Supporting Information

for Adv. Mater. Interfaces, DOI: 10.1002/admi.202101677

Hydrophobin-Coated Solid Fluorinated Nanoparticles for $^{19}$F-MRI

Nazeeha Ayaz, Valentina Dichiarante, Claudia Pigliacelli, Jacopo Repossi, Lara Gazzera, Marta Borreggio, Daniele Maiolo, Cristina Chirizzi, Greta Bergamaschi, Linda Chaabane, Elisa Fasoli, Pierangelo Metrangolo,* and Francesca Baldelli Bombelli*
Hydrophobin-coated Solid Fluorinated Nanoparticles for $^{19}$F-MRI

Nazeeha Ayaz, Valentina Dichiarante, Claudia Pigliacelli*, Jacopo Repossi, Marta Borreggio, Daniele Maiolo, Cristina Chirizzi, Greta Bergamaschi, Linda Chaabane, Elisa Fasoli, Pierangelo Metrangolo* and Francesca Baldelli Bombelli*
Supporting Information

**Biocompatible Superfluorinated $^{19}$F-MRI Nanoprobes with a Hydrophobin Surface Shell**

By Nazeeha Ayaz, Valentina Dichiarante, Claudia Pigliacelli, Jacopo Repossi, Marta Borreggio, Daniele Maiolo, Cristina Chirizzi, Greta Bergamaschi, Linda Chaabane, Elisa Fasoli, Pierangelo Metrangolo* and Francesca Baldelli Bombelli*

N. Ayaz, Dr. V. Dichiarante, Dr. C. Pigliacelli& J. Repossi, M. Borreggio, Dr. D. Maiolo, Dr. C. Chirizzi, Prof. E. Fasoli, Prof. P. Metrangolo, Prof. F. Baldelli Bombelli
Department of Chemistry, Materials, and Chemical Engineering "Giulio Natta", Politecnico di Milano, Milano, 20131, Italy
E-mail: pierangelo.metrangolo@polimi.it, francesca.baldelli@polimi.it

Dr G. Bergamaschi
Istituto di Scienze e Tecnologie Chimiche “Giulio Natta” – National Research Council of Italy (SCITEC-CNR), 20131 Milan (Italy)

Dr. L. Chaabane
Institute of Experimental Neurology (INSpe) and Experimental Imaging Center (CIS), IRCCS San Raffaele Scientific Institute, Milano, 20132, Italy

&Affiliation at time of experiments

Dr. C. Pigliacelli
Hyber Center of Excellence, Department of Applied Physics, Aalto University, Puumiehenkuja 2, FI-00076 Espoo, Finland.
**Figure S1**: Water droplets profiles on: (top left) flat pristine Au chips; (top right) F$_{27}$-SH coated Au chips; (bottom left) Au chips coated with F$_{27}$-SH and HFBII and (bottom right) PEG-SH coated Au chips.
Figure S2: QCM-D experiments illustrating a, b) HFBII (first) and FBS (second) deposition on F27-SH functionalized Au QCM crystals; c, d) PEG-SH deposition on Au QCM crystals and further FBS deposition e, f) FBS deposition on pristine Au QCM crystals and g, h) FBS deposition on F27-SH functionalized Au QCM crystal. Frequency changes are displayed in blue, and dissipation changes are displayed in red, while the vertical black lines indicate start of rinsing.
Figure S3: Adsorbed mass (ng cm$^{-2}$) of a) PEG-SH on Au QCM crystals with further FBS binding on the formed PEG-SH layer; FBS binding on b) pristine Au QCM crystals and c) on F$_{27}$-SH functionalized Au QCM crystals.
Figure S4: a) Intensity weighted size distribution of HFB-FNPs as measured by dynamic light scattering at $\theta = 90^\circ$; b) Stability of HFB-FNPs formulations monitored over a period of 7 days at 4°C; c) Comparison among FTIR spectra of PERFECTA, HFBII and HFB-FNPs formulation.
Figure S5: Estimation of PERFECTA concentration present in HFB-FNPs by $^{19}$F NMR (peak at -72.4 ppm) by comparison with an external standard of 5.5μM solution of TFA in D$_2$O (peak at -75.48 ppm).

Figure S6: $^{19}$F NMR spectra of standard HFB-FNPs at pH 7.4 (red line) and the reduction in $^{19}$F signal observed in case of HFB-FNPs at pH 3.7 (green line).

Table S1: Comparison between DLS and NMR characterization of HFB-FNPs at pH 7.4 and pH 3.7.

|                  | pH 7.4  | pH 3.7  |
|------------------|---------|---------|
| HFB-FNPs         |         |         |
| $<R_H>$ [nm]     | 88±4    | 198±10  |
| PDI              | 0.14±0.1| 0.29±0.2|
| Zeta Potential [mV] | -50.8   | 42.1    |
| $^{19}$F atoms/cell | 2.25×10$^{19}$ | 1.08×10$^{19}$ |
Figure S7: a) Intensity weighted DLS size distributions at $\theta = 90^\circ$ of HFB-FNPs in PB, *in situ* and isolated as HC NPs in 10HP; b) Intensity weighted DLS size distributions at $\theta = 90^\circ$ of HFB-FNPs in PB, *in situ* and isolated as HC NPs in 55HP; c) Intensity weighted DLS size distributions at $\theta = 90^\circ$ of HFB-FNPs in PB, *in situ* and isolated as HC NPs in 10HS d, e, f) Main representative classes of proteins identified in the HCs of HFB-FNP isolated from 10HP, 55HP and 10HS, respectively, with corresponding % abundances.
Figure S8: SDS-PAGE of HC isolated after incubation of HFB-FNPs with 10%, 55% HS (v/v). Bands corresponding to control samples (HFB-FNPs in PB, 10HS and 55HS without NPs) can also be observed. The arrow indicate the bands relative to HFBII which is not present in the NP free controls.

Figure S9: Percentage of increase in the number of microglia (BV-2) (with standard deviation expressed as grey lines) cells with respect to the initial number of cells plated, as a function of time.
Figure S10: Molecular structure of poly(ethylene glycol) methyl ether thiol (PEG-SH, average MW: 2000).