Supporting information

Antifouling fluoropolymer-coated nanomaterials for ^{19}F MRI

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1. Materials

(2-Fluoroethyl)acrylamide (FEAM) was synthesized according to a reference.[1] 2-(((butylthio)carbonothioyl)thio) propanoic acid (PABTC) was synthesized according to the literature procedure.[2] 2-Fluoroethylamine hydrochloride was purchased from Fluorochem Ltd. All other chemicals, including 4,4′-azobis(4-cyanovaleric acid) (ACVA), *N*-(3-dimethylaminopropyl)-*N*′-ethylcarbodiimide hydrochloride (EDC), 4-(dimethylamino)pyridine (DMAP), gold(III) chloride hydrate (50% Au basis), lithium borohydride, diethyl ether, methanol and phosphate-buffered saline (PBS) tablets were purchased from Sigma-Aldrich Ltd. (Prague, Czech Republic) and were used as received. Water was deionized with a Millipore Milli-Q water purification system.

2. Synthesis of PFEAM polymers F1-5

In a Schlenk flask, FEAM monomer (500 mg, 4.27 mmol), PABTC chain transfer agent ($[FEAM]_0/[PABTC]_0$ ratios in **Table S1**, e.g., 20.4 mg, 85.5 μmol for F1) and ACVA initiator ($[PABTC]_0/[ACVA]_0 = 3:1$) were dissolved in DMSO. The flask was sealed, purged with argon and stirred at 70 °C for 8 h (polymers F1-2), respectively 16 h (polymers F3-5). The polymer was isolated by precipitation into diethyl ether, filtered and dried under vacuum. The crude polymer was purified by gel filtration using Sephadex LH-20 column using methanol as the eluent to obtain polymers F1-5 as yellowish powders (yield 409 – 451 mg).

3. Polymer characterizations

Size exclusion chromatography (SEC)

SEC was used to determine the molar masses (M_w = weight-averaged molar mass, M_n = number-averaged molar mass) and the dispersity ($Đ = M_w/M_n$) of the prepared polymers. This was performed by using an Agilent 1260-series HPLC system equipped with a 1260 ISO-pump, a 1260 automatic liquid sampler, a thermostated column compartment at 50 °C equipped with two PLgel 5 μm mixed-D columns and a precolumn in series, a 1260 diode array detector, and a 1260 RI detector with *N*,*N*-dimethylformamide as the mobile phase (flow rate of 0.5 mL min^{-1}). The molar masses and dispersities were calculated against narrow dispersity poly(methylmethacrylate) standards.

Nuclear magnetic resonance (NMR)

The chemical structure of the polymers was confirmed by ^1H, ^{19}F and ^{13}C NMR spectroscopy with a Bruker Avance MSL 400 MHz spectrometer. All chemical shifts are given in ppm.

4. Magnetic resonance (MR) properties

Relaxometry

The ^{19}F relaxation times of F3 in PBS (pH 7.4, c_{pol} = 20 mg mL^{-1}) were measured by using a 1.5 T Minispec 60 MHz relaxometer (Bruker Biospin, Germany) at 37 °C equipped with a fluorine probe (resonance frequency for fluorine was 54 MHz). The T_1 relaxation times were measured with the inversion recovery sequence (repetition time (TR) = 0.1–10000 ms, recycle delay = 4 s, scans = 4, echo time (TE) = 0.05 ms, monoexponential fitting, 16 points per fitting). The T_2 relaxation times were measured with the Carr–Purcell–Meiboom–Gill sequence (TR = 10000 ms, recycle delay = 2 s, scans = 8, TE = 0.05 ms, monoexponential fitting, 20000 points per fitting).

Imaging

$^{1}H/^{19}F$ MR properties of fluorinated polymer F3 in PBS, respectively F1@Au nanoparticles in water, were measured by ^{19}F MR spectroscopy (MRS) and ^{19}F MR imaging (MRI) using a 4.7 T (Bruker Biospec 47/20, Ettlingen, Germany) scanner. The scanner was equipped with a $^{1}H/^{19}F$ custom-made radiofrequency surface coil. T_2-weighted ^{1}H MR images were acquired for reference using a Rapid Acquisition with Relaxation Enhancement (RARE) sequence with the following parameters: TR = 3000 ms, TE = 12 ms, effective echo time TE_{eff} = 36 ms, turbo factor = 8, bandwidth = 34722 Hz, spatial resolution = 0.137 × 0.137 mm^2, slice thickness = 0.85 mm, number of acquisitions NA = 1, and scan time = 1 min 12 s.

The fluorine MRI experiment was performed using RARE sequence with the following parameters: TR = 500 ms, TE = 7.25 ms, TE_{eff} = 43.50 ms, turbo factor = 16, bandwidth = 34722 Hz, spatial resolution = 0.547 × 0.547 mm^2, slice thickness = 7 mm, NA = 1-1024, and scan time = 2 s – 34 m 8 s. The imaging experiment was performed at 25 °C in 0.5 mL Eppendorf tubes containing various concentrations of the polymer (c_{pol} = 0 - 40 mg mL^{-1}), respectively single concentration F1@Au nanoparticles (c = 5 mg.mL^{-1}) with the cross-sections of the tubes shown in the phantom images. The ^{19}F image was overlapped with the anatomic ^{1}H image with spin echo based contrast. Fig. 1E shows the image taken using 1024 acquisitions. ^{19}F MRI of F1@Au nanoparticles was performed with these parameters: TR = 2500 ms, TE = 6. 5 ms, TE_{eff} = 13 ms, turbo factor = 16, bandwidth = 34722 Hz, spatial resolution = 0.547 × 0.547 mm^2, slice thickness = 7 mm, NA = 8, and scan time = 10 m 40 s. This experiment was performed at 25 °C.

5. *In vitro* polymer cytotoxicity

Cell culture

Primary human fibroblasts (HFs) were kindly provided by Dr. P. Jendelova, Institute of Experimental Medicine, Czech Academy of Sciences, Prague, Czech Republic. Cells were cultured in Dulbecco's modified Eagle's medium (DMEM; Gibco) supplemented with 10% (v/v) fetal bovine serum (FBS; Sigma-Aldrich) and 100 units mL^{-1} penicillin-streptomycin (Gibco). Cells were maintained at 37°C in the humidified atmosphere with 5% CO_2.

Cytotoxicity assay

HFs were seeded on 96-well plates in concentration 8×10^3 cells per well. After 24 h of cultivation, cells were treated with different final concentrations of polymer F3 dissolved in MilliQ water (two-fold serial dilutions from 2000 to 7.8 μg mL^{-1}). The amount of MilliQ water with a dissolved sample in each well was adjusted to make the final MilliQ concentration 0.1% (v/v) for each tested polymer concentration, including vehicle control (no polymer F3). After 72 h of incubation, PrestoBlue Cell Viability Reagent (Thermo Fisher Scientific) was added into each well and performed according to the manufacturers' instructions. The fluorescence intensity was measured with excitation/emission 550/590 nm using a Synergy H1 Hybrid reader (BioTek Instruments, Inc.). Cytotoxicity of the tested F3 polymer was derived from the decrease of cell viability calculated as a percentage of control cells.

6. Gold surface coating by polymers

Substrate preparation

Substrates of size 1×2 cm^2 were cut from double-side polished silicon wafers (CZ, orientation <100>, B-doped, resistivity 15-25 Ω cm) with ~1 μm wet silicon dioxide (SiO_2) (Siegert Wafer GmbH, Germany). Gold-coated substrates for surface plasmon resonance (SPR) analysis and infrared spectroscopy were purchased from the Institute of Photonics and Electronics, CAS (Czech Republic) and consisted of glass support, ~2 nm of the titanium adhesion layer and ~50 nm of the gold layer. All samples were thoroughly washed with ethanol and deionized water (Milli Q system, Millipore). Dry samples were exposed to ozone for 20 min just before spin coating or polymer grafting.

Grafting of F1 and reference PEG layers

The grafting of trithiocarbonate chain-end containing F1, respectively thiol chain-end containing PEG chains was performed for 48 h from 2 mg mL^{-1} ethanol solutions. Polymers of the same degree of polymerization were chosen for the direct comparison of the non-fouling properties of the resulting polymer layers.

F1 chain-end functionalization on surface

The conversion of F1 chain-end carboxylic acid group to methyl ester was performed on surface. F1-coated gold wafers were incubated in 0.5 wt.% solution of EDC in dry methanol, catalyzed by 0.1 wt.% DMAP at room temperature overnight. The modified wafers were thoroughly washed with deionizd water, ethanol and dried under vacuum. The methyl ester-functionalized polymer is herein denoted as $F1_{Me}$.

7. Characterization of polymer coatings

Spectroscopic ellipsometry (SE)

J.A. Woollam M-2000X spectroscopic ellipsometer was used to measure the dry thickness of the polymer layers. Ellipsometric data were obtained in the air at room temperature in the wavelength range λ = 245–1000 nm at angles of incidence of 60, 65, and 70°. The obtained data were analyzed with CompleteEASE software.

One of the main problems associated with SE analysis of ultra-thin coatings (films of thickness lower than one-tenth of the wavelength of used probing light) is the strong correlation between the thickness and optical properties. To increase the precision of the F1 thickness determination utilizing spectroscopic ellipsometry, the optical dispersion function of the F1 polymer layer were determined by multiple sample analysis combined with interference enhancement method.[3, 4] The SE measurements (**Fig. S7-11**) were performed on F1 polymer layers spin-coated (Conditions: 2500 rpm; solutions concentrations: 1.25; 2.5; 5; 10; 20 mg mL^{-1}) on previously characterized SiO_2 (1010.0 ± 1.5 nm)/Si substrates. In this case, a two-layer optical model was used consisting of the silicon substrate, SiO_2 and F1 layer, and ambient air. The presence of a thick transparent SiO_2 layer between the silicon substrate and F1 films causes damped interference oscillations in measured ellipsometric spectra (Ψ and Δ), enhancing the sensitivity of the measurement/data extraction to both film thickness and the film optical constants. The optical dispersion function of the F1 films was modeled with the Cauchy dispersion function (**Table S2**). The ellipsometric data recorded from the five differently thick F1 films were simultaneously fitted while independently varying thicknesses and the parameters of the Cauchy dispersion function ($n(\lambda)=A_n+B_n/\lambda^2+C_n/\lambda^4$). As expected, the F1 films were transparent, i.e., showed no extinction coefficient (k = 0) in the measured UV-VIS region.

The extracted optical dispersion function was utilized for the determination of F1 layer thickness on SPR sensors (**Fig. S12**). Reported ellipsometric thicknesses of brushes represent the mean ± standard deviation of six independent measurements on different samples. The Cauchy dispersion function of PEG was taken from a previous study.[5] In this case, a one-layer optical model was used consisting of gold (note that the Au layer is 50 nm thick and can be considered as optically opaque for the used UV-VIS range, its optical dispersion function was obtained through B-Spline function utilizing complex refractive indices of gold crystal as starting values for the fit), F1 or PEG layer, and ambient air. In all modeling cases, mean-squared error (*MSE*) values lower than 1 were obtained, showing an excellent match between measured and modeled SE spectra.

Calculation of grafting density, the distance between grafting sites, and structural state of F1 and PEG polymer chains

The density $\sigma = \frac{h\rho N_A}{M_n}$ and the distance between grafting sites supposing hexagonal packing $D = \sqrt{\frac{2}{\sqrt{3}\sigma}}$ were estimated utilizing the layer thickness in the dry state as determined by ellipsometry (h), the bulk densities of F1 and PEG were taken to be 1.07 and 1.09 g cm^{-3}, respectively, and N_A is the Avogadro constant. The radius of gyration R_g for F1 and PEG polymers in water was calculated according to literature reports.[6, 7] The overlap parameter $\frac{D}{2R_g}$, can be utilized to describe the state of tethered polymer chains. Values of (i) $\frac{D}{2R_g} > 1.0$ indicate that the polymer chains are in a "mushroom" state; (ii) $\frac{D}{2R_g} = 1.0$ are characteristic for a mushroom-to-brush transition; whereas $\frac{D}{2R_g} < 1.0$ are indicative that the chains stretch away from the surface and attain brush conformation.

Contact angle goniometry

The static water contact angles were measured with contact angle goniometer OCA 20 (Dataphysics, Germany) equipped with SCA 21 software. 2 µL drops were deposited on tested surfaces and their profiles were extracted after 30 s. The extracted profiles were fitted with the tangent leaning method. Reported values represent the mean ± standard deviation of six independent measurements on different samples.

Infrared reflection-absorption spectroscopy (IRRAS)

The infrared spectra of the dry polymer layers anchored to SPR sensors were recorded using a Thermo Nicolet NEXUS 870 FTIR Spectrometer equipped with a deuterated triglycine sulfate thermoelectric-cooled detector and a Smart SAGA grazing angle (80°, *p*-polarization) reflection spectroscopy accessory (Thermo Fisher Scientific). The measurement chamber was continuously purged with dry air. The spectra are averages of 128 scans taken at a resolution of 2 cm^{-1}. The acquisition time was around 15 min. All IRRAS spectra are reported as $-\log(R/R_0)$, where R is the reflectance of the sample and R_0 is the reflectance of bare SPR sensor.

X-ray photoelectron spectroscopy (XPS)

Measurements were carried out with a K-Alpha$^+$ spectrometer (ThermoFisher Scientific, East Grinstead, UK). The samples were analyzed using a micro-focused, monochromated Al Kα X-ray source at an angle of incidence of 30° (measured from the surface) and an emission angle normal to the surface. The kinetic energy of the electrons was measured using a 180° hemispherical energy analyzer operated in the constant analyzer energy mode (CAE) at 200 eV and 50 eV pass energy for the survey and high-resolution spectra respectively. To limit the X-ray induced destruction of the thin polymer films and maximize the signal to noise ratio, 20 individual points were measured within areas covering 4 × 8 mm^2. At each point, high-resolution Au 4f, S 2p, C 1s, N 1s, O 1s and F 1s core level and survey spectra were measured. Spectral resolutions of 0.1 and 1.0 eV were used for the high-resolution and survey spectra, respectively. All reported XPS spectra are averages of the twenty individual measurements referenced to the C1s peak of hydrocarbons at 285.0 eV. Data acquisition and processing were performed using Thermo Advantage software. The XPS spectra were fitted with Voigt profiles obtained by convolving Lorentzian and Gaussian functions. The analyzer transmission function, Scofield sensitivity factors, and effective attenuation lengths (EALs) for photoelectrons were applied for quantification. EALs were calculated using the standard TPP-2M formalism. The BE scale was controlled by the well-known position of the photoelectron C-C and C-H, C-O and C(=O)-O C 1s peaks of polyethylene terephthalate and Cu 2p, Ag 3d, and Au 4f peaks of metallic Cu, Ag and Au, respectively. The BE uncertainty of the reported measurements and analysis is in the range of ±0.1 eV.

Surface plasmon resonance spectroscopy (SPR)

A custom-built SPR instrument (Institute of Photonics and Electronics, Academy of Sciences of the Czech Republic, Prague) based on the Kretschmann geometry of the attenuated total reflection method and spectral interrogation of the SPR conditions was used. The tested solutions of proteins or body fluids were driven by a peristaltic pump through four independent channels of a flow cell for 15 min., in which the SPR responses were simultaneously measured as shifts in the resonant wavelength, λ_{res}. The sensor response ($\Delta\lambda_{res}$) was obtained as the difference between the baselines in phosphate-buffered saline (PBS) before and after the injection of the human blood plasma. Reported values represent the mean ± standard deviation of three independent measurements on different samples.

8. Synthesis of F1-coated gold nanoparticles

A solution of gold chloride hydrate (50% gold basis, 5 mg, 12.7 μmol) in methanol (0.5 mL) was added to the rapidly stirred solution of F1 polymer (42.5 mg, 6.35 μmol) in dichloromethane (100 mL) at room temperature, followed by addition of a freshly prepared sodium borohydride (2.4 mg, 63.5 μmol) solution in methanol (0.6 mL) was added dropwise. After 2 minutes of stirring, the reaction mixture was allowed to stand for 16 hours. The solvent was evaporated under vacuum at room temperature, and the as-prepared coated gold NPs were resuspended in distilled water. The NPs were purified by extensive dialysis against distilled water (molecular weight cut-off 14 - 16 kDa) for 10 days with daily replacement of the water reservoir to remove any unbound polymer. The purified NPs were isolated by freeze-drying.

9. Characterization of F1-coated gold nanoparticles

Scanning transmission electron microscopy (STEM)

STEM imaging was performed using JEOL NeoARM 200Foperated at 200 kV. Images were collected using an annular dark field (ADF) and annular bright field (ABF) detectors. The alignment of the microscope was performed using the standard Ronchigram adjustment method. Size measurement was performed using ImageJ software.

Dynamic light scattering (DLS) measurements

Hydrodynamic diameters of the NPs were measured in PBS (pH = 7.4) by DLS using a Zetasizer NanoZS instrument, Model ZEN3600 (Malvern Instruments, UK). The nanoparticle solution was filtered prior to measurement through a 0.22 μm PVDF syringe filter. The apparent volume-weighted mean hydrodynamic diameter of the particles, D_h, was determined at a

scattering angle of θ = 173°, and the DTS (Nano) program was used to evaluate the data. The reported value represents the mean ± standard deviation of five independent measurements.

10. Statistics

All data are presented as mean ± standard deviation. Unless otherwise stated, the statistical analysis was performed by Origin (Originlab, U.S.) software using one-way ANOVA with $P < 0.05$ indicating statistically significant difference.

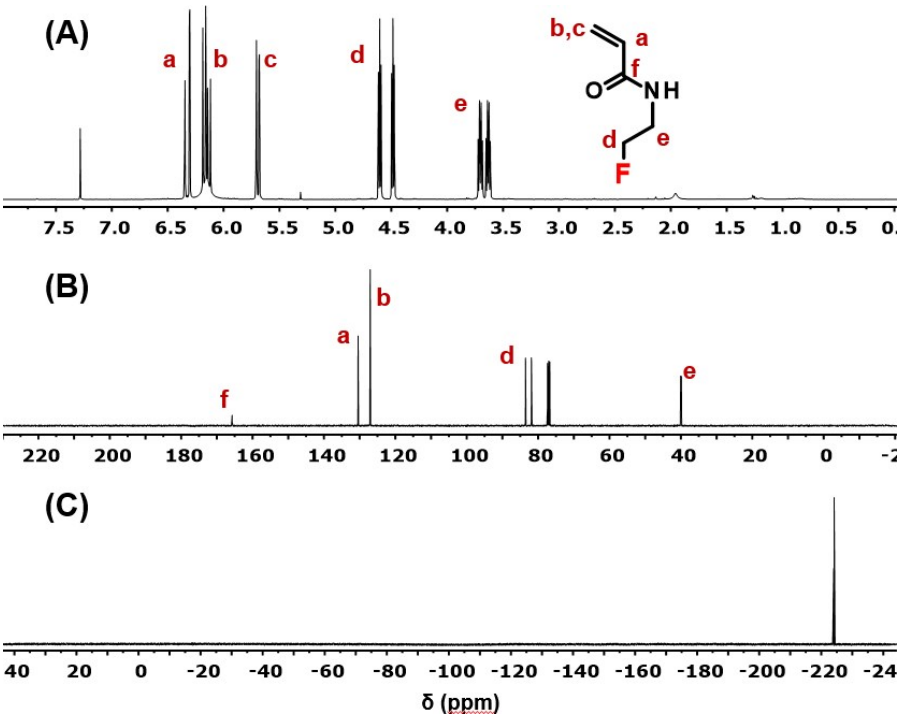

Fig. S1. ^{1}H (A), ^{13}C (B), and ^{19}F (C) NMR spectra of FEAM monomer in $CDCl_3$.

Table S1. Characteristics of synthesized PFEAM.

Polymer	$[M]_0/[CTA]_0$	M_n[a] (kDa)	$Đ$[a]	D_h[b] *(nm)*
F1	50	6.7	1.08	3.43±0.07
F2	100	11.0	1.09	5.21±0.28
F3	200	21.1	1.09	8.08±0.11
F4	400	39.2	1.10	11.13±0.09
F5	600	49.8	1.08	12.83±0.23

[a]Determined by SEC. [b]Hydrodynamic diameter measured by DLS

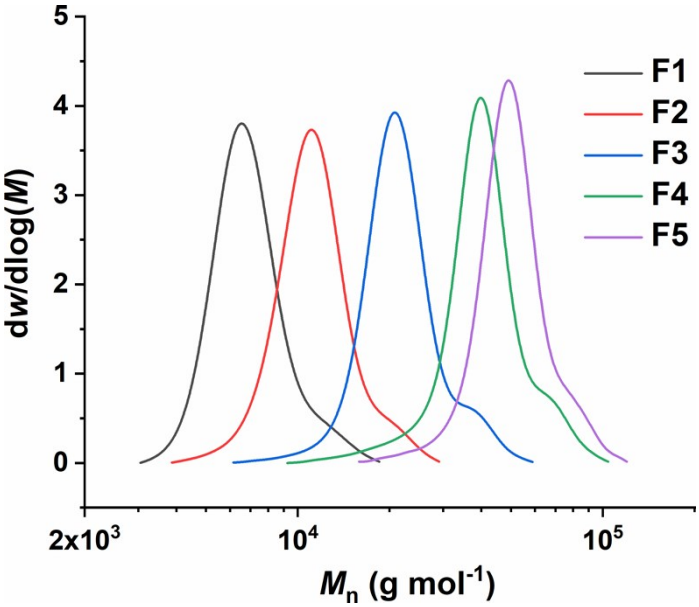

Fig. S2. Molar mass distributions of polymers F1-5 measured by SEC with PMMA calibration.

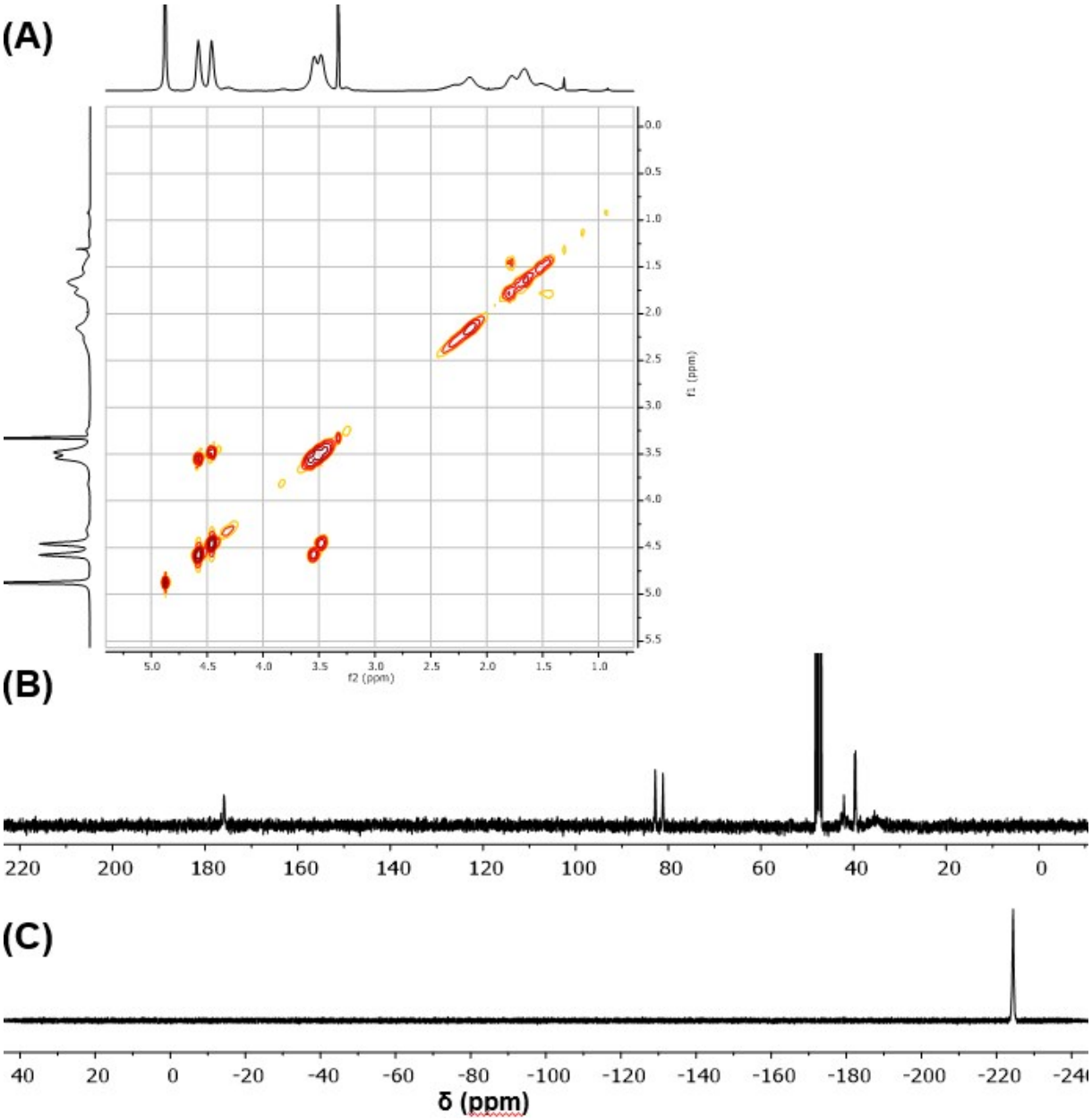

Fig. S3. ^{1}H COSY (A), ^{13}C and ^{19}F (C) NMR spectra of F3 in CD_3OD.

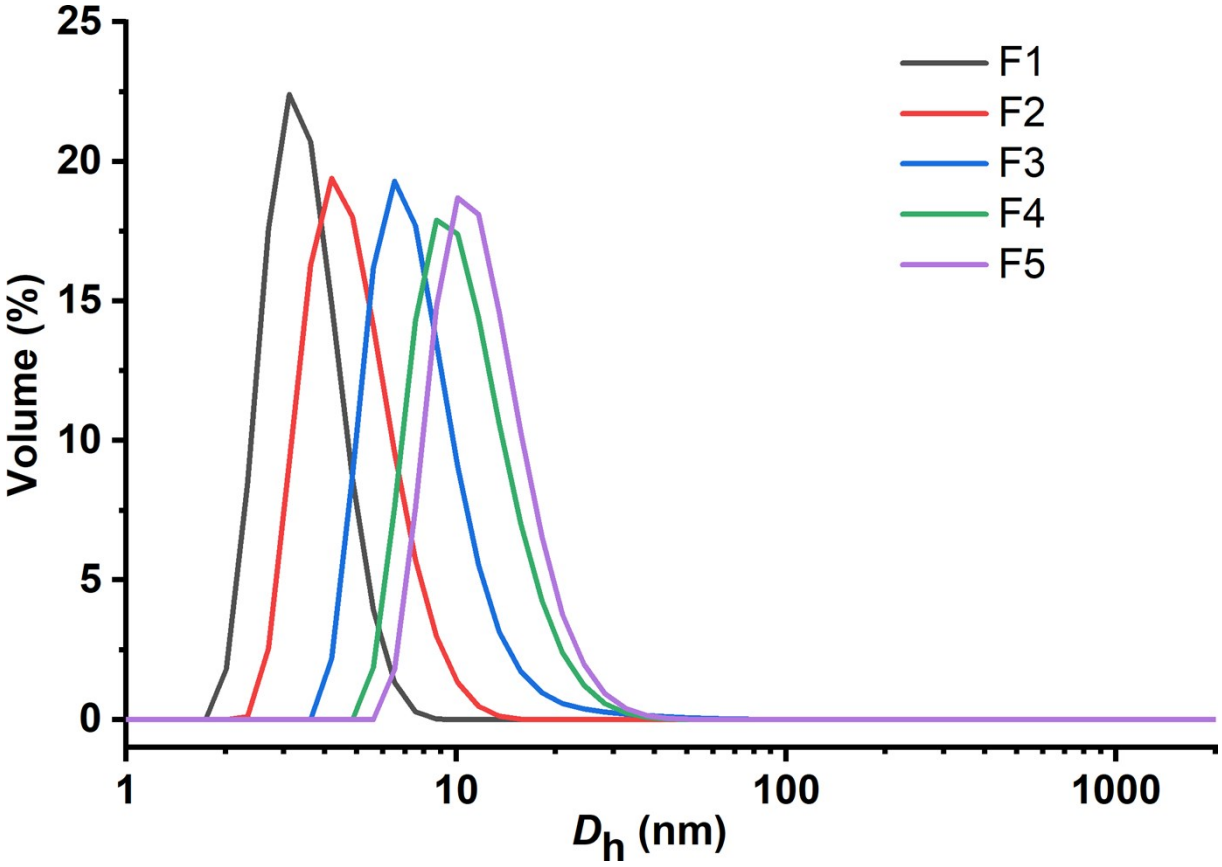

Fig. S4. Hydrodynamic diameter distribution of F1-5 polymers in water (c = 5 mg mL^{-1}) measured by DLS.

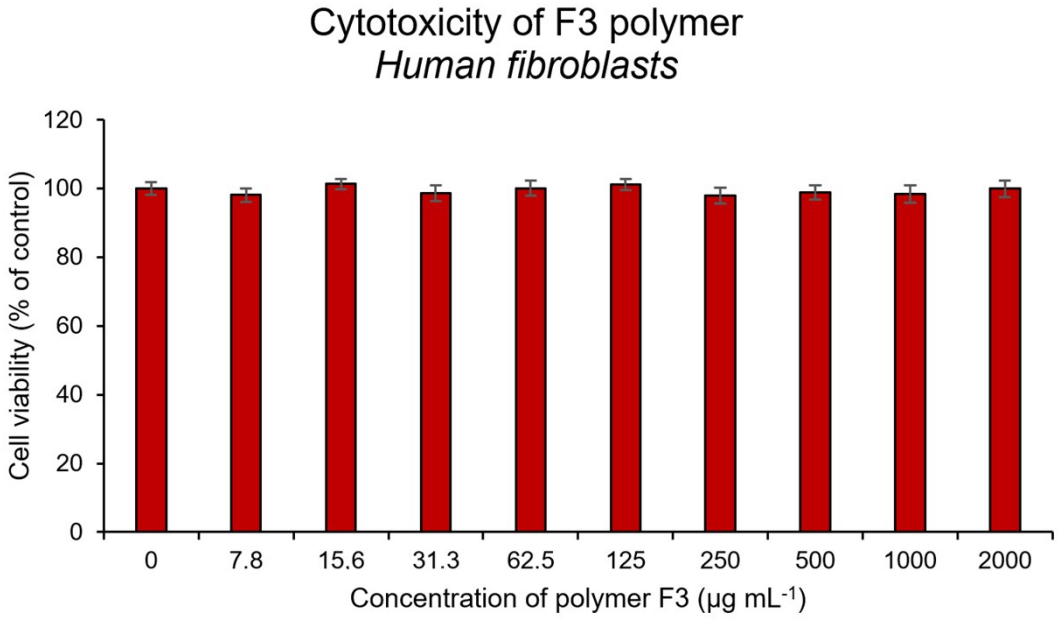

Fig. S5. Viability of primary human fibroblasts after 72 h of incubation with different concentrations of F3.

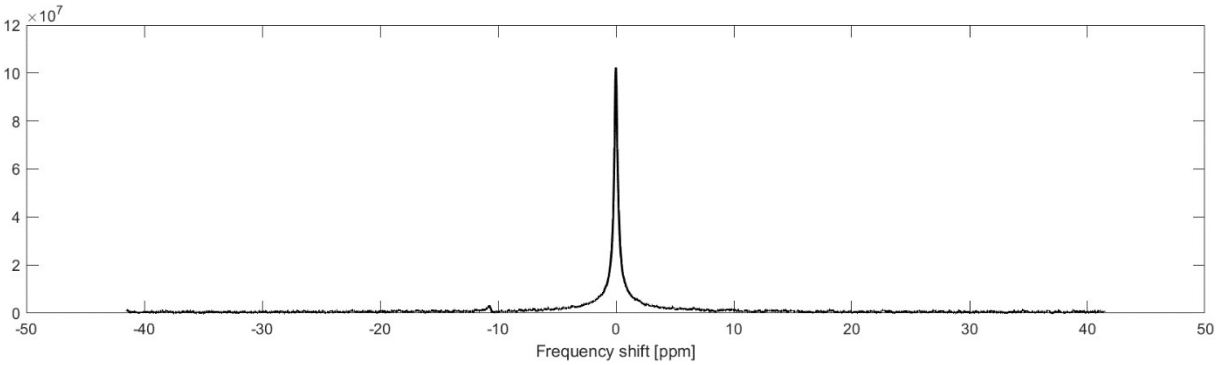

Fig. S6. ^{19}F MRS spectrum of F3 in PBS (pH = 7.4, c_{pol} = 20 mg mL^{-1}) recorded by 4.7 T scanner and centered for MRI experiments.

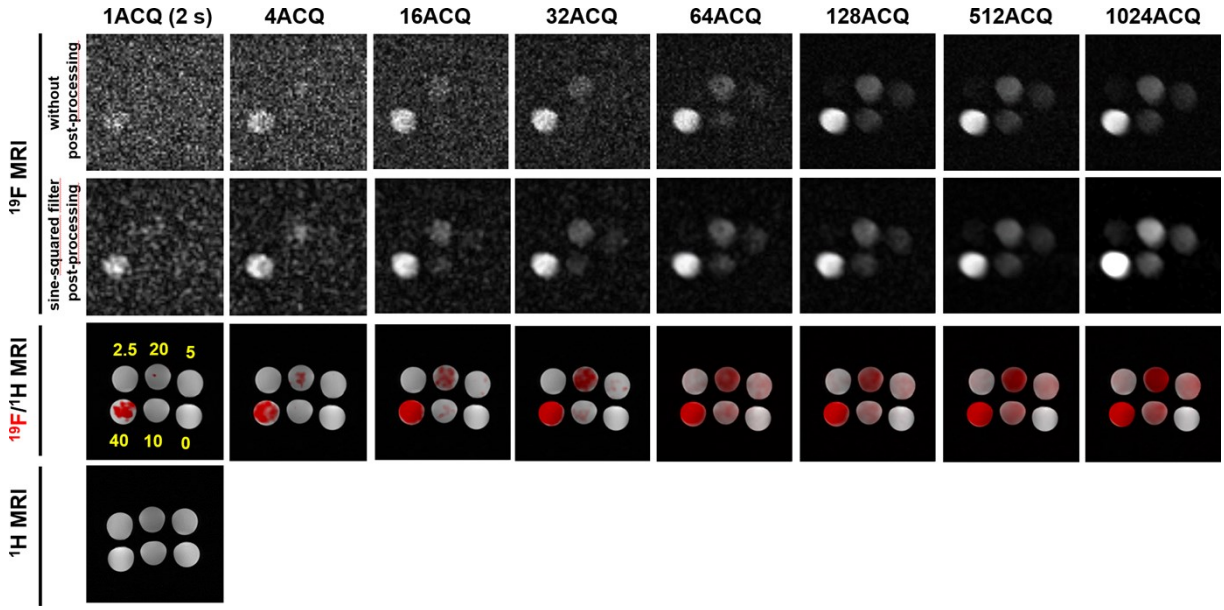

Fig. S7. ^{19}F RARE and ^{1}H MRI images (4.7 T) of F3 in PBS (pH) at different polymer concentrations (yellow numbers represent c_{pol} in mg mL^{-1}) and a different number of acquisitions (1 - 1024). Overlay images (row 3) show ^{19}F MRI signal in red color.

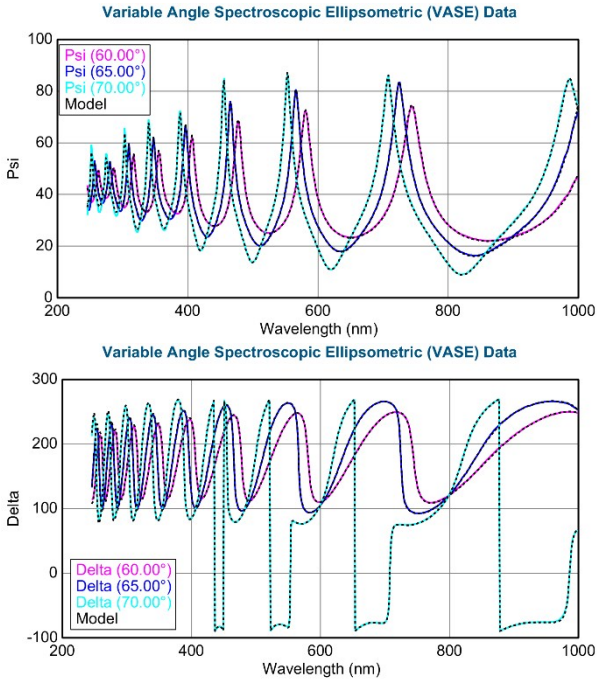

Fig. S8. Experimental (full lines) and fitted (dotted lines) psi and delta spectra at various AOI of F1 Sample 1 (93.7 nm)

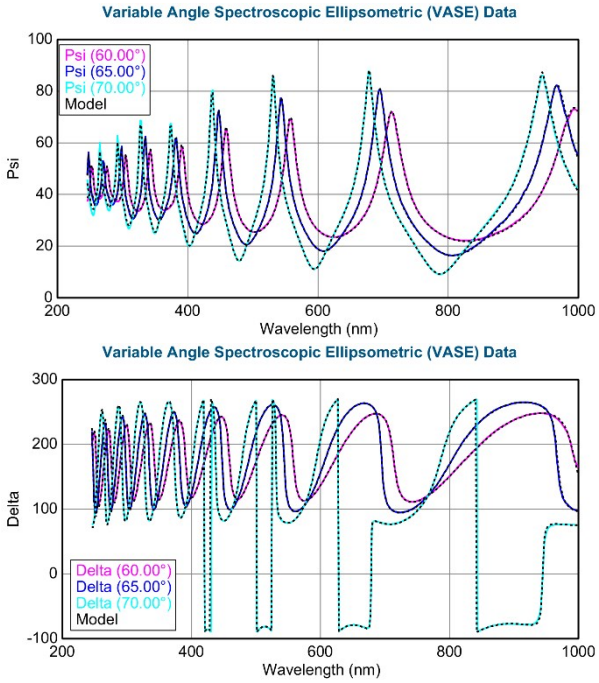

Fig. S9. Experimental (full lines) and fitted (dotted lines) psi and delta spectra at various AOI of F1 Sample 2 (49.8 nm)

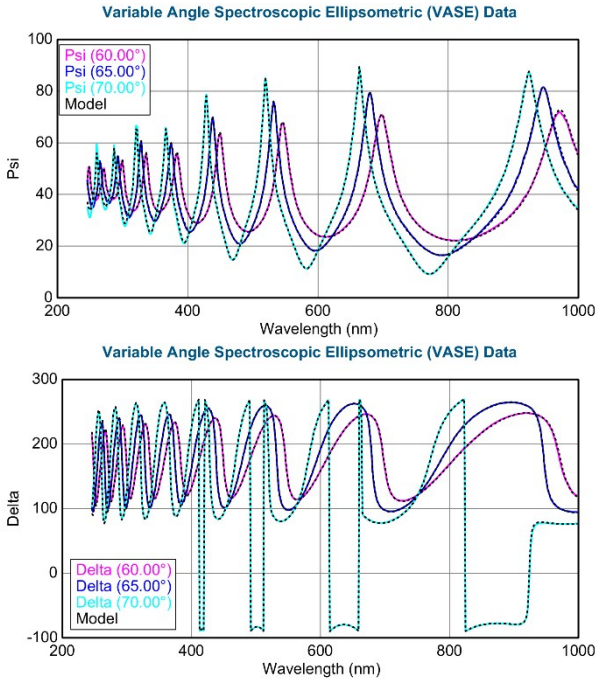

Fig. S10. Experimental (full lines) and fitted (dotted lines) psi and delta spectra at various AOI of F1 Sample 3 (27.7 nm).

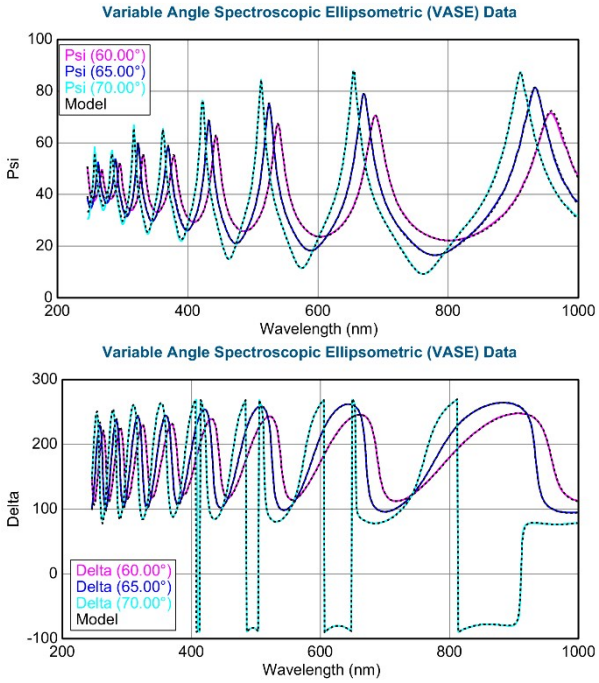

Fig. S11. Experimental (full lines) and fitted (dotted lines) psi and delta spectra at various AOI of F1 Sample 4 (14.4 nm).

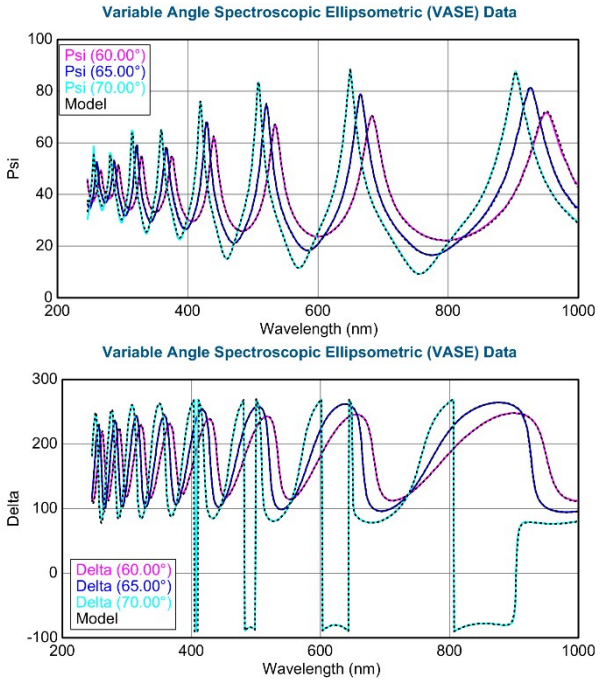

Fig. S12. Experimental (full lines) and fitted (dotted lines) psi and delta spectra at various AOI of F1 Sample 5 (7.4 nm).

Table S2. Thicknesses and Cauchy parameters of F1 films obtained from the combined multiple sample analysis of the ellipsometric data.

Sample 1 thickness:	**93.7 nm**
Sample 2 thickness:	**49.8 nm**
Sample 3 thickness:	**27.7 nm**
Sample 4 thickness:	**14.4 nm**
Sample 5 thickness	**7.4 nm**
Cauchy parameters	
A_n	**1.492**
B_n	**3.2×10^3 [nm^2]**
C_n	**1.9×10^8 [nm^4]**
k = 0	
MSE	**0.8**

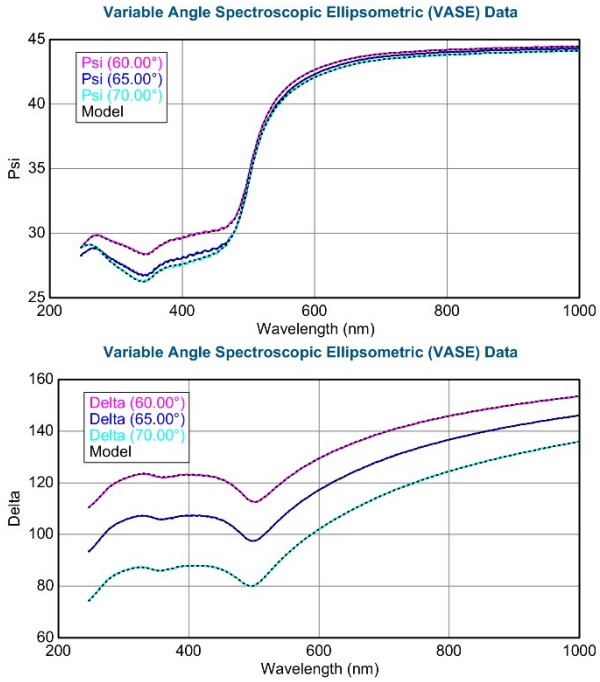

Fig. S13. Experimental (full lines) and fitted (dotted lines) psi and delta spectra at various AOI of SPR chip coated with 1.5 nm F1.

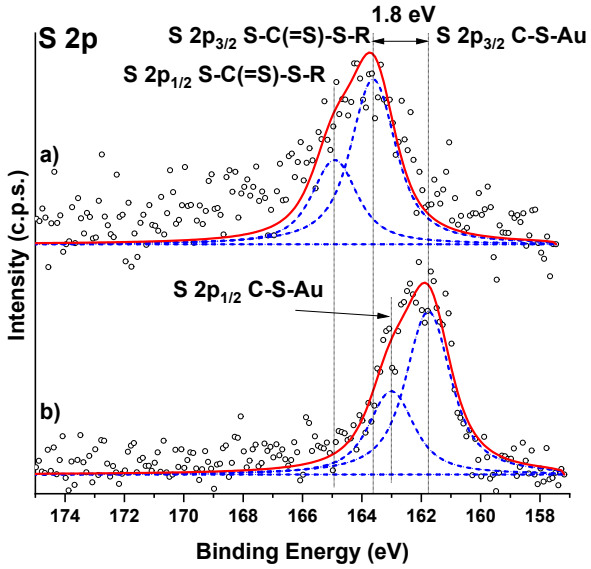

Fig. S14. XPS analysis proves the covalent binding of F1 chains to the gold substrate through the C–S–Au bond formation. High-resolution core-level S 2p XPS spectra of free trithiocarbonate end-group in unbound F1 (a) and corresponding reacted C–S–Au anchoring moiety (b). Measured spectra are presented with open circles, while their corresponding fitted envelopes are presented with red lines. The individual contributions of different functional groups are represented with blue lines.

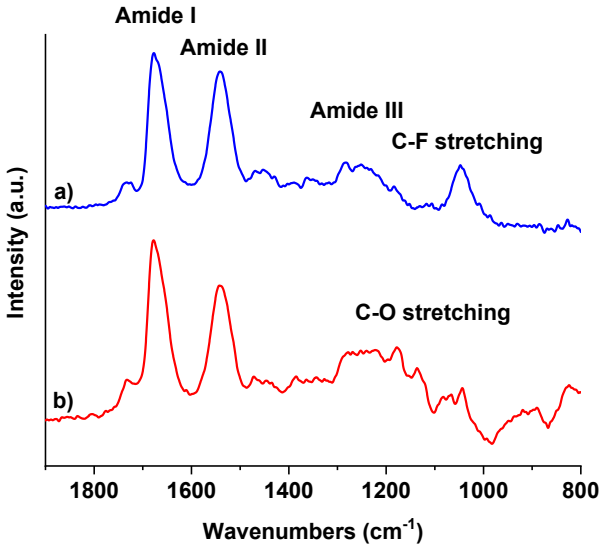

Fig. S15. IRRAS spectra of F1-coated (a) and $F1_{Me}$-coated (b) gold surface.

Table S3. Surface parameters and fouling of blood plasma on polymer-coated gold wafers

Polymer	DP[a]	h^b [nm]	σ^c [nm^{-2}]	D^d [nm]	$D/2R_g{}^e$	θ^f [°]	Plasma fouling[h] [%]
F1	50	1.5±0.2	0.17±0.02	2.6	0.91	33±1	9±2
$F1_{Me}$	50	1.7±0.1	0.19±0.01	2.5	0.87	31±2	18±1
PEG	45	1.4±0.2	0.46±0.07	1.6	0.63	24±1	43±3

[a]Degree of polymerization. [b]Ellipsometric thickness. [c]Grafting density in number of chains per area. [d]Distance between grafting sites. [e]Values $D/2R_g < 1$ indicate that the polymer chains have attained the brush regime; R_g - radius of gyration. [f]Static water contact angle. [h]Plasma protein fouling by polymer coated gold surfaces compared to that of bare gold.

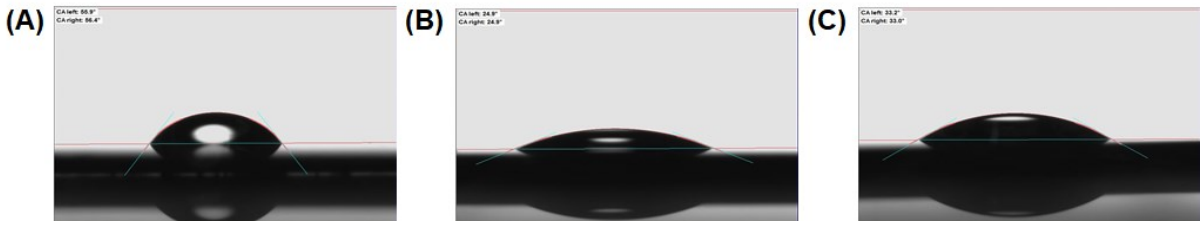

Fig. S16. Representative images of static water contact angles of (A) bare gold (60±4°), (B) reference PEG layer (24±1°) and (C) F1 (33±1°) layer (n = 6).

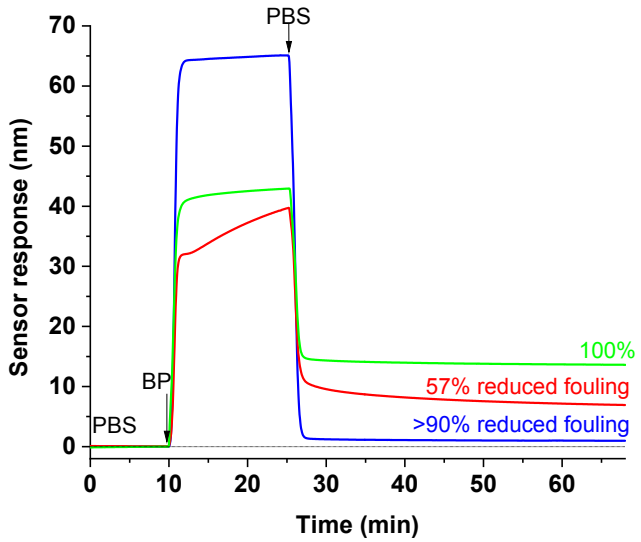

Fig. S17. Representative SPR sensorgrams of blood plasma fouling on bare gold (green), reference PEG (red) and F1 (blue) polymer brush layers.

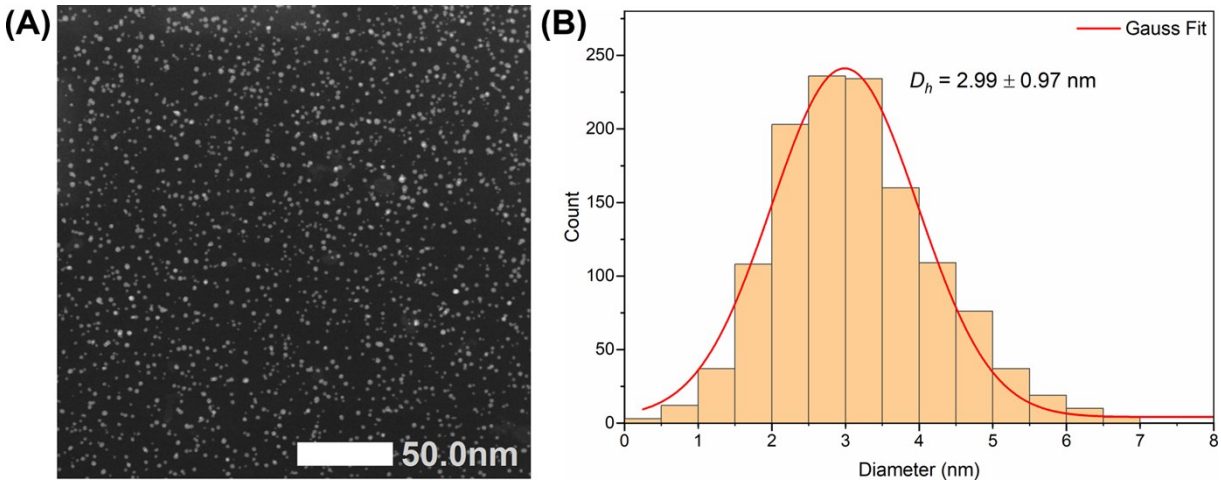

Fig. S18. (A) STEM micrograph of F1@Au NPs. (B) Size distribution histogram of the F1@Au NP cores.

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