Electronic Supplementary Material (ESI) for Physical Chemistry Chemical Physics

Probing the Conformation and 2D-Distribution of Pyrene-Terminated Redox-Labeled Poly(Ethylene Glycol) Chains End-Adsorbed on HOPG using Cyclic Voltammetry and Atomic Force Electrochemical Microscopy

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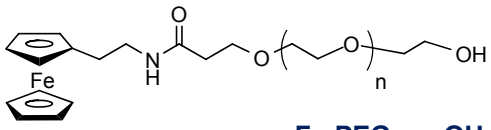
Supplementary Information

PEG Derivatives and Materials. The ferrocene-end labeled linear poly(ethylene glycol) PEG chain Fc-PEG-OH (PEG = $(CH_2CH_2O)n$ with $n \approx 79$, avg. M_W PEG 3400) was synthesized and obtained as an analytical sample as described previously.[1,2] The Fc-PEG_{3400}-NHS bearing an active succinimidyl carbonate for preparation of Fc-PEG-Pyrene (see Figure S1) was prepared from Fc-PEG-OH molecule as described previously.[2]

Spectroscopic Instrumentation. ^1H NMR spectra were recorded on a Bruker AM-400 spectrometer operating at 400 MHz at room temperature in $CDCl_3$. Chemical shifts are reported in parts per million relative to the solvent residual proton signal δ =7.26 ppm.

Matrix-assisted laser desorption/ionisation time of flight (MALDI-TOF) mass spectra were obtained from the Small Molecule Mass Spectrometry platform of IMAGIF (Centre de Recherche de Gif, France). A Voyager DE-STR MALDI-TOF mass spectrometer (AB Sciex, Les Ulis, France), equipped with a 337-nm pulsed nitrogen laser (20 Hz) and a Acqiris® 2 GHz digitizer board, was used for all experiments. Mass spectra were obtained in reflectron positive ion mode with the following settings: accelerating voltage 20kV, grid voltage 62 % of accelerating voltage, extraction delay time of 300 ns. The laser intensity was set just above the ion generation threshold to obtain peaks with the highest possible signal-to-noise (S/N) ratio without significant peak broadening. The polymer solution was prepared at a concentration of 0.6 mM in THF. The matrix solution (2-[(2E)-3-(4-tert-Butylphenyl)-2-methylprop-2-enylidene]malonitrile (DCTB) was prepared at a concentration of 6 mM. The sample for MALDI analysis was prepared by mixing the polymer solution with matrix solution at a volume ratio of 1:9. The mass spectrometer was externally calibrated using PEG4500. Data were processed using the Data Explorer software package (AB Sciex).

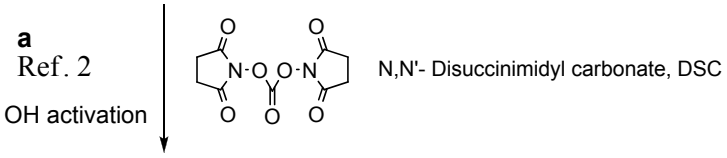
Synthesis of Fc-PEG$_{3400}$-Pyrene starting from analytically pure Fc-PEG$_{3400}$-OH

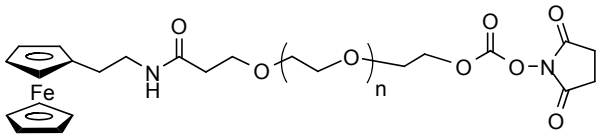


Synthesis Ref. 1-2

Fc-PEG$_{3400}$-OH

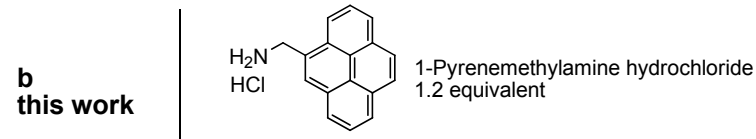
Purity > 95 % . Polydispersity1.01. Average n 79
^{1}H NMR spectrum, Fig. S2
Maldi-TOF MS analysis, Table S1, Fig. S4 and Fig. S5

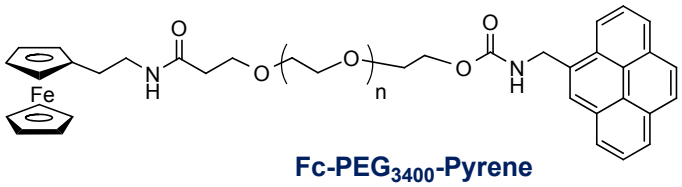




Fc-PEG$_{3400}$-NHS

NHS availability > 95%





Fc-PEG$_{3400}$-Pyrene

Purity Pyrene-end functionality > 99%
Polydispersity1.01. Average n 79
^{1}H NMR spectrum, Fig. S2
Maldi-TOF MS analysis, Table S1, Fig. S3 and Fig. S5

Figure S1. Schematic overview of the preparation of the pyrene end-functionalized Fc-PEG, Fc-PEG-Pyrene via the amine reactive succinimidyl carbonate Fc-PEG-NHS derived from analytically pure Fc-PEG-OH. Detailed chemical structures and some specifications are given**.**

Fc-PEG$_{3400}$-Pyrene: Synthesis and Characterization Data

The pyrene-end functionalized Fc-PEG conjugate Fc-PEG-Pyrene, was prepared by coupling the Fc-PEG-NHS succinimidyl carbonate (derived from Fc-PEG-OH) with the amino group of 1-pyrene-methylamine, following a method used previously for the conjugation of Fc-PEG-NHS to amino groups of cysteamine.[3]

A 50 mg sample of Fc-PEG-NHS active ester (~13 μmol of available active carbonate NHS groups) was dissolved in 180 μL of chloroform and treated with pyrene methylamine hydrochloride (4.23 mg, 15.8 μmol, 1.2 excess), and triethylamine (4.4 μL, 31.2 μmol, 2.4 excess). The reaction mixture was protected from light and stirred for 24 h at room temperature. After solvent removal, the resulting crude product was purified by LC silica chromatography (Magerey Nagel silica gel, particle size 0.063-0.2 mm) (eluent: chloroform/methanol, 90:10). The LC purification, run in order to remove unreacted pyrene methylamine, was followed by UV-detection at 280 nm. The fractions of the desired product, corresponding to the first eluted band, were collected, pooled, and filtered through a 0.2-μm Teflon (VWR) unit before solvent removal. Drying in vacuo yielded the Fc-PEG$_{3400}$-pyrene conjugate as a pale yellow solid (32 mg, isolated yield ~60%), which was successfully evaluated for its identity and purity by ^{1}H NMR and MALDI-TOF mass spectrometry.

^{1}H-NMR (400 MHz, CDCl$_3$) δ 8.30 (d, J = 9.3 Hz, C*H* of pyrene), 8.25 - 8.11 (m, 4H, C*H* of pyrene), 8.11 - 7.96 (m, 4H, C*H* of pyrene), 6.51 (br s, 1H, N*H* amide), 5.43 (br s, 1H, N*H* urethane), 5.07 (d, *J* = 5.55 Hz, 2H, pyrene-C*H*$_2$-NHCOO-), 4.27 (app t, 2H,-C*H*$_2$COONH-), 4.09 (br s, 5 *H*Fc), 4.03-4.07 (m, 4 *H*Fc), 3.65-3.55, (m, ~340H, PEG backbone (C*H*$_2$C*H*$_2$O)$_n$), plus 2H, -C*H*$_2$CH$_2$COONH-), 3, 35 (m, 2H,), 2.53 (t, *J* = 7.15 Hz, 2H, -NHCOC*H*$_2$-), 2.44 (t, *J* = 5.8 Hz, 2H, -C*H*$_2$ α to ferrocene Fc). The ^{1}NMR spectrum of Fc-PEG-Pyrene is shown in Figure S2 with the ^{1}HNMR spectrum of Fc-PEG-OH given for comparison.

^{1} H NMR spectra of Fc-PEG-Pyrene and of its precursor Fc-PEG-OH

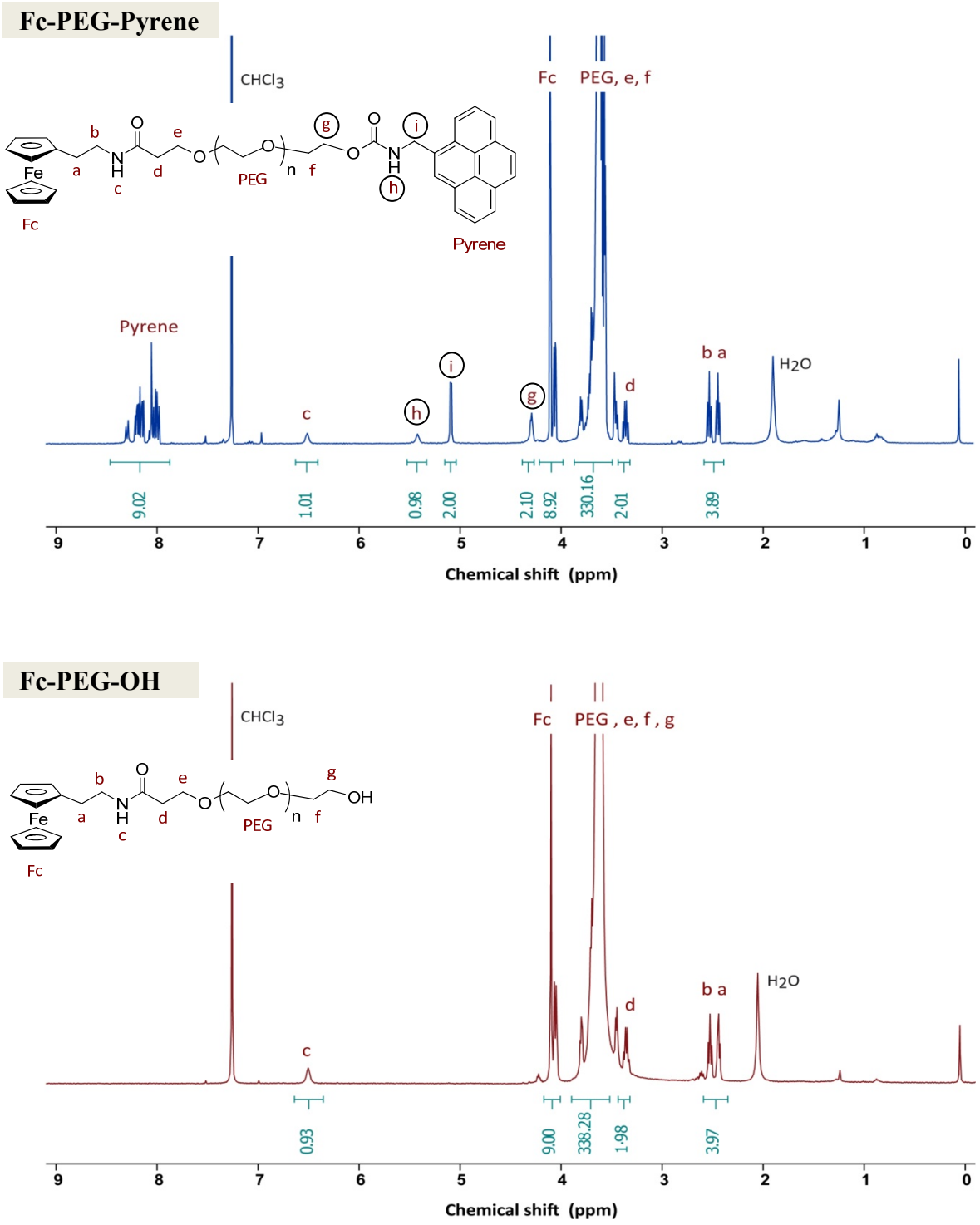


Figure S2. **The ^{1}H NMR spectra (400 MHz, $CDCl_3$) of Fc-PEG-Pyrene (top) and of its precursor Fc-PEG-OH (bottom)** (previously characterized in ref. 1, and given for comparison). For Fc-PEG-Pyrene, the ratio of the integral intensity of the peaks confirms the high degree of pyrene–end group functionalization. Highlighted by round circles the three distinguishable signals characterizing the urethane bond linking the Fc-PEG segment to the pyrene unit (aromatic protons region).

MALDI-TOF MS Analysis and Results.

The successful coupling approach linking Fc-PEG succinimidyl carbonate Fc-PEG-NHS (starting from Fc-PEG-OH) to 1-pyrene methylamine (see Scheme1) was demonstrated by MALDI-TOF MS analysis in positive ion reflectron mode. In particular, this technique ascertained that the desired Fc-PEG-Pyrene product used in this work *was basically free of* Fc-PEG-OH impurities (that would result from incomplete NHS activation or from hydrolysis (inactivation in the moisture) of Fc-PEG-NHS).

(1) **Molecular weight** *data analysis.* The spectra for Fc-PEG-Pyrene, and for its precursor Fc-PEG-OH, (Figure S3 and Figure S4 respectively) exhibited a main series of peaks due to $[M+Na]^+$ adducts and a lower series due to $[M+K]^+$ adducts. For both samples, each series can be identified to polymeric chains formed by the same number of repetitive oxyethylene CH_2CH_2O units, n value unit ΔM = 44.05 Da (see partial expansion of the spectra).

The data and results of analysis for the Fc-PEG samples; i.e. molecular weight M_p derived from the top mass peak MNa and number average molecular weight M_n are ***given below***.

- **Fc-PEG-OH** Molecular Formula = $C_{17}H_{23}NO_3Fe$+ nC_2H_4O

  Measured Fc-PEG-OH.Na^+, 3760.21;

  Calculated (MNa), ***n =77***, Iso : C171H331NO80FeNa, 3760.21.

  Results *Molecular weight* for Fc-PEG-OH : M_p 3737 ; M_n 3815.

- **Fc-PEG-Pyrene** Molecular Formula = $C_{35}H_{34}N_2O_4Fe$ + nC_2H_4O

  Measured Fc-PEG-Pyrene.Na^+, 4017.20;

  Calculated (MNa), ***n=77***, Iso: C189H342N2O81FeNa, 4017.20.

  Results *Molecular weight* for Fc-PEG-Pyrene : M_p 3994; M_n 4015.

(2) **Analysis of purity of Fc-PEG-Pyrene**. Isotopic abundance spectra of Fc-PEG-OH and Fc-PEG-Pyrene terminated species were compared to confirm the absence of Fc-PEG-OH in the Fc-PEG-Pyrene sample (see Figure S5).

MALDI-TOF MS spectrum and analysis of Fc-PEG-Pyrene

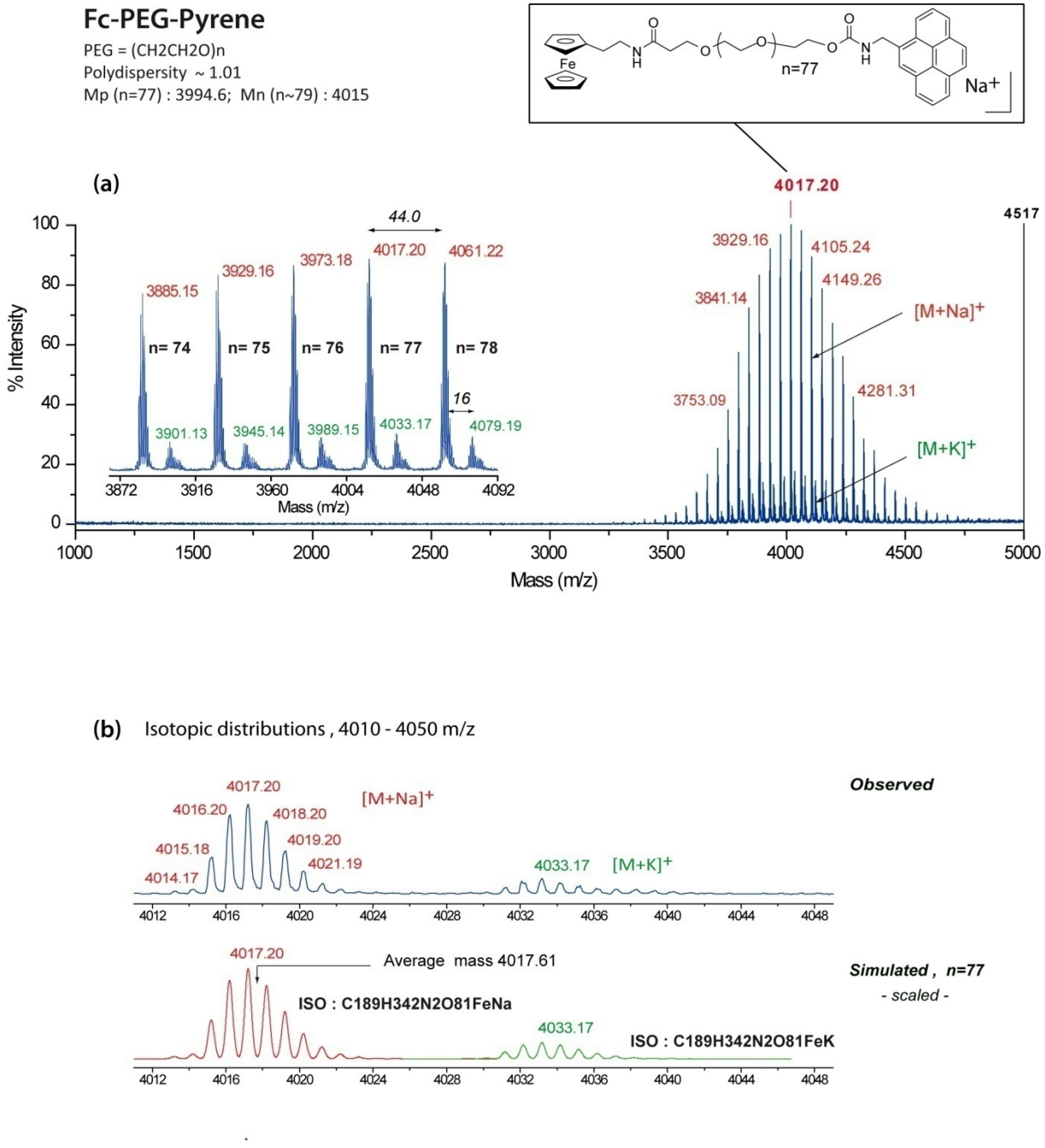


Figure S3. **Positive ion reflectron mode MALDI-TOF-MS spectrum of the Fc-PEG-Pyrene conjugate.** (a) The spectrum exhibits a main mass series of peaks corresponding to Fc-PEG-Pyrene cationized by sodium, $[M+Na]^+$ adducts (red font color), and a minor series corresponding to potassium adducts $[M+K]^+$ (green font color). The two different series of peaks differ by 44.0 amu, the mass of one repeat oxyethylene unit of PEG, (CH_2CH_2O) and are shifted to one another by 16 Da which is in agreement with the atomic masses of Na and K. The most abundant ion (4017.20) is the apex of the isotope distribution of sodium adduct for Fc-PEG-Pyrene molecule with PEG n=77. Inset: Enlarged MALDI-TOF MS of the Fc-PEG-Pyrene in the mass range corresponding to repeat units n = 73-77. (b) Zoom view of the MALDI-TOF mass spectrum (mass range 4010-4050 m/z) of the Fc-PEG-Pyrene molecule with n =78, with its corresponding isotopic simulation. The observed isotopic patterns for the $[M+Na]^+$ and $[M+K]^+$ adducts are in excellent agreement with the calculated (intensity scaled) isotopic distributions. Simulation was done with Mike Senko's IsoPro 3.1 freeware.

MALDI-TOF MS spectrum analysis of Fc-PEG-OH

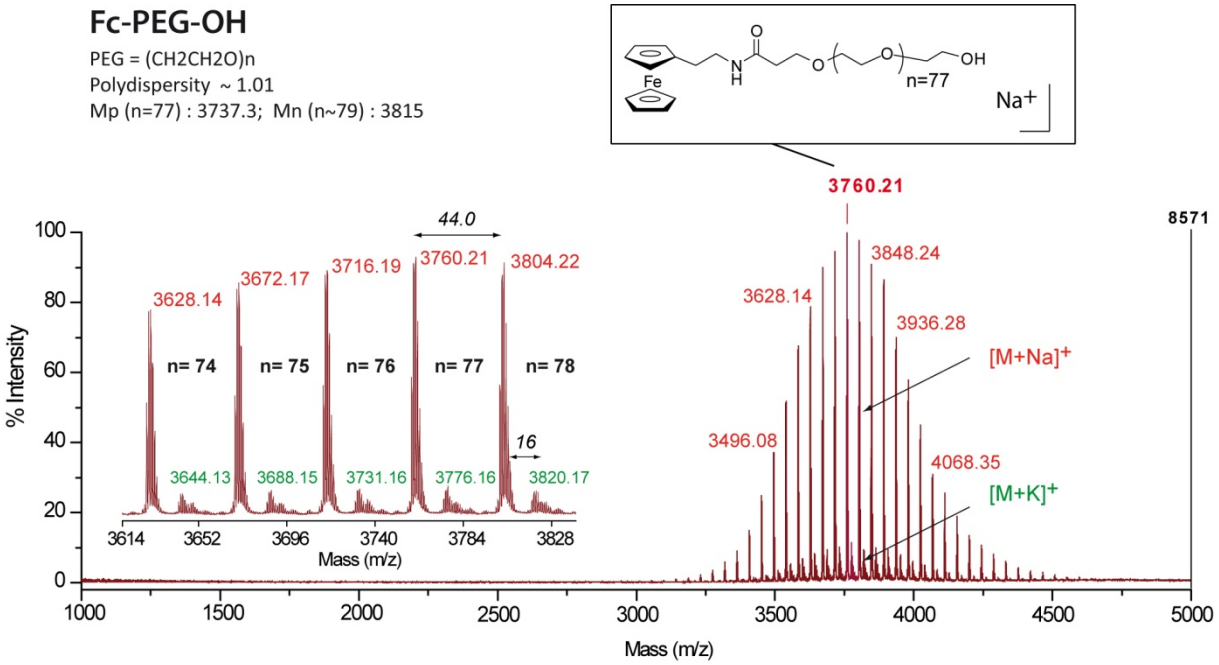


Figure S4. **Positive ion reflectron mode MALDI-TOF-MS spectrum of Fc-PEG-OH.** A main mass series of peaks corresponding to sodium cationized adduct ($[M+Na]^+$ (red font), and a minor one due to potassium adduct $[M+K]^+$ (green font) are observed. The most abundant ion (3760.21) is the apex of the isotope distribution of sodium adduct for Fc-PEG-OH molecule with PEG n=77. Inset: Enlarged MALDI-TOF MS of Fc-PEG-OH in the mass range corresponding to n = 74-78.

MALDI-TOF MS : *no detectable Fc-PEG-OH in Fc-PEG-Pyrene*

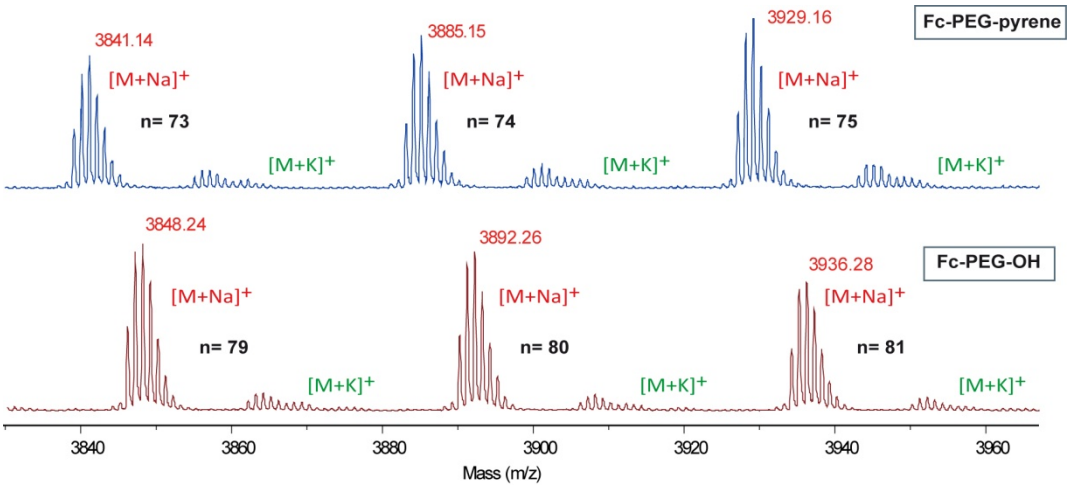


Figure S5. **Comparison of MALDI-TOF-MS spectra of Fc-PEG-Pyrene (top) and its starting construct Fc-PEG-OH (bottom) in the expanded mass range m/z 3830-3970.** The experimental isotopic distributions of the Fc-PEG-Pyrene sample (n =73-75) clearly show no isotope overlap with those of its precursor derivative Fc-PEG-OH (n=79-81), exemplifying that the Fc-PEG-Pyrene sample is absolutely free of Fc-PEG-OH chains. The spacing between adjacent isotopic peaks in all isotopic adducts ($[M+Na]^+$, and $[M+K]^+$) was ~1 Da.

Scanning electron microscopy images of the combined AFM-SECM probes

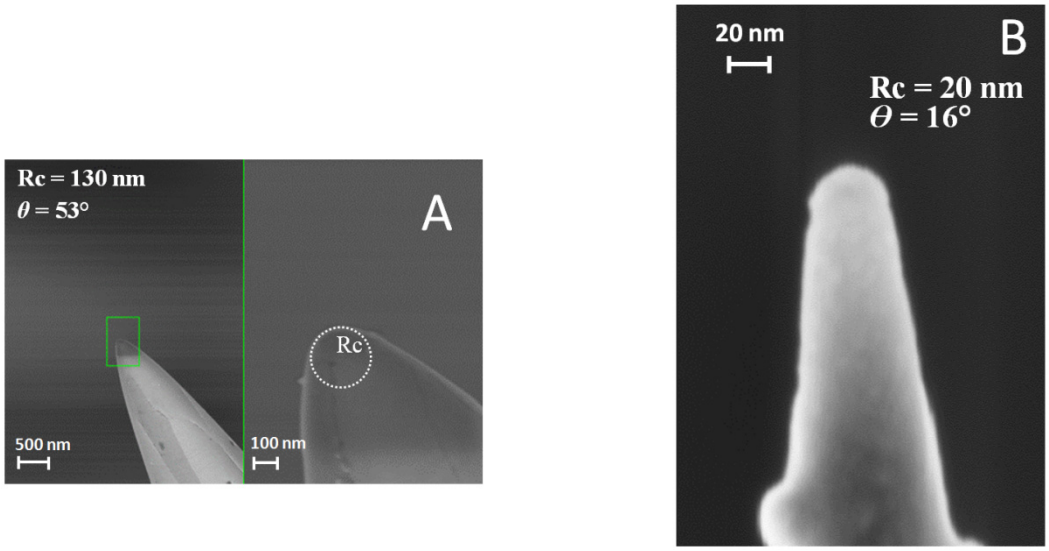


Figure S6. Scanning electron microscopy images of the tip of combined AFM-SECM probes such as those used in the present work. (A) Probe fabricated using a previously reported etching process (ref [4]), characterized by a tip radius in the order of ~100 mn, and used here for contact mode Mt/AFM-SECM experiments. (B) Sharper probe fabricated using an improved process (to be reported), characterized by tip radius in the order of ~20 nm, and used here for Mt/AFM-SECM imaging.

Tapping mode Mt/AFM-SECM amplitude and current approach curves

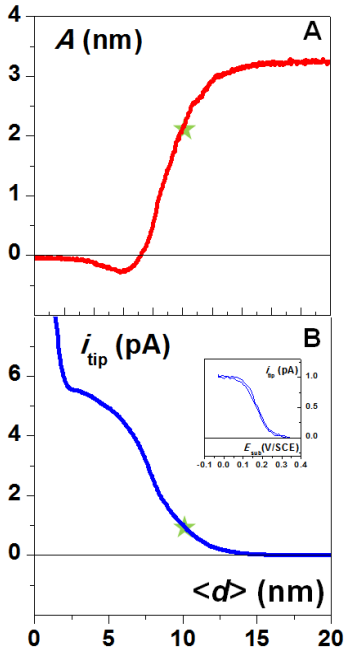


Figure S7. Tapping mode Mt/AFM-SECM amplitude (A) and current (B) approach curves simultaneously recorded upon approaching an oscillating AFM-SECM probe from a HOPG surface bearing a Fc-PEG-Pyrene layer. The abscissa in (A) and (B) is the time-averaged tip-substrate distance <*d*>. The inset in (B) shows the tip current vs. substrate potential dependence recorded by holding the tip at the position denoted by a green star in (A) and (B). The S-shaped curve obtained, characterized by a mid-height potential of ~0.17 V/SCE, ascertains that the current is due to the tip electrochemically interrogating the Fc heads of the surface attached PEG chains.

References

[1] Anne, A.; Moiroux, J. Quantitative Characterization of the Flexibility of Poly(ethylene glycol) Chains Attached to a Glassy Carbon Electrode. *Macromolecules* **1999,** 32, 5829-5835.

[2] Abbou, A.; Anne, A.; Demaille, C. Probing the Structure and Dynamics of End-Grafted Flexible Polymer Chain Layers by Combined Atomic Force - Electrochemical Microscopy. Cyclic Voltammetry within Nanometer-Thick Macromolecular Poly(ethylene glycol) Layers. *J. Am. Chem. Soc.* **2004**, *126*, 10095-10108.

[3] Anne, A.; Demaille, C.; Goyer, C. Electrochemical Atomic-Force Microscopy Using a Tip-Attached Redox Mmediator. Proof-of-Concept and Perspectives for Functional Probing of Nanosystems. *ACS Nano* **2009**, *3*, 819-827.

[4] Abbou, J.; Demaille, C.; Druet, M.; Moiroux, Electrodes of Controlled Geometry for Scanning Electrochemical-Atomic Force Microscopy. J. *Anal. Chem.* **2002**, *74*, 6355-6363.