Supporting Information for

The *para*-Fluoro–Thiol Ligation in Water

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Experimental Section

Materials

N,*N*-Dimethylacrylamide (DMAAm; 99%, Acros), and 2,3,4,5,6-pentafluorostyrene (PFS; 98%, ABCR) were passed through a basic alumina (Roth) column to remove the inhibitor prior to use. 1,8-Diazabicyclo[5.4.0]undec-7-ene (DBU; > 98%, Merck), mercaptoethanol (99%, Roth), 4-benzoyl benzylamine hydrochloride (ABCR), Tween 20 (VWR, molecular biology grade), ethanol (99.8%, Acros), sodium hydrochloride (NaOH; ≥ 99%, Roth), hydrochloric acid (HCl; 37%, Roth), disodium hydrogen phosphate (Na_2HPO_4; ≥ 99%, Roth), and glycine (≥ 99%, Roth) were used as received. Toluene (VWR) and methanol (VWR) were used as received. Tissue culture petri dishes (100% USP VI crystal virgin polystyrene, Ø 35 mm, ref. no. 734-2317) and pH standards for calibration were purchased from VWR and Roth, respectively.

N-*tert*-Butyl-*N*-[1-diethylphosphono-(2,2-dimethylpropyl)] nitroxide (SG1)[1] and 2-methyl-2-[*N*-*tert*-butyl-*N*-(1-diethoxyphosphoryl-2,2-dimethylpropyl)aminoxy]propionic acid (MAMA-SG1)[2] were synthesized according to the literature.

All reagents and chemicals required for peptide synthesis were purchased either from Novabiochem or from Iris Biotech and were used without prior purification. Solvents for peptide synthesis and HPLC purification were obtained from either from Biosolve B.V. or from Actu-All Chemicals B.V.

Characterization

NMR spectroscopy NMR spectroscopy measurements were performed on a Bruker AM 500 spectrometer at 500 MHz. The analytes were dissolved in $CDCl_3$ and the residual solvent signals were employed for shift correction. On ^{19}F CPD NMR spectra, at least one of the following was made: Baseline correction with the method Bernstein polynomial fit (with polynomial order of 14), manual correction, multipoint baseline correction.

Size-exclusion chromatography (SEC) Size-exclusion measurements were performed on a Polymer Laboratories/Varian PLGPC 50 Plus system comprising a Polymer Laboratories 5.0 mm bead-size guard column (50 × 7.5 mm^2), followed by three PL columns and a differential refractive index detector. The eluent was *N*,*N*'-dimethylacetamide (DMAc) at 50 °C with a flow rate of 1 mL min^{-1}. The SEC system was calibrated using linear poly(styrene) (PS) standards ranging from 160 to 6 × 10^6 g mol^{-1} and linear poly(methyl methacrylate) standards ranging from 700 to 2 × 10^6 g mol^{-1}. The resulting molecular weight distributions were determined by universal calibration using Mark–Houwink parameters for PMMA, that is K = 129.8 x 10^{-3} mL g^{-1}, α = 0.688.

pH-metry Measurements of the pH were conducted using a Mettler Toledo SevenCompact™ pH/Ion S220 pH-meter, calibrated using standard solutions of pH 2.0, pH 4.0, pH 7.0, pH 9.0, and pH 12.0.

Liquid chromatography coupled to electrospray ionization mass spectrometry (LC-ESI-MS) The identity of the Cys-PGLa was established by using analytical LC (1100 series, Agilent Technologies) connected to ESI-mass spectrometer (QTOF, Bruker Daltonics). The presence of a single peak with the expected mass confirmed that the purified peptides were over 95% pure.

Time-of-flight secondary-ion mass spectrometry (ToF-SIMS) ToF-SIMS was performed on a TOF.SIMS[5] instrument (ION-TOF GmbH, Münster, Germany). This spectrometer is equipped with a bismuth cluster primary ion source and a reflectron type time-of-flight analyzer. UHV base pressure was < 5×10^{-9} mbar. For high mass resolution, the Bi source was operated in the "high current bunched" mode providing short Bi_3^+ primary ion pulses at 25 keV energy and a lateral resolution of approx. 4 µm. The short pulse length of 1.1 to 1.3 ns allowed for high mass resolution. The primary ion beam was rastered across a 500 × 500 μm^2 field of view on the sample, and 128 × 128 data points were recorded. Primary ion doses were kept at 10^{11} ions/cm^2 (static SIMS limit) for all measurements. Due to the highly insulating nature of the use polystyrene substrates charge compensation during spectrometry was necessary. Therefore, an electron flood gun providing electrons of 21 eV was applied, and the secondary ion reflectron tuned accordingly. Spectra were calibrated on the omnipresent C^-, CH^-, CH_2^-, C_2^-, C_3^-; or on the C^+, CH^+, CH_2^+, and CH_3^+ peaks. Based on these datasets the chemical assignments for characteristic fragments were determined.

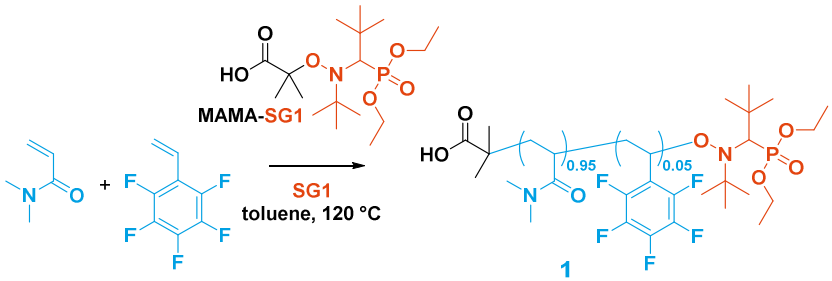
Preparation of the buffered solutions

The buffer solutions employed for the PFTR experiments were prepared by mixing the following components.

pH 11: 0.1 M NaOH (5.5 mL) + 0.05 M Na_2HPO_4 (100 mL); exact pH value = 10.98.
pH 12: 0.1 M NaOH (53.8 mL) + 0.05 M Na_2HPO_4 (100 mL); exact pH value = 11.99.
pH 12.5: 0.1 M NaOH (100 mL) + a solution of glycine (7.52 g L^{-1}) and NaCl (5.86 g L^{-1}) (60 mL); exact pH value = 12.50.
pH 13: 0.4 M NaOH (90 mL) + 140 mL of a solution of glycine (7.52 g L^{-1}) and NaCl (5.86 g L^{-1}) (140 mL); exact pH value = 13.04.

Syntheses

Synthesis of Poly(DMAAm-stat-PFS) ***1***



DMAAm (5.00 g, 50.4 mmol), PFS (0.49 g, 2.5 mmol), MAMA-SG1 (150 mg, 0.39 mmol), free SG1 (17.3 µL, 0.039 mmol), 1,3,5-trioxane (0.4 g, 4.4 mmol), and toluene (5.8 mL) were placed in a 25-mL round-bottom flask, which was sealed with a rubber septum. A t_0 sample was taken and the mixture was deoxygenated by purging with nitrogen for 45 minutes. The flask was then placed in a pre-heated oil bath at 120 °C and stirred for 5 hours. After taking a t_{end} sample, toluene was evaporated and a ^{1}H NMR spectrum was measured for conversion calculation (x_{DMAAm} = 0.83; x_{PFS} = 0.95) and indirect calculation of the PFS content (F_{PFS} = 0.054; see Figure S1). The residue was redissolved in THF and precipitated twice in cold diethyl ether to yield **1** as a white powder which was finally dried in vacuum. SEC (DMAc): M_n = 9700 g mol^{-1}; $Đ$ = 1.35 (see Figure 3 in the main text).

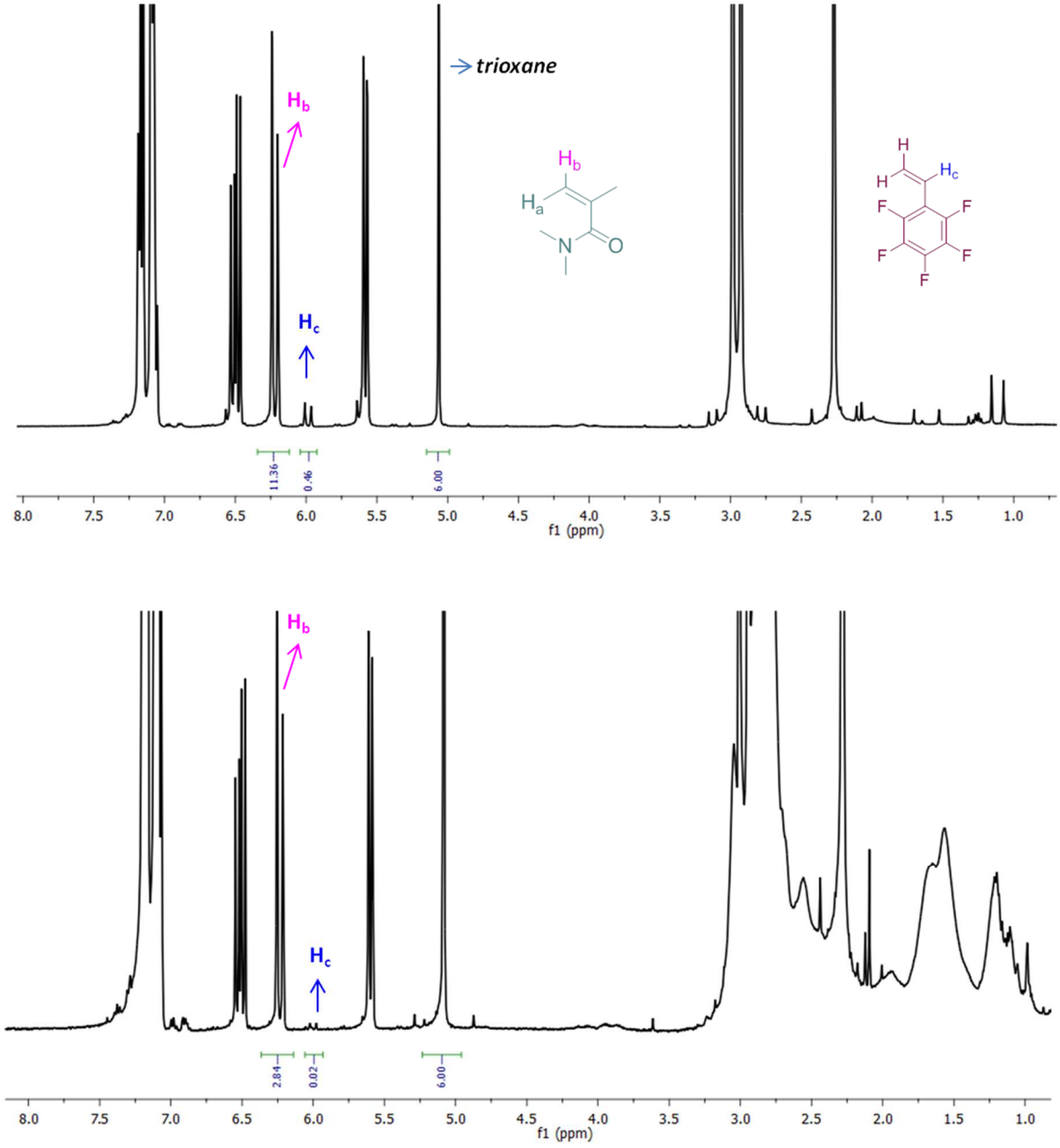


Figure S1. ^{1}H NMR spectra of the t_0 sample (top) and t_{end} sample (bottom) of the synthesis of **1**. The PFS content in **1** was calculated indirectly – due to the absence of non-overlapping characteristic proton for PFS units – by taking into account the original feed and the independent monomer consumption.

Exemplary PFTR on Poly(DMAAm-stat-PFS) with mercaptoethanol in a buffered pH 12 aqueous solution

It must be noted that for the establishment of each complete kinetic curve, reactions were carried out in 2 to 3 independent flasks for practical handling, especially with respect to sampling times.

1 (68 mg, 1 eq. PFS units) was dissolved in the pH 12 buffer (3.6 mL) in a 5-mL round-bottom flask and stirred for 15 minutes at room temperature until complete dissolution. Afterwards, 2-mercaptoethanol (25.9 µL, 0.368 mmol, 10 eq. with respect to PFS units) was added and the flask was sealed with a rubber septum. The flask was placed in an ice bath and the mixture was deoxygenated by purging with nitrogen for 30 minutes. The flask was then placed in a pre-heated oil

bath at 40 °C. Samples were withdrawn regularly using deoxygenated syringes (1 mL per sample). The reaction was quenched by adding 0.2 mL of pH 1 solution per 1 mL sample taken from the reaction flask. They were then dried prior to ^{19}F NMR measurements.
Further experiments were set in the same way with various pH solutions, temperatures, and mercaptoethanol equivalents. Samples taken from pH 13 solutions (1 mL each) were quenched with 2 mL pH 1 and those taken from pH 11 solutions were quenched with 0.02 mL of a HCl solution at pH 1.
An experiment at pH 13 and 40 °C was carried out in a bigger batch in order to allow purification of the final product by precipitation. For this purpose, after quenching, the solution was dried. The residue was dissolved in THF, filtered, and precipitated twice in cold diethyl ether to yield a white powder (M_n = 10 800 g mol^{-1}; $Đ$ = 1.36).

Control experiment with ethanol
1 (30 mg) was dissolved in pH 13 buffer (1.6 mL) in a 5-mL round-bottom flask and stirred for 15 minutes at room temperature until complete dissolution. Ethanol (9.5 µL, 0.16 mmol, 10 eq. with respect to PFS units) was then added and the flask was sealed with a rubber septum. The flask was placed in an ice bath and the mixture was deoxygenated by purging with nitrogen for 30 minutes. The flask placed in an oil bath at 40 °C for 4 days. Finally, the sample was dried prior to NMR analysis.

Control experiment with aminoethanol
1 (30 mg) was dissolved in pH 13 buffer (1.6 mL) in a 5-mL round-bottom flask and stirred for 15 minutes at room temperature until complete dissolution. Ethanolamine (9.9 µL, 0.16 mmol, 10 eq. with respect to PFS units) was then added and the flask was sealed with a rubber septum. The flask was placed in an ice bath and the mixture was deoxygenated by purging with nitrogen for 30 minutes. The flask placed in an oil bath at 40 °C for 1 day. Finally, the sample was dried prior to NMR analysis.

Synthesis of the Cys-PGLa peptide
Peptide synthesis was performed on an automated peptide synthesizer (Syro II, Multisyntech) using Fmoc/O^{t}Bu solid phase peptide synthesis protocols described previously.[3, 4] Cysteine-modified PGLa (Cys-PGLa: H_2N-C-GMASKAGAIAGKIAKVALKAL-$CONH_2$amide) was synthesized on a rink amide MBHA resin as described previously, yet with an additional Cys residue which was coupled at the *N*-terminus. Peptides were cleaved from the resin using TFA:TIS:H_2O (92:4:4 v/v/v) and were purified on a C18 semi-preparative HPLC column using water:acetonitrile gradients, each containing 5 mM HCl, as previously described.[4]

Modification of Petri dishes via photografting
4-Benzoyl benzylamine hydrochloride (24.7 mg, 0.01 moles) was mixed with **1** (100 mg) in water (1 mL) and the resulting solution was poured into a Petri dish. This preparation was then irradiated for 30 min with a Philips Cleo PL-L lamp (310–400 nm, max. 350 nm, 36 W) in a custom-built UV reactor. As a control experiment, the same preparation was made, at the exception that 4-benzoyl benzylamine hydrochloride was omitted. After the reaction, solutions were removed from the Petri dishes and the latter were rinsed with water. To minimize materials present due to unspecific adsorption, an excessive washing step was applied. Each Petri dish was placed in a separate beaker filled with water (150 mL) and placed on a benchtop shaker at 150 rpm. Water was changed three times in a total time of 24 hours. At the end of this time, the Petri dishes were dried with an air gun. The modified Petri dishes were cut into pieces (ca. 1 × 1 cm^2) for the next step.

Peptide grafting on modified Petri dishes

Cys-PGLa peptide (1 mg) was dissolved in pH 12 buffer (1 mL). 100 µL of this peptide solution was placed on a piece which was cut from the previously modified Petri dish. For the control experiment, the pH 12 buffer was replaced by deionized water. Both samples were left stationary at room temperature, covered with Al foil for 24 hours. Subsequently, both pieces were first rinsed with water for 10 seconds. Tween 20 (0.01 vol%) was used to wash the surfaces in order to remove unspecific adsorption. Each piece was taken into a 5 mL vial, filled with the surfactant solution, and sonicated for 5 minutes. This was repeated three times per sample, eventually followed by sonication with pure water and drying in a stream of air.

Note on preliminary investigations

As presented in one of our previous studies, 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) is a strong base used in the presence of aliphatic thiols to mediate PFTR and allows the reaction to proceed to full completion in a few minutes in DMF.[5] Hence, to obtain a reference sample, a DBU-catalyzed reaction was performed with **1** in DMF: 83% conversion was observed at RT after 24 h with only 1.1 eq. thiol and 1.1 eq. DBU, while full conversion was reached with 5 eq. thiol and 5 eq. DBU (Figure S2, A and B, respectively). In water, however, we observed no reaction in the same conditions. The ^{19}F NMR spectrum of **1** showed no change, even with 5 eq. DBU (Figure S2, C and D). This can be explained by immediate protonation of the base by water,[6] leading to the impossibility to generate the reactive thiolate species. Likewise, no reaction took place in methanol or ethanol (Figure S3). As the pK_a of thiols is typically not larger than 11,[7, 8] we envisaged that simply working at pH higher than this value would provide a means to produce thiolates, hence to trigger the reaction. The pH was initially adjusted by simple addition of NaOH. While reaction finally did occur, high yields could not be reached. At this point, it was found that the pH of the solution was not stable and steadily decreased. Consequently, buffered pH solutions were used for subsequent experiments. For a similar initial pH, much faster kinetics were observed with a buffered solution (Figure S4, Table S1). The rest of the study was therefore conducted with buffers set to a predetermined pH value. Importantly, PFTR is theoretically not an oxygen sensitive reaction and could thus be performed under ambient conditions. Nevertheless, since thiols are prone to oxidation and may lead to the formation of disulfide bonds over long time periods, we conducted all studies in deoxygenated media as our initial studies yielded lower conversions under ambient conditions (data not shown).

Additional NMR spectra and kinetics data

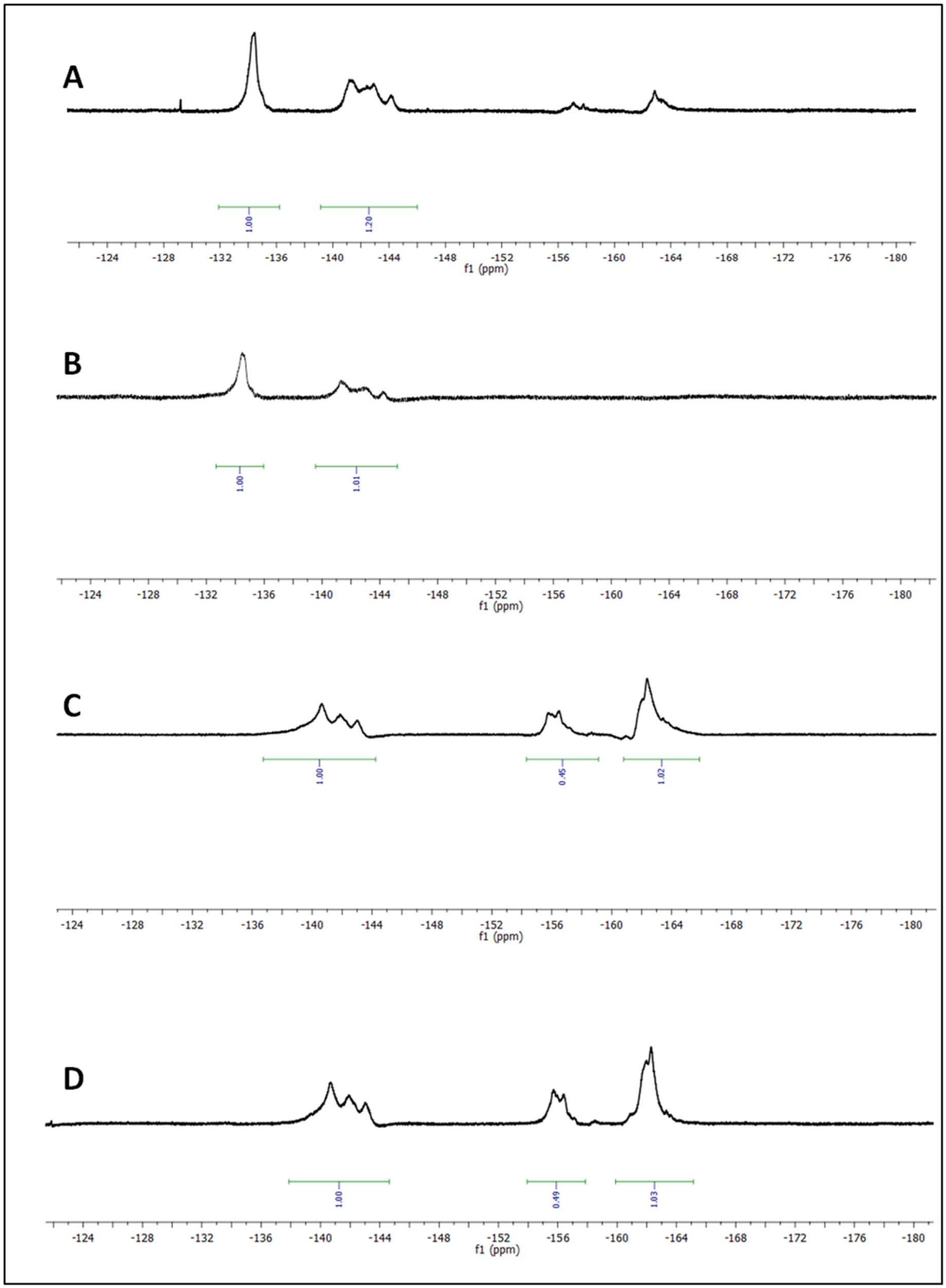


Figure S2. ^{19}F NMR spectra of **1** after 24 hours in various PFTR conditions: (A) in DMF with 1.1 eq. DBU and 1.1 eq. mercaptoethanol, RT, 83% conversion; (B) in DMF with 5 eq. DBU and 5 eq. mercaptoethanol, RT, 100% conversion; (C) in water with 1.1 eq. DBU and 1.1 eq. mercaptoethanol, RT, no conversion; (D) in water with 5 eq. DBU and 1.1 eq. mercaptoethanol, RT, no conversion.

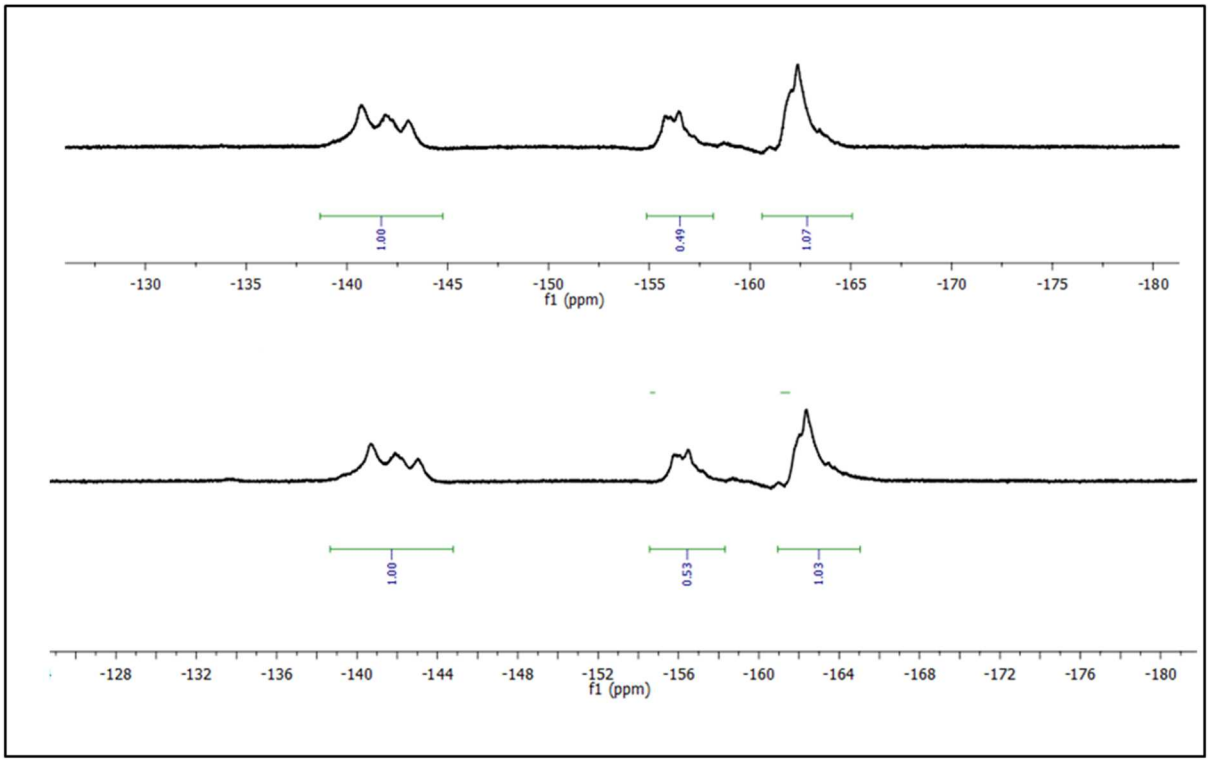


Figure S3. ^{19}F NMR spectra of **1** after PFTR conducted with 1.1 eq. DBU and 1.1 eq. mercaptoethanol in methanol (top) and in ethanol (bottom), both showing no conversion.

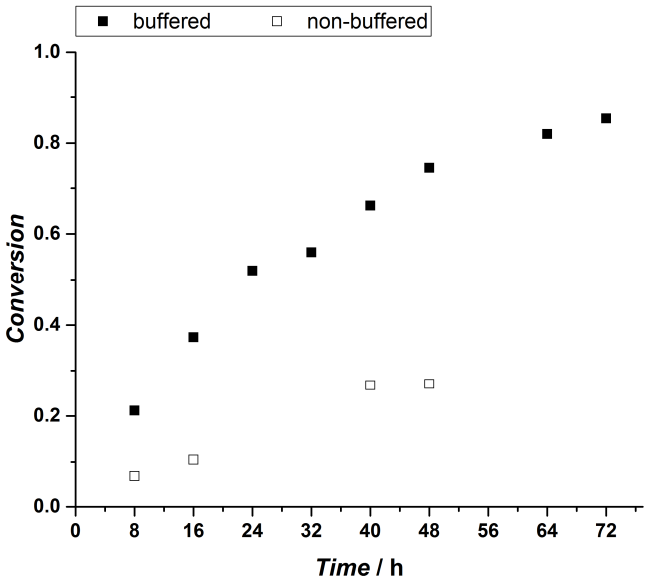


Figure S4. Conversion vs. time plot for the PFTR of **1** in buffered and non-buffered pH 12 solutions, both conducted at 40 ˚C with 10 eq. of mercaptoethanol.

Table S1. Detailed conversion values for the PFTR of **1** in buffered and non-buffered pH 12 solutions at 40 ˚C with 10 equivalents of mercaptoethanol.

Time / h	Conversion	
	Buffered	Non-buffered
8	0.21	0.07
16	0.37	0.10
24	0.52	
32	0.56	
40	0.66	0.26
48	0.75	0.26

Table S2. Detailed conversion values for the PFTR of **1** in buffered pH 12 solution at 40 ˚C with various equivalents of mercaptoethanol (Figure 1A).

Time / h	Conversion		
	5 eq.	10 eq.	20 eq.
8	0.08	0.21	0.35
16	0.17	0.37	0.45
24	0.24	0.52	0.61
32	0.24	0.56	0.66
48	0.36	0.75	0.79
72	0.40	0.85	0.89

Table S3. Detailed conversion values for the PFTR of **1** in buffered solution of various pH at 40 ˚C with 10 equivalents of mercaptoethanol (Figure 1B).

Time / h	Conversion			
	pH 11	pH 12	pH 12.5	pH 13
4				0.41
8		0.21	0.41	0.67
12				0.75
16		0.37	0.60	0.83
20				0.87
24	0.26	0.52	0.78	0.95
32		0.56	0.85	
40		0.66	0.89	
48	0.43	0.75	0.93	
64		0.82		
72	0.44	0.85		
96	0.49			
120				
144	0.54			
168	0.54			

Table S4. Detailed conversion values for the PFTR of **1** at different temperatures in buffered pH 13 solution with 10 equivalents of mercaptoethanol (Figure 1C).

Time / h	Conversion		
	RT	40 ˚C	50 ˚C
2			0.36
4		0.41	0.65
6			0.77
8		0.67	
12		0.75	
13			0.91
16		0.83	> 0.99
20		0.87	
24		0.95	
48	0.57		
72	0.64		
96	0.66		
120	0.68		

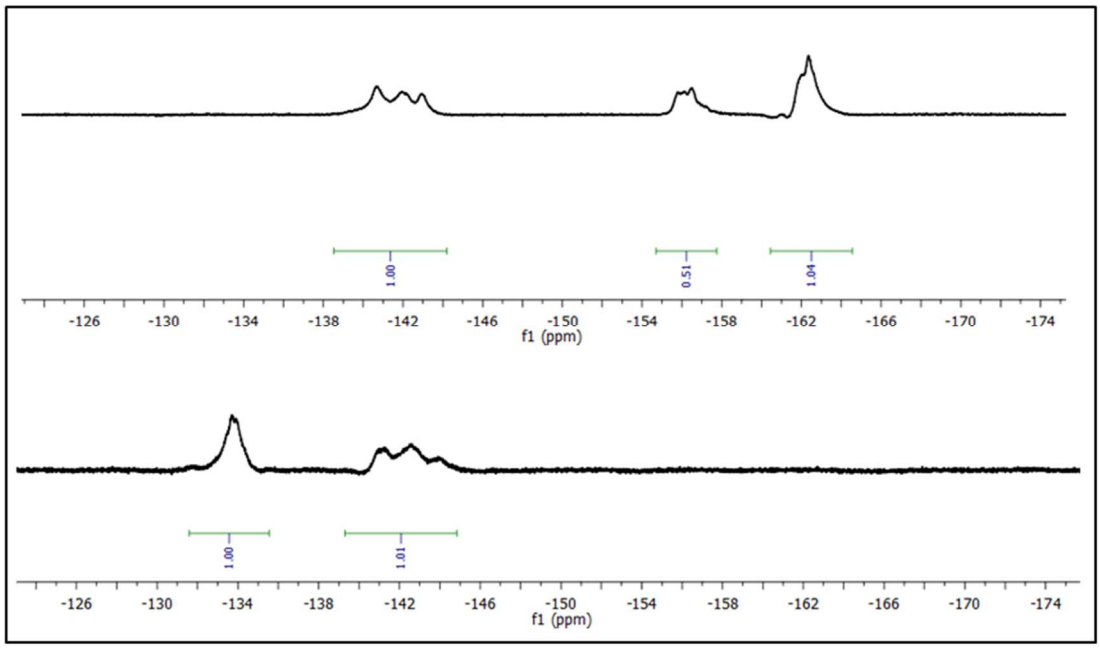


Figure S5. ^{19}F NMR spectra of (top) **1** and (bottom) **2** obtained by full PFTR on **1**, with peak integrations.

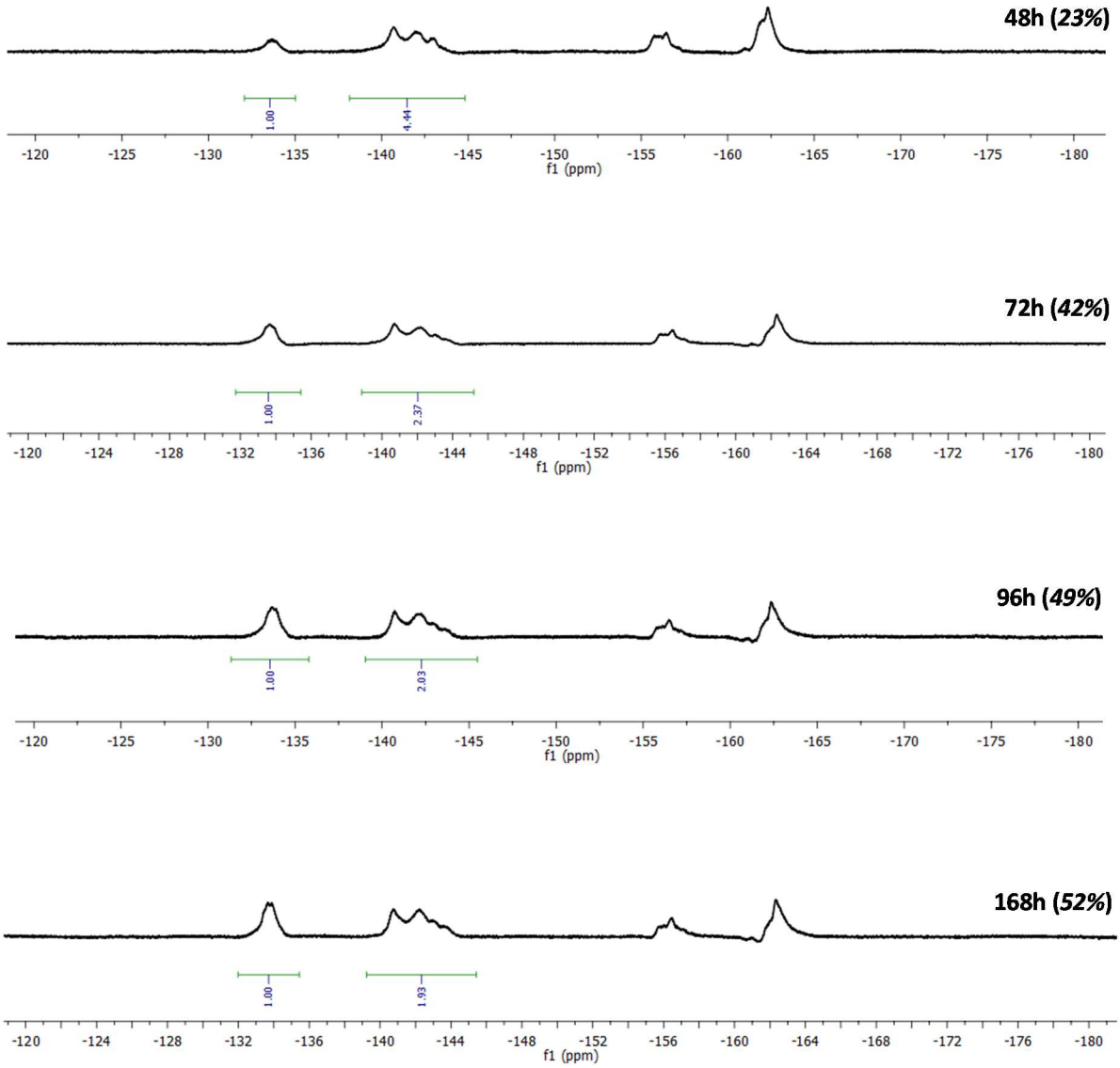


Figure S6. ^{19}F NMR spectra of samples withdrawn from PFTR conducted at pH 11, 40 ˚C, with 10 equivalents of mercaptoethanol with peak integrations showing 23% to 52% conversions.

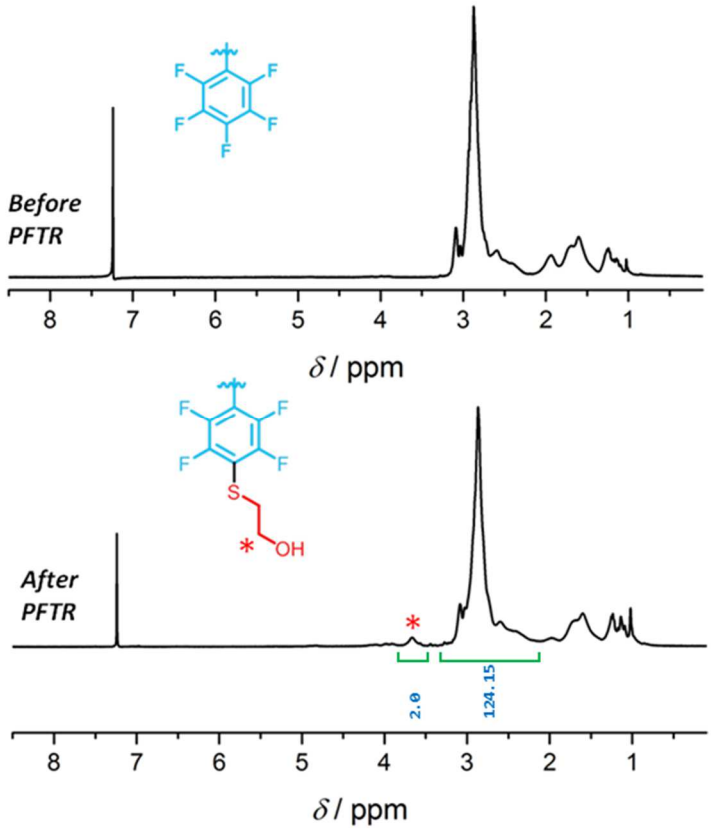


Figure S7. ^{1}H NMR spectra of **1** (top) and **2** (bottom).

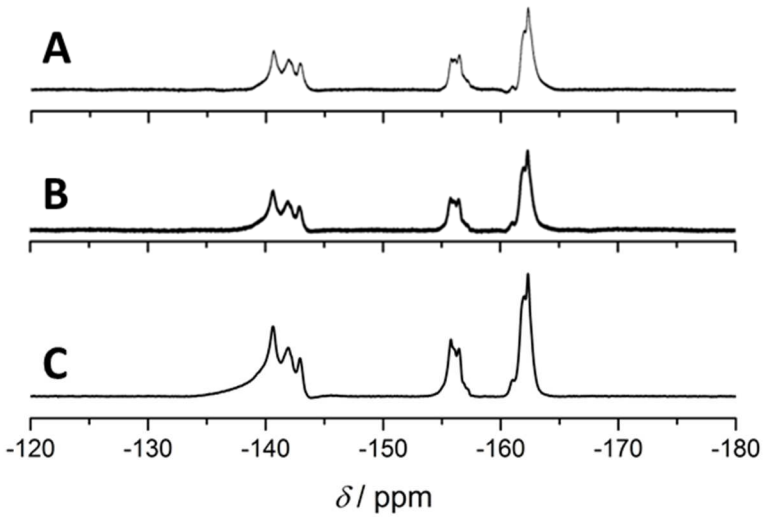


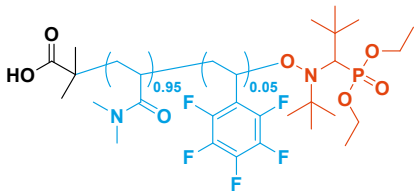
Figure S8. ^{19}F NMR spectra of (A) **1**, (B) **1** after incubation during 4 days with 10 eq. ethanol at pH 13 and 40 °C, and (C) **1** after incubation during one day with 10 eq. ethanolamine at pH 13 and 40 °C.

ToF-SIMS Analysis

Photografting Step

In the presence of a benzophenone derivative irradiated with UV light, radicals can be created at the surface of the TCPS Petri dish, as well as on the backbone of polymer **1**, by hydrogen abstraction. This results in a crosslinked layer of **1** immobilized at the Petri dish surface. The photografting step was assessed by ToF-SIMS. Ion fragments specific for the pentafluorophenyl moiety (Figure S9) and the phosphorus-containing polymer cap (Figure S10) can be detected. The control experiment carried out with simple incubation of **1** on top of a Petri dish in the absence of light revealed some unspecific adsorption, leading however to less material immobilized.

Chemical structure of **1**:



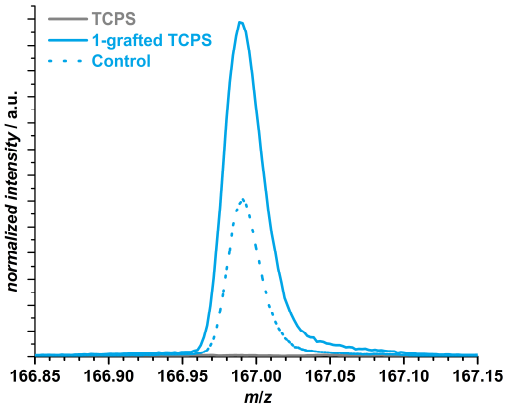


Figure S9. Pentafluorophenyl-specific region of the secondary ion mass spectra obtained for the original TCPS Petri dish (grey, solid), after photografting of **1** (blue, solid), and the control experiment of the photografting step (blue, dashed). Theoretical *m*/*z* value ($C_6F_5^-$) 166.99.

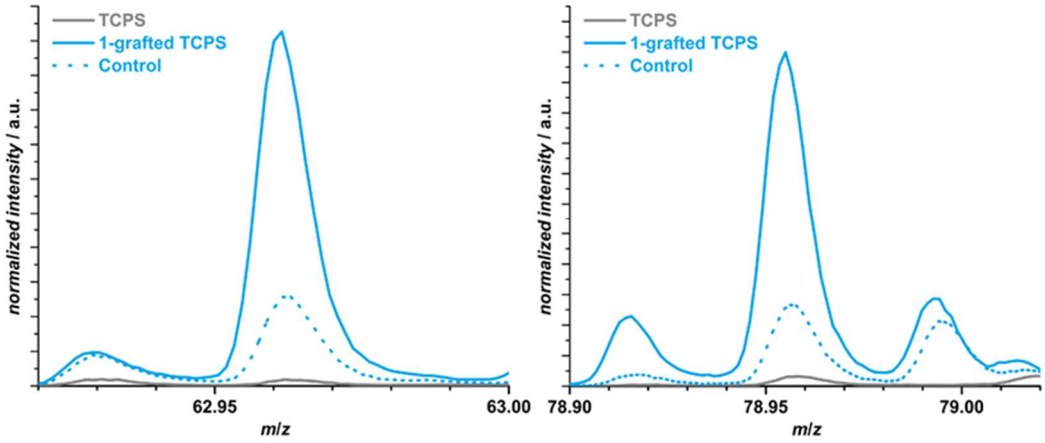


Figure S10. Phosphonate-specific regions (left, PO_2^-; right, PO_3^-) of the secondary ion mass spectra obtained for the original TCPS Petri dish (grey, solid), after photografting of **1** (blue, solid), and the control experiment of the photografting step (blue, dashed). Theoretical *m*/*z* values: (PO_2^-) 62.96; (PO_3^-) 78.96.

Peptide Functionalization

See description in the main text.

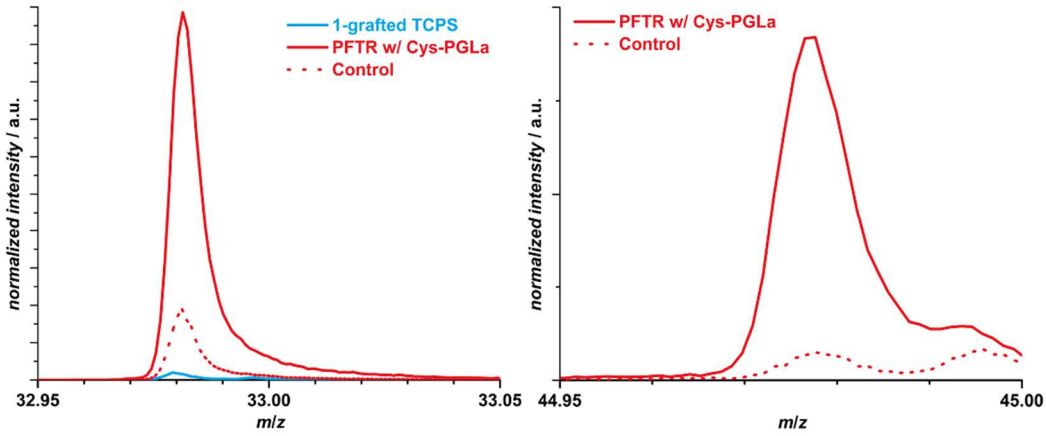


Figure S11. Cysteine-specific regions (left, HS^-; right, CHS^+) of the secondary ion mass spectra obtained after photografting of **1** (blue, solid), PFTR with peptide Cys-PGLa (red, solid), and PFTR control (red, dashed). Theoretical *m*/*z* values: (HS^-) 32.98; (CHS^+) 44.98.

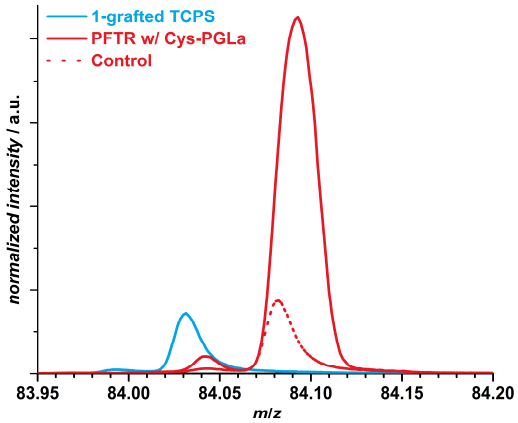


Figure S12. Lysine-specific region of the secondary ion mass spectra obtained after photografting of **1** (blue, solid), PFTR with peptide Cys-PGLa (red, solid), and PFTR control (red, dashed). Theoretical *m*/*z* value ($C_5H_{10}N^+$) 84.08.

Note on non-specific peptide adsorption

Non-specific peptide adsorption was evidenced by comparing washings with pure water or with surfactant solutions (Tween 20 or sodium dodecylsulfonate) after PFTR-based peptide grafting. While sodium dodecylsulfonate allowed an efficient removal of simply adsorbed, non-grafted peptide, it was not adequate as possible crosstalk in the thiol specific region could not be discarded. However, Tween 20 yielded unambiguous results (Figure S13): In all regions of interest, the signal decreased after washing with this surfactant (red solid line), in comparison to washing with pure water (purple solid line). The remaining signal was however significantly stronger than that of the control sample washed with Tween 20 as well (red dashed line), demonstrating the success of the peptide grafting by PFTR.

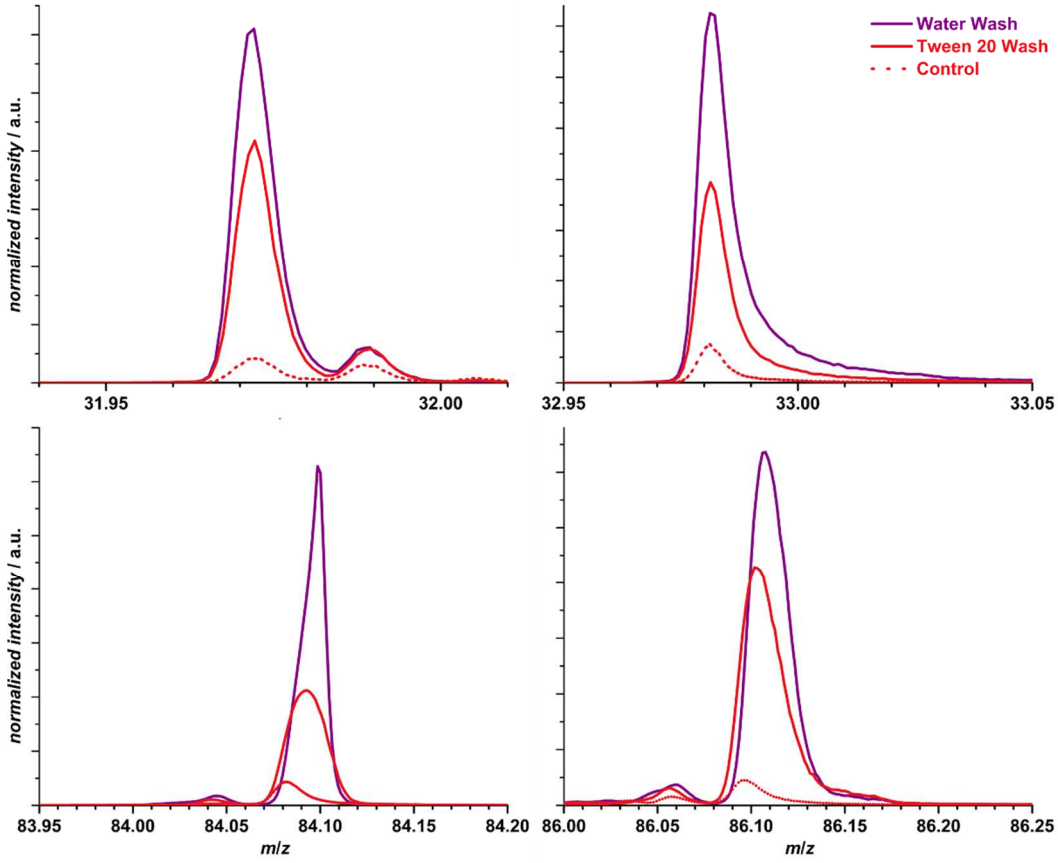


Figure S13. Selected regions of the secondary ion mass spectra obtained after PFTR with peptide Cys-PGLa followed by washing with water (purple, solid) or Tween 20 solution (red, solid), and PFTR control experiment (red, dashed).

References

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