Functionalized Fluorescent silica nanoparticles for bioimaging of cancer cells

Ruth Prieto-Montero¹, Alberto Katsumiti², Miren Cajaraville², Íñigo López Arbeloa³ and Virginia Martinez-Martinez ¹*

¹Departamento de Química Física, Universidad del País Vasco/Euskal Herriko Unibertsitatea (UPV/EHU) Apartado 644, 48080, Bilbao, Spain.

²Departamento de Zoología y Biología Celular Animal, Universidad del País Vasco/Euskal Herriko Unibertsitatea (UPV/EHU) Apartado 644, 48080, Bilbao, Spain.

*Correspondence: virginia,martinez@ehu.eus; Tel.: +34 946015969
**Figure S1.** SEM image of MSNs

**Figure S2.** TEM images for ORMOSIL nanoparticles using different second silica source and proportions
Figure S3. TEM images for MSN-C-R101-80

Figure S4: FT-IR spectra of MSN (black) and MSN-PEG (red). Most of the peaks from 400 cm$^{-1}$ to 1200 cm$^{-1}$, and particularly the main vibration band at 1100 cm$^{-1}$ are attributed to Si-O-Si vibrations, are present in both samples. The presence of PEG molecules can be verified by the IR band at 2960 cm$^{-1}$ assigned to stretching vibration of CH$_2$ groups of the alkyl chains and the peak at 1460 cm$^{-1}$ to their deformation vibration. The broad band at 2875 cm$^{-1}$ can be assigned to stretching modes of the CH$_3$ groups. The shoulder at 1260 cm$^{-1}$ that appears in the main band may be assigned to stretching vibrations of C-O-C ether bonds.
**Figure S5.** Normalized absorption spectra to the absorption peak of R101 for MSN-S-R101-60-PEG-FA (black), and MSN-S-R101-60-PEG (brown) and FA (blue) (right) and emission spectra for MSN-S-R101-60-PEG-FA under 355 nm excitation wavelength (left). Black and red arrows correspond to absorption (left) and emission (right) bands of folic acid and rhodamine 101, respectively.

**Video 1.** Fluorescence images of MSN-S-R101-60-PEG internalized into lysosomes of HeLa cells; images show lysosomes (green), rhodamine 101 from (red). Scale br 10 μm.

**Video 2.** Fluorescence images of MSN-S-R101-60-PEG internalized into lysosomes of HeLa cells; images show lysosomes (green), rhodamine 101 from (red). Scale br 10 μm.
Technical note:

The relative brightness of the nanoparticles with respect to free dye in solution is calculated following the equation[1]:

$$\text{Relative Brightness} = \frac{(I_{fl}^{NP}) / C^{NP}}{(I_{fl}^{R101}) / C^{R101}}$$

Being $I_{fl}^{NP}$ and $I_{fl}^{R101}$ the fluorescence intensity of the nanoparticles suspension and dye solution, respectively and $C^{NP}$ and $C^{R101}$ the number of particles and R101 dye molecules in suspension and diluted solution, respectively.

The number of particles is estimated by the concentration of the particles in solution (mg mL$^{-1}$), the density of the porous silica nanoparticles (1.6 g cm$^{-3}$) and the average diameter of the nanoparticles (d = 60 nm).

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[1] Cho, E.B.; Volkov, D.O.; Sokolov, I. Ultrabright fluorescent silica mesoporous silica nanoparticles: Control of particle size and dye loading. *Adv. Funct. Mater.* **2011**, *21*, 3129–3135, doi:10.1002/adfm.201100311.