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Electronic Supplementary Material

In vivo evaluation of riboflavin receptor targeted fluorescent USPIO in mice with prostate cancer xenografts

Jabadurai Jayapaul[1,2,3] (✉), Susanne Arns[1], Matt Bunker[4,5], Marek Weiler[1], Sandra Rutherford[5], Peter Comba[2], and Fabian Kiessling[1] (✉)

[1] *Institute for Experimental Molecular Imaging, RWTH Aachen University, Pauwelsstrasse 30, Aachen 52074, Germany*
[2] *Anorganisch-Chemisches Institut, Universität Heidelberg, Im Neuenheimer Feld 270, Heidelberg 69120, Germany*
[3] *Molecular Imaging Group, Department of Structural Biology, Leibniz Institut für Molekulare Pharmakologie (FMP), Robert-Rössle-Str. 10, Berlin 13125, Germany*
[4] *AstraZeneca, Pharmaceutical Development, Charter Way, Macclesfield, Cheshire SK10 2NA, UK*
[5] *Molecular Profiles Ltd., 8 Orchard Place, Nottingham Business Park, Nottingham NG8 6PX, UK*

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Contents

Address correspondence to Jabadurai Jayapaul, jayapaul@fmp-berlin.de; Fabian Kiessling, fkiessling@ukaachen.de

S1 Schematics of FLUSPIO synthesis

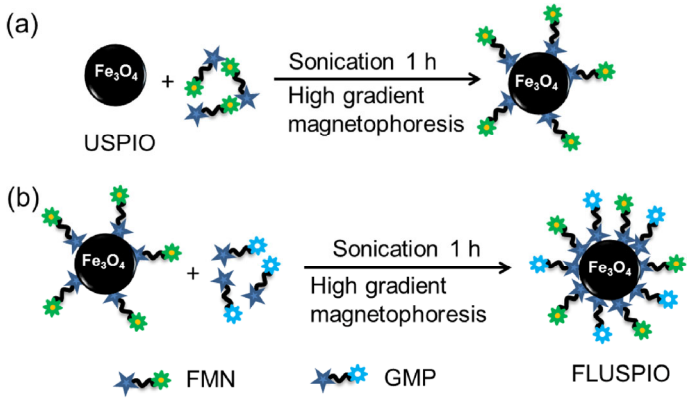

Figure S1 Schematic sketch of the synthetic steps involved in the preparation of FLUSPIO ((a) and (b)). FMN was adsorbed onto USPIO cores by sonication for 1 h at ambient temperature. Then, the particles were purified from excess FMN by high gradient magnetophoresis assisted washing with water (a). Similarly, the FMN-coated USPIO were subjected to coating with GMP for 1 h and purified with magnetophoresis to yield the final FLUSPIO particles (b).

S2 Theoretical calculation of number of FMN per USPIO

The topological polar surface area of FMN has been quoted as 202 Å^2 [S1].

From this, we can calculate the theoretical number of ligands for one mono-layer on the surface of 5.5 nm diameter USPIO nanoparticle.

Surface area (SA) of a 5.5 nm USPIO

$$\begin{aligned} SA_{USPIO} &= 4\pi r^2 \\ &= 4 \times 3.14 \times (2.75\ nm)^2 \\ &= 94.99\ nm^2 \end{aligned}$$

Theoretical prediction of number of FMN ligands that can fit as one monolayer on a 5.5 nm USPIO (L)

$$\begin{aligned} L &= SA_{USPIO}/SA_{FMN} \\ &= 94.99\ nm^2/20.2\ nm^2 \\ &= 4.7\ ligands \end{aligned}$$

Based on a 100% magnetite USPIO, it is possible to calculate the number of (Fe_3O_4) units per USPIO

$$\begin{aligned} \text{Mass of one USPIO } (M_{USPIO}) &= \rho_{Fe_3O_4} \times (4/3\pi r^3) \\ &= 5.175\ g{\cdot}cm^{-3} \times (4/3 \times 3.14 \times (2.75\ nm)^3) \\ &= 5.175\ g{\cdot}cm^{-3} \times (87.069 \times (10^{-7})^3\ cm^3) \\ &= 450.585 \times 10^{-21}\ g \end{aligned}$$

$$\begin{aligned} N_{Fe_3O_4} &= M_{USPIO} \times N_A / M_{WFe_3O_4} \\ &= [(450.585 \times 10^{-21}\ g) \times (6.023 \times 10^{23})]/231.533\ g{\cdot}mol^{-1} \\ &= 1172 \end{aligned}$$

The predicted molar composition of USPIO with one monolayer of FMN would be $(Fe_3O_4)_1(FMN)_{0.004}$.

Considering the topological polar surface area of GMP (202 Å^2) [S2], the theoretical composition of FLUSPIO with one monolayer of FMN and GMP will be $(Fe_3O_4)_1(FMN)_{0.004}(GMP)_{0.004}$.

S3 TEM images of FLUSPIO

The morphology, size and size distribution of the nanoparticles were characterized by TEM (EM 400 T, Philips, Eindhoven, The Netherlands). FMN-USPIO and FLUSPIO were embedded in 2% agarose, rinsed with distilled

water and dehydrated with ethanol (30%, 50%, 70%, 90%, and 100%). Subsequently, they were embedded in Epon, polymerized 8 h at 37 ˚C, 56 h at 60 ˚C, and then cut into 70–100 nm thick slices. The particle size and size distributions were calculated using an image analysis program (iTEM software, Olympus, Germany) and by measuring the diameter of at least 100 particles.

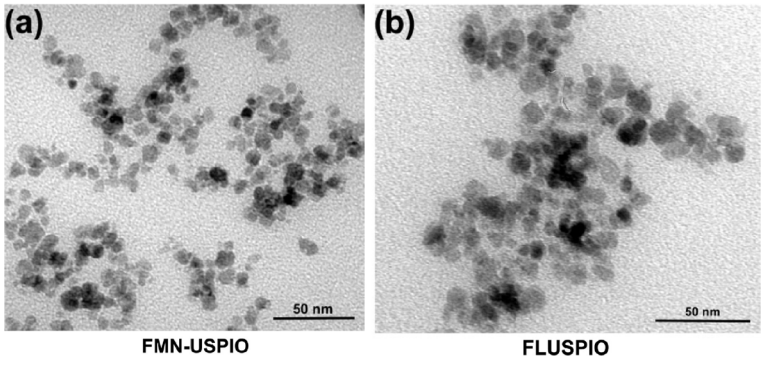

Figure S2 TEM images of FMN-coated USPIO and FLUSPIO ((a) and (b)). TEM image of FMN-coated USPIO prepared during the first synthetic step. Quantitative analysis of the images revealed particle size of 5.6 ± 1.3 nm (a). Similar quantitative analysis of the final FLUSPIO particles revealed particle size of 5.6 ± 1.7 nm (b).

S4 Fluorescence spectroscopy

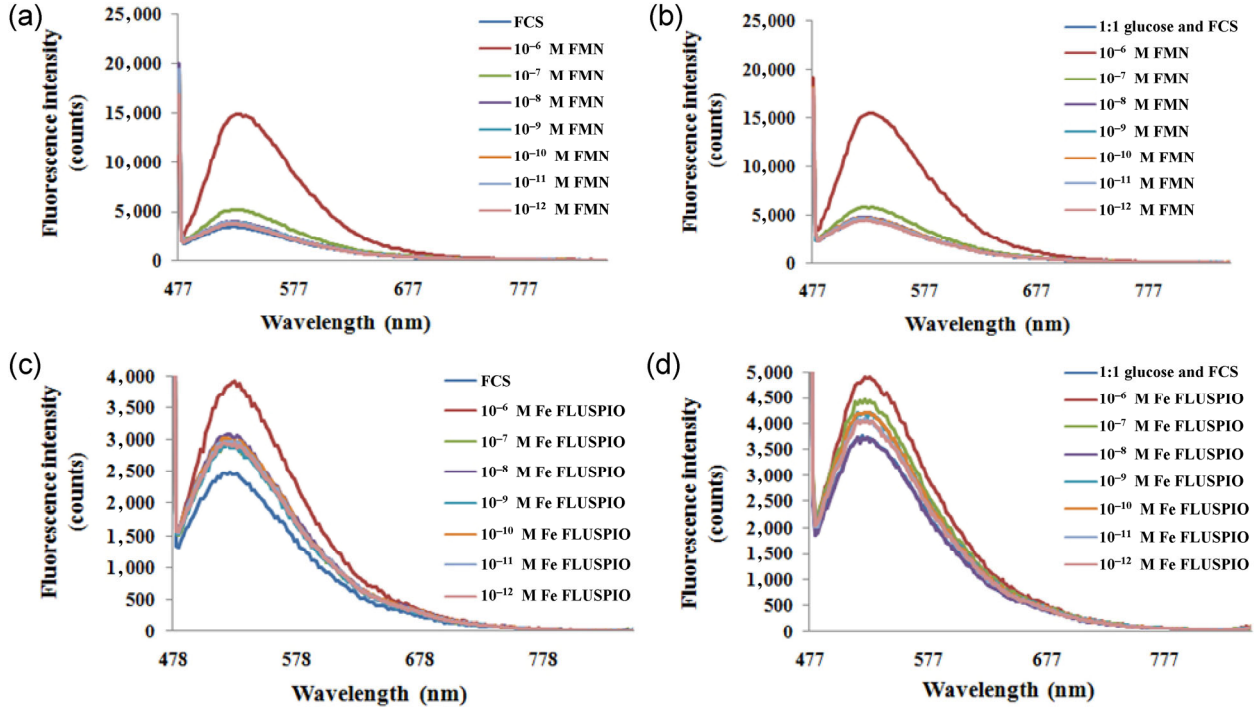

Figure S3 Fluorescence spectra of FMN ((a) and (b)) and FLUSPIO ((c) and (d)) recorded in FCS ((a) and (c)) and in a 1:1 mixture of FCS and 5% glucose solution ((b) and (d)) at different concentrations. No significant changes in fluorescence intensity of FMN are observed in FCS alone and in 1:1 mixture of FCS and glucose solution. However, there is a moderate decrease in fluorescence intensity of FMN adsorbed on FLUSPIOs' surface compared to free FMN which may be explained by partial quenching occurring due to densely packed FMN on USPIO surface and also due to intrinsic absorption/scattering contributions from USPIO.

S5 Cytotoxicity of FLUSPIO (Trypan blue staining)

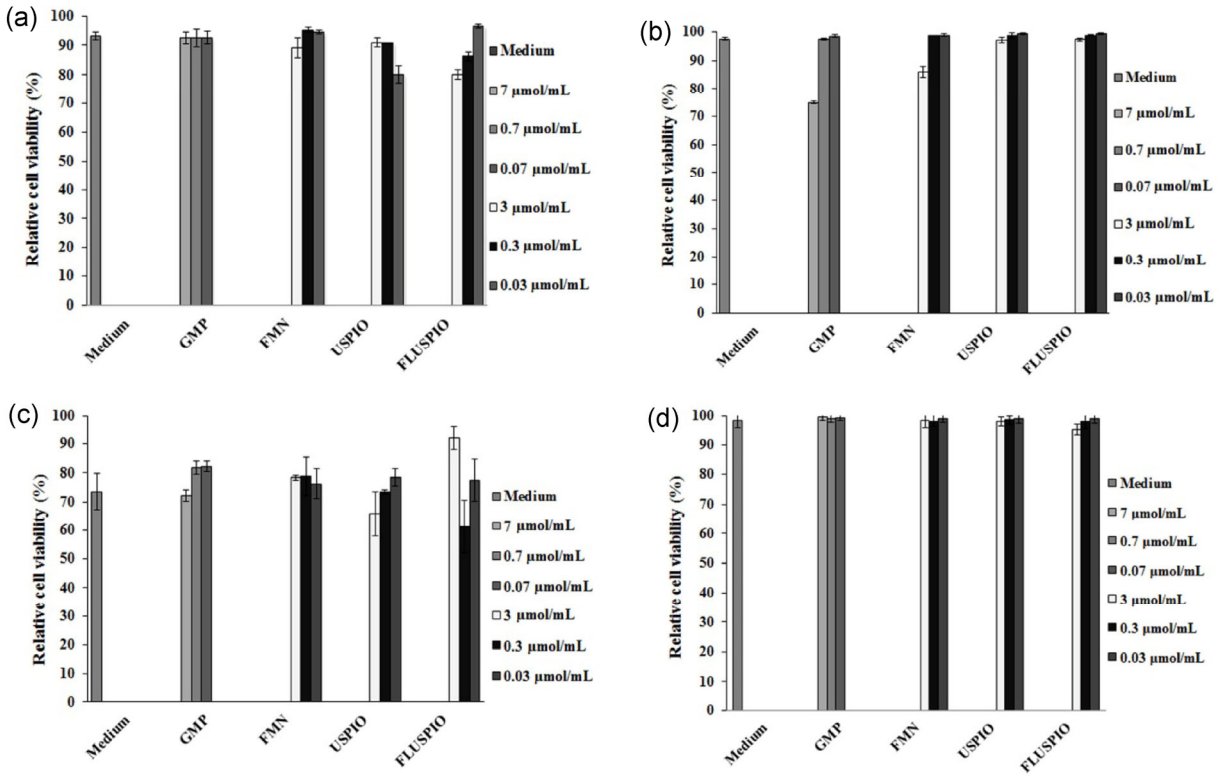

Figure S4 Cytotoxicity (trypan blue staining) of GMP, FMN, USPIO, and FLUSPIO on LnCap cells ((a) and (b)) and HUVEC ((c) and (d)) after incubation for 3 h ((a) and (c)) and 24 h ((b) and (d)). No significant reduction in viability of LnCap cells and HUVEC was found.

S6 Cellular labeling with FLUSPIO (Prussian blue staining)

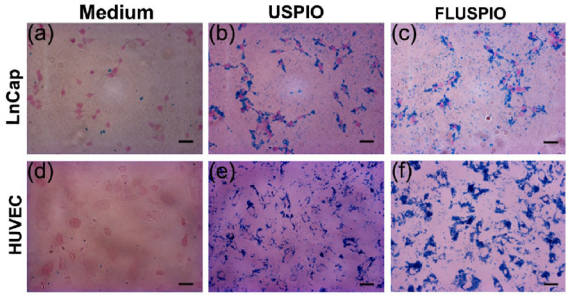

Figure S5 Cellular uptake of FLUSPIO by LnCap cells and HUVEC as investigated by Prussian blue staining. LnCap cells and HUVEC were incubated (3 h) with medium ((a) and (d)), 0.3 μmol Fe/mL of USPIO ((b) and (e)) and FLUSPIO ((c) and (f)). Cells were counterstained with nuclear fast red. Prussian blue images clearly indicate higher internalization of FLUSPIO by HUVEC than LnCap cells and than USPIO ((b) and (e)). Microscopic images were acquired at 20× magnification. Scale bar: 50 μm.

References

[S1] *Flavin Mononucleotide*; U.S. National Library of Medicine: Bethesda, MD, Sep 16, 2004. http://pubchem.ncbi.nlm.nih.gov/compound/Riboflavin_5_-phosphate#section=Chemical-and-Physical-Properties (accessed Oct 30, 2015).

[S2] *5′-Guanylic Acid*; U.S. National Library of Medicine: Bethesda, MD, Sep 16, 2004. http://pubchem.ncbi.nlm.nih.gov/compound/6804#section=Chemical-and-Physical-Properties (accessed Oct 30, 2015).