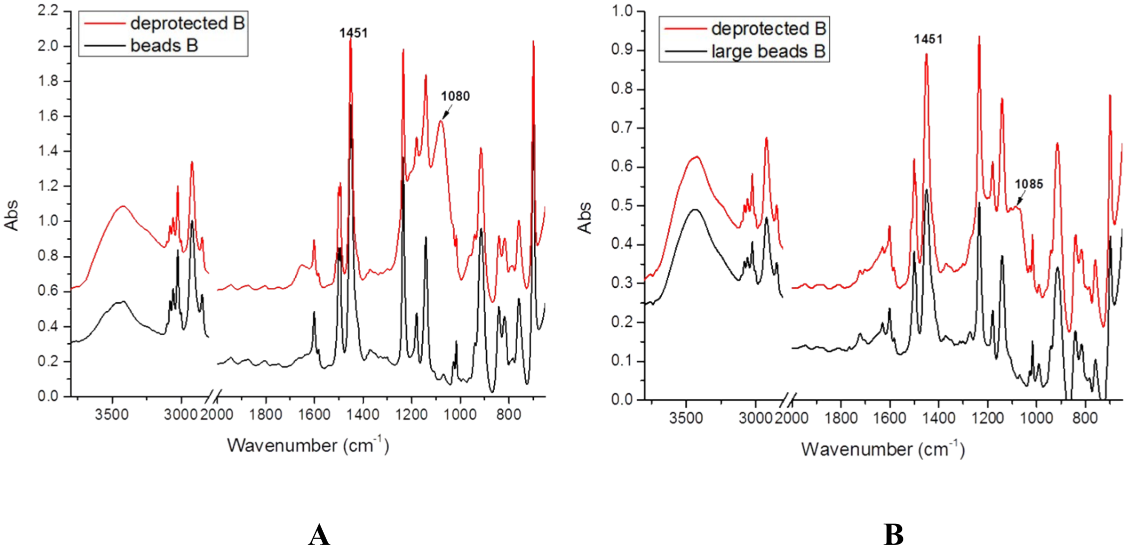


Figure S5. IR analysis of KOH hydrolysis reactions. (A) Small fluorosulfonated polymer beads **6b** (B) Large fluorosulfonated polymer beads **7b**.

Table S1. Zeta potential and DLS data for fluorosulfonated polymers **6a-c** and hydrolyzed products **9a-c**

Entry	Polymer	Original diameter (nm)	Hydrolyzed diameter (nm)	Zeta potential (mV)
a	**6a**	120	--	-30
b	**6b**	67	--	-30
c	**6c**	80	--	-30
d	**9a**	--	153	-38
e	**9b**	--	111	-33
f	**9c**	--	118	-28

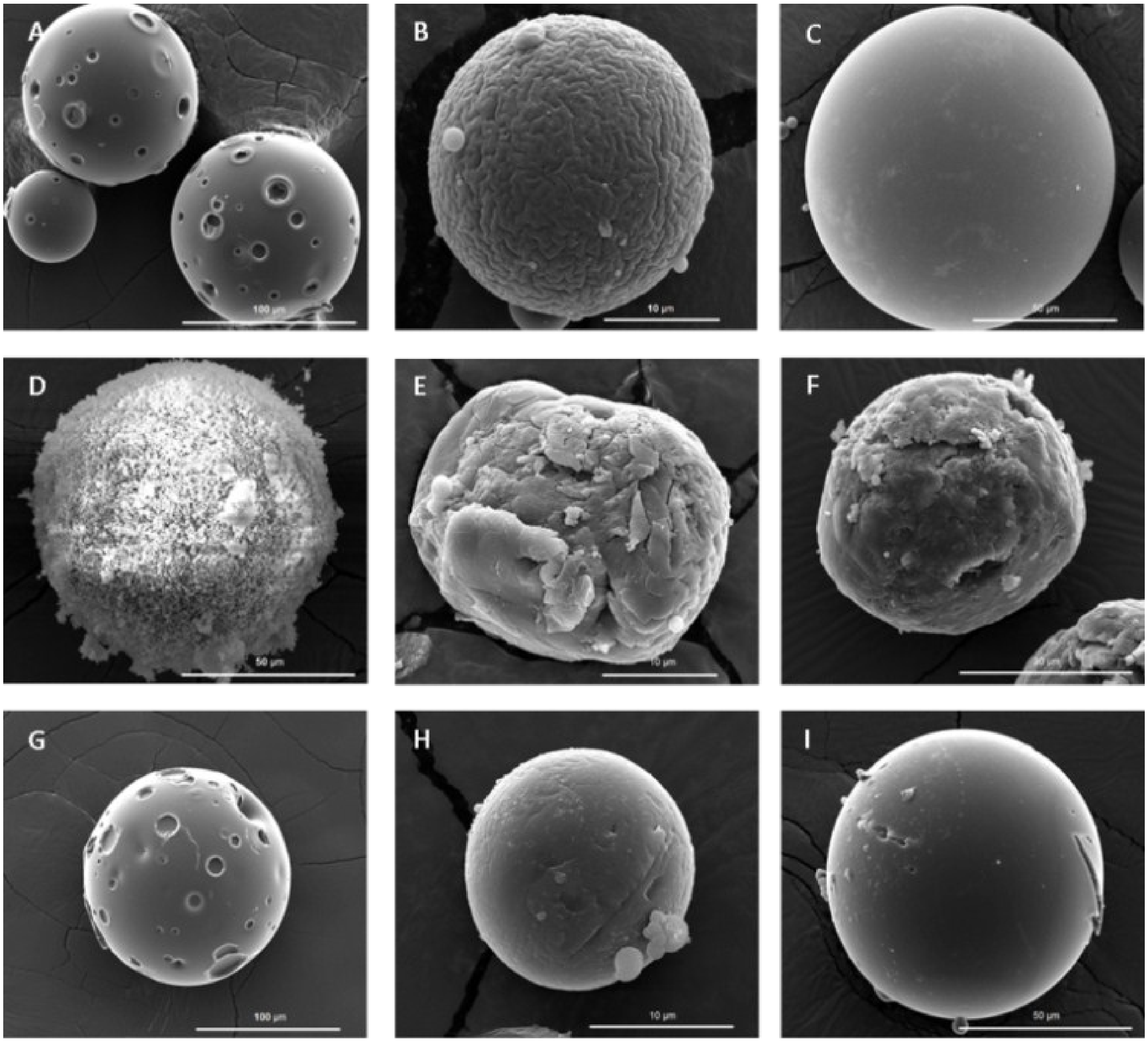


Fig. S6 Scanning electron micrographs of original and deprotected large fluorosulfonated beads. (A-C) Large fluorosulfonated beads **7a-c** before deprotection, (D-F) deprotected beads from aqueous base hydrolysis, and (G-I) deprotected beads via TBD-catalyzed hydrolysis.

Table S2: Elemental analysis of large fluorosulfonated beads **7a-c** and corresponding hydrolyzed resins **10a-c**

	C%	H%	S%	F%	Conversion[b]
7a	52.3	4.1	14.1	8.1	
7b	73.2	6.1	5.9	3.5	
7c	86.4	7.1	1.7	1.1	
			2 M KOH at 85 °C		
10a	52.6	4.1	13.9	7.7	5%
10b	74.1	6.2	5.8	3.2	9%
10c	86.7	7.3	1.7	1.0	9%
			TBS-benzyl alcohol/triazabicyclodecene (TBD) at 70 °C		
10a	55.2	5.8	9.7	1.5	81%
10b	73.9	6.9	3.7	Trace[a]	~100%[c]
10c	85.0	7.6	1.5	Trace[a]	~100%[c]

[a]Trace: < 0.25%; [b]Conversion = [F%(original) – F%(deprotected)]/ F%(original); [c]Full conversion within limit of detection (less than 0.25% F).

Table S3: Ion exchange capacities (IECs) for hydrolyzed beads **10a-c**

	IEC (mequiv g^{-1})		Composition (% wt of VPSF)	
	Calculated	*Measured*	*Theoretical*	*Experimental*[a]
10a	3.5	2.7	90	89
10b	1.7	1.2	27	39
10c	0.53	0.45	9	11

[a]Calculated from elemental analysis results

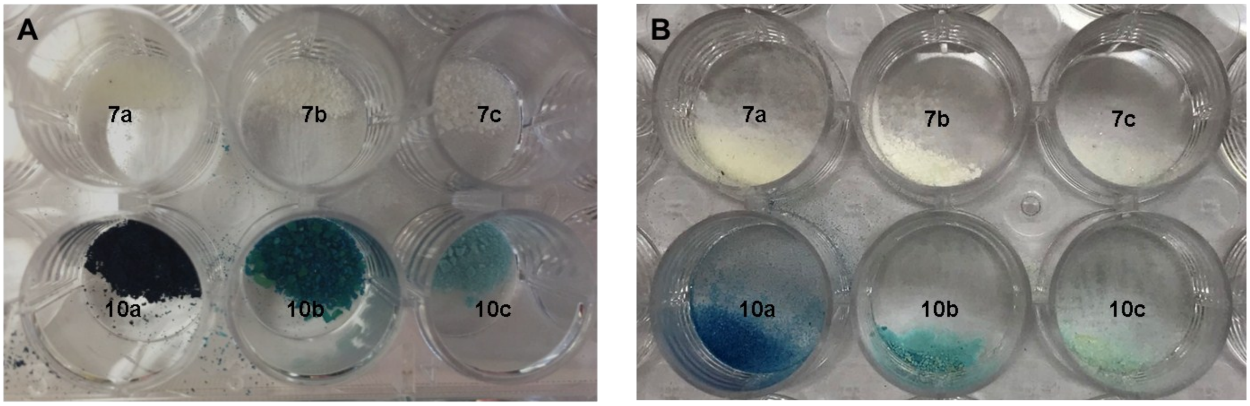
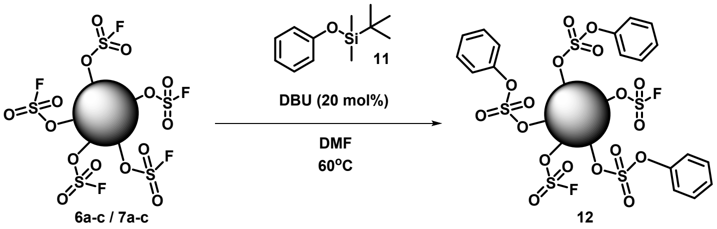
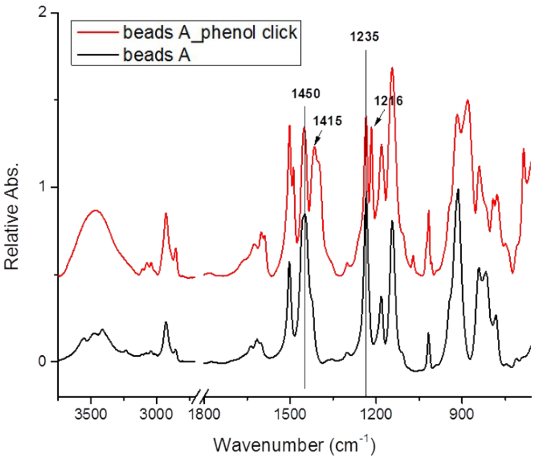
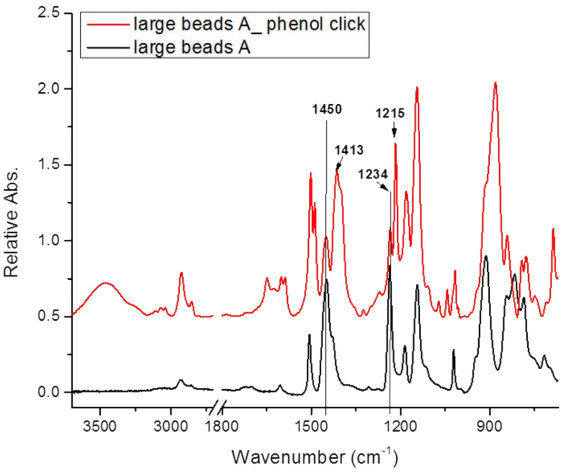


Figure S7. Methylene blue capture experiment. (A) Parent and hydrolyzed DVB-crosslinked polymer beads obtained via TBD-catalyzed hydrolysis following aqueous methylene blue treatment. (B) Parent and hydrolyzed DVB-crosslinked polymer beads obtained via aqueous basic hydrolysis following aqueous methylene blue treatment.



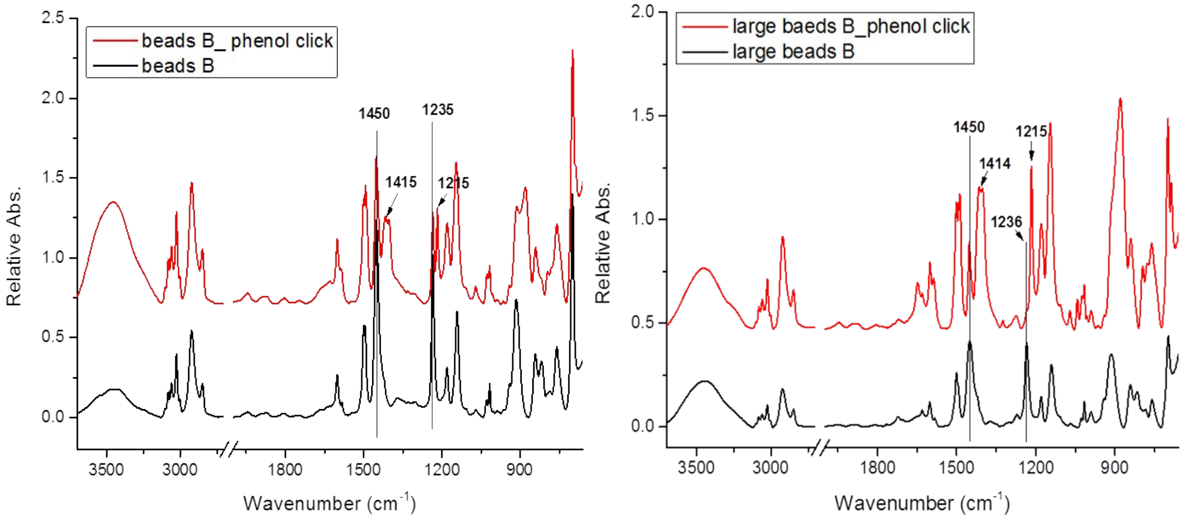
Scheme S2. TBS-phenol SuFEx click on fluorosulfonated polymer beads


beads A_phenol click
beads A
1235
1450
1415
1216
Relative Abs.
2
1
0
3500
3000
1800
1500
1200
900
Wavenumber (cm^{-1})


large beads A_ phenol click
large beads A
1450
1215
1413
1234
Relative Abs.
2.5
2.0
1.5
1.0
0.5
0.0
3500
3000
1800
1500
1200
900
Wavenumber (cm^{-1})

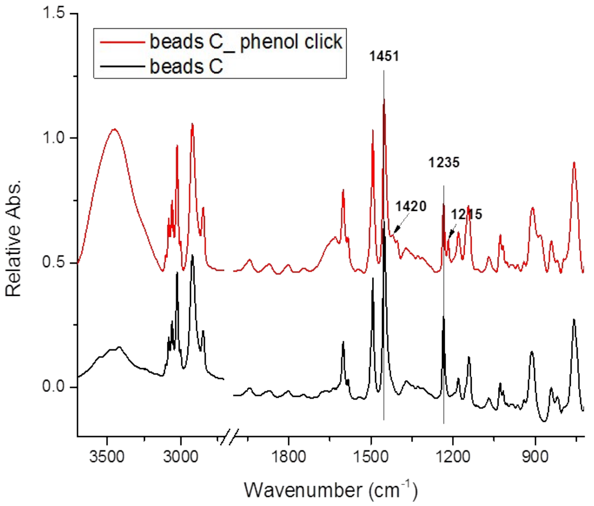
A **B**

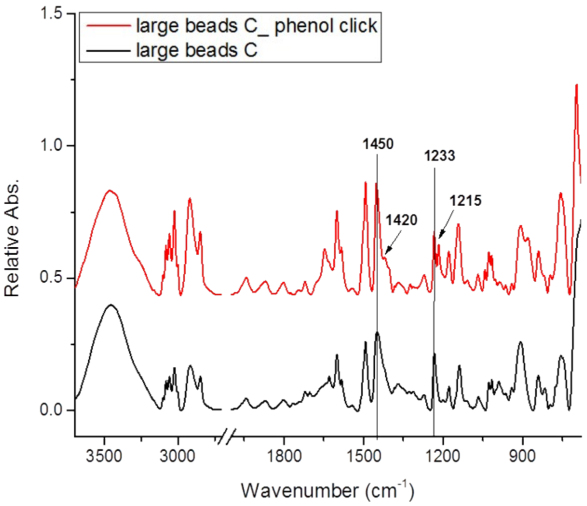
Figure S8. IR analysis of TBS-phenol click reactions. (A) Small fluorosulfonated polymer beads **6a** (B) Large fluorosulfonated polymer beads **7a**.



A **B**

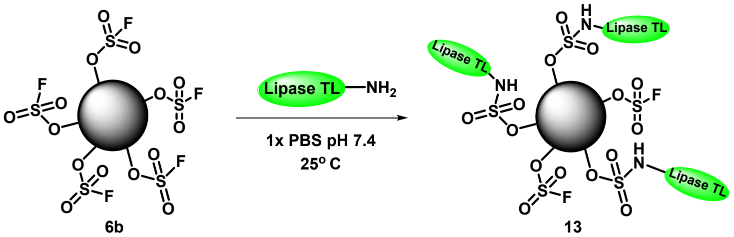
Figure S9. IR analysis of TBS-phenol click reactions. (A) Small fluorosulfonated polymer beads **6b** (B) Large fluorosulfonated polymer beads **7b**.


beads C_ phenol click
beads C
1451
1235
1420
1215
Relative Abs.
1.5
1.0
0.5
0.0
3500
3000
1800
1500
1200
900
Wavenumber (cm^{-1})


large beads C_ phenol click
large beads C
1450
1233
1420
1215
Relative Abs.
1.5
1.0
0.5
0.0
3500
3000
1800
1500
1200
900
Wavenumber (cm^{-1})

A **B**

Figure S10. IR analysis of TBS-phenol click reactions. (A) Small fluorosulfonated polymerbeads **6c** (B) Large fluorosulfonated polymer beads **7c**.



Scheme S3. Immobilization of Lipase TL on fluorosulfonated polymer beads

A **B**

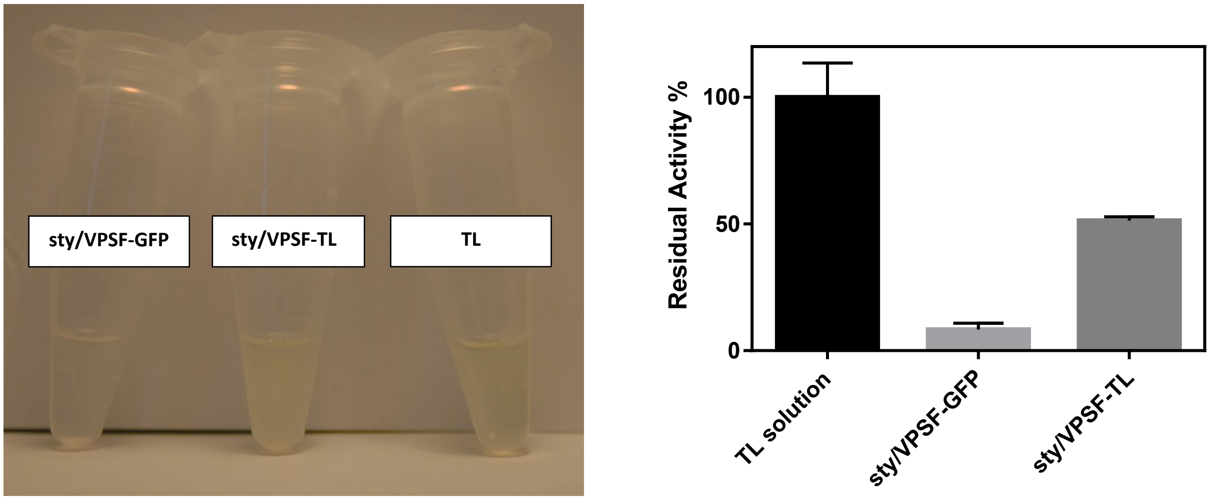


Figure S11. Residual lipolytic activity of immobilized lipase TL (sty/VPSF-TL) compared to lipase TL in solution and immobilized GFP as a control. (A) Reaction mixture; (B) Residual activity.

References

1.K. Brooks, J. Yatvin, M. Kovaliov, G. H. Crane, J. Horn, S. Averick and J. Locklin, *Macromolecules*, 2018, **51**, 297-305.
2.K. W. Pepper, *J. Appl. Chem.*, 1951, **1**, 124-132.