Electronic Supplementary Material (ESI) for Journal of Materials Chemistry B.
This journal is © The Royal Society of Chemistry 2014

Supporting information for "Silica Nanoreactors from Silylated Riboflavin for Efficient Singlet Oxygen Delivery"

Natalia C. Angeluzzi, Marcelo Muñoz, Daniela T. Marquez, Mauricio Baptista, Ana Maria Edwards, Emilio I. Alarcon, and Juan C. Scaiano

Experimental

Chemicals: Riboflavin, Igepal-CO, tetraethyl ortosilicate, N, N-Dimethyl formamide, cyclohexane, dihydroethidium, sodium azide, and triethylamine were purchased from Sigma-Aldrich (>95%). Methanol, ammonium hydroxide, 3-(Triethoxysilyl)propyl isocyanate were purchased from Fisher Scientific and used as received.

Synthesis and characterization of RF-TPI$_4$: To a 0.27 mmol of riboflavin, 1.2 mmol of 3-(Triethoxysilyl)propyl isocyanate (TPI) were added in 50 ml dry DMF in the presence of 250 μl of triethylamine (Et_3N). The reaction was performed at 70ºC for 48 h in a dry N_2 saturated atmosphere in the dark. Note that the RF-TPI$_4$ product showed good solubility in organic solvents such as chloroform, something that also indicates the actual modification of the ribityl chain, *vide infra*. ^{1}H-NMR ($CDCl_3$; 300 MHz): δ 0.55 (m, 8H), δ 1.31 (m, 36H), δ 1.53 (m, 8H), δ 2.33 (s, 3H), δ 2.47 (s, 3H), δ 3.07 (m, 8H), δ 3.72 (m, 24H), δ 3.99 (dd, 1H), δ 4.25 (dd, 1H), δ 4.97 (m, 1H), δ 5.19 (m, 1H), δ 5.51 (m, 2H), δ 5.72 (m, 2H), δ 7.84 (m, 2H). IR ν/cm^{-1}= 3400 (N-H stretching), 3100 (-CO-NH- stretching), 1680-1670 (CO vibration), 1520 (CO vibration).

RF-TPI was prepared using a third of the TPI reagent employed for the synthesis of RF-TPI$_4$.

Synthesis of RF-TPI$_4$@NP: Spherical silica nanoparticles were prepared based on the procedure described on the literature by Philipse et al.[1] Briefly, 640 mg IGEPAL–CO were added to 10 ml cyclohexane and sonicated for 10 minutes, then while stirring, 300 μl of RF-TPI$_4$ (1.0 mg/ml in toluene), 136 μl NH4OH, and 110 ul TEOS were added and then left stirring for 8 hours in the dark. The solution was centrifuged at 10000 rpm (F14-6X250LE rotor; 15316 G) for 5 minutes and then washed with 5.0 ml of methanol, this procedure was repeated three times. The particles and the RF-TPI$_4$ not incorporated are washed out in the methanolic phase. The product was isolated by centrifugation (12250 G, 10 min) and then washed with methanol until no RF-TPI$_4$ was detectable in the UV-Vis. Control samples prepared without RF-TPI$_4$ were prepared following the same protocol described above. Measurements of the unbound RF-TPI$_4$ in the methanolic phase render that ≈ 20% of the initial concentration of the dye was incorporated into the nanoparticles.

Physical characterization of the nanomaterials: RF-TPI$_4$@NP were also characterized using a JSM-7500F field emission scanning microscope (SEM) from Jeol Ltd. The SEM samples were prepared depositing 10 µl of the nanoparticles suspension onto a Cu grid and dried under vacuum in a desiccator for 24h. Suspension of the nanoparticles were also characterized by using dynamic light scattering in a Malvern Zetasizer Nano ZS at 20ºC in 1.0 cm pathlength disposable cuvettes. Zeta potential for the nanoparticles was measured in a 0.5 ml zeta potential organic solvent compatible cuvette (from Malvern).

Photophysical characterization of RF-TPI$_4$ and RF-TPI$_4$@NP: Absorption spectra were recorded in a Cary-100 UV-Visible spectrophotometer using 1x1 cm quartz cuvettes. Fluorescence emission measurements were carried out in a Photon Technology International (PTI) spectrofluorometer at room temperature. Emission spectra were performed at 1000 nm/min speed to avoid dye photobleaching. Fluorescence quantum yields were calculated from the integration of fluorescence emission spectra and expressed as the area under curve (AUC) are related to the sample fluorescence quantum yiel (Φ_{Fl_sample}) as:

$$\Phi_{FL\_Sample} = \Phi_{RF-EtOH} \frac{\left(\frac{AUC_{sample}}{A_{450nm\_Sample}}\right) \times \eta^2_{sample}}{\left(\frac{AUC_{RF-EtOH}}{A_{450nm\_RF-EtOH}}\right) \times \eta^2_{RF-EtOH}} \quad (1)$$

with Φ_{Fl} RF-EtOH = 0.32.[2] Fluorescence lifetimes were measured in an Easy-Life (from PTI) system using a 350 nm LED pulse excitation and the lifetimes calculated in the Easy-Life integrated software.

Time resolved measurements: Nanosecond laser flash photolysis measurements were carried out in a LFP 111 laser-flash photolysis system (Luzchem Inc., Ottawa, Canada) by using a Nd-YAG 355 nm at 10 mJ/pulse in 1.0 cm path-length fussed silica cuvettes. Triplet quantum yields were calculated on the basis that the RF electronic transitions in the triplet-excited state were not modified upon TPI incorporation, using pure RF as standard. Note that the values reported in this work are expressed as relative to the pure RF dye. Finally, the generation of singlet oxygen and its lifetime were determined from phosphorescence decay curves at 1270 nm. Data were recorded with a Hamamatsu NIR detector (peltier cooled at -62.8ºC operating at 800 V, coupled to a grating monochromator) upon excitation with a 355 nm Nd:YAG laser. A customized Luzchem Research LFP-111 system was employed to collect and process the data.

Photodegradation studies: Photostability of RF, RF-TPI_4 and RF-TPI_4@NP, was assessed by measuring changes in the absorption spectra at different irradiation times upon UVA exposure in a CCP-4V computer controlled photoreactor (Luzchem®, Ottawa, ON, Canada) with 14 UVA lamps.

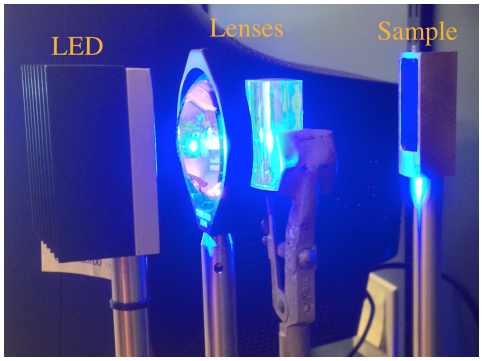
Protein oxidation and crosslinking: In all cases, 10 mM pH 7.4 phosphate buffer solutions containing 50 μM human serum albumin and the RF derivative was irradiated in a CCP-4V computer controlled photoreactor (Luzchem®, Ottawa, ON, Canada) with 14 UVA lamps for incremental time intervals. At each time interval microliter aliquots of solution were taken and stored at -80°C in the dark for their subsequential peroxides, carbonyl, or LDS electrophoresis. Oxygen consumption measurements were also carried out using a MI-730 mMicro-oxygen electrode Bedford (Microelectrodes, Inc.) and the effect of sodium azide also assessed.

Peroxides: Briefly, total peroxide content was determined by using a colorimetric Peroxo-Quant (from Thermo Scientific), which is based on the Fe(II)/(III) oxidation upon Fenton reaction with the peroxides. The resulting Xylenol orange/Fe(III) complex produce a new absorption measured at 595 nm[3] in a microplate reader, which is proportional to a given concentration of peroxides.

Carbonyls: Protein oxidation was studied using a protein carbonyl colorimetric assay kit, which is based on the reaction between 2,4-dinitrophenylhygrazine (DNPH) and protein carbonyls. DNPH reacts with protein carbonyls forming a Schiff base to produce the corresponding hydrazone, which can be analyzed spectrophotometrically by measuring its absorbance at 370 nm. [4]

Oxidation of dihydroethidium: 20 μM dihydroethidium was employed as superoxide target.[5, 6] The samples were irradiated using a 465±10 nm LED system equipped with a set of lenses that allowed to homogeneously expand the LED beam and give to it a rectangular shape as seen in the picture below. Fluorescence emission was

detected upon 518 nm excitation and recorded between 525-690 nm with a scan speed of 600 nm/min in a LS-50 Perkin Elmer fluorometer.



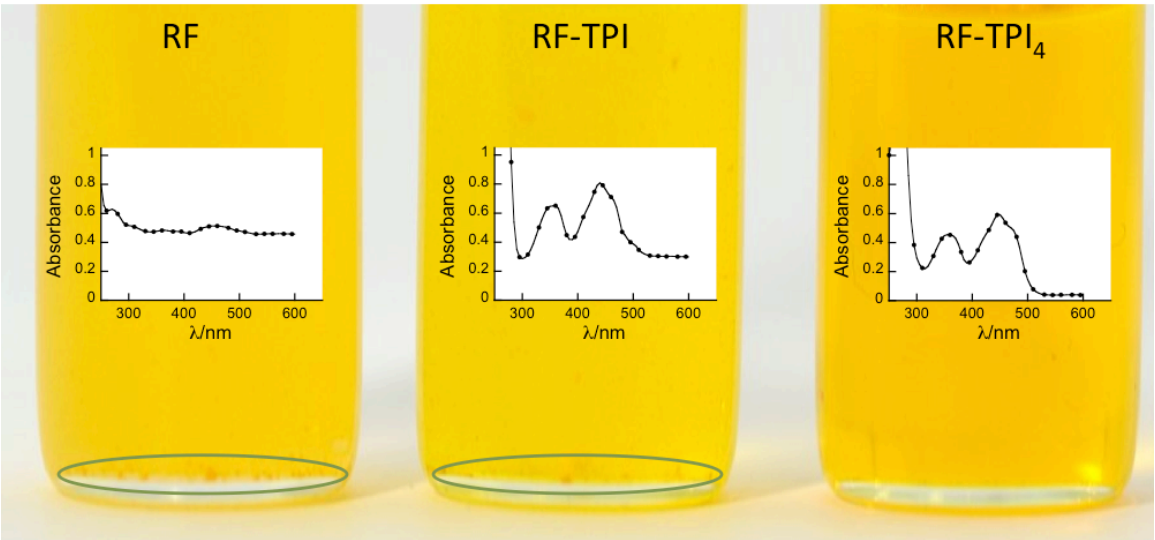


Figure S1. Actual transmission digital pictures of chloroform solutions (1.0 mg/ml) for RF and the RF-TPI derivatives. Note that RF forms a colloidal suspension in chloroform that spontaneously will form precipitate (green circled). Upon the incorporation of only one TPI chain, the tendency to form those precipitate decreases but it is still present (see middle picture). However, further addition of TPI leads to a complete solubilization of the compound in chloroform (right picture). Absorption spectra for the samples shown here have also been included where it can also be seen the reduction of the light scattering as the number of TPI chains increases (from left to right).

1. A. P. Philipse, M. P. B. van Bruggen and C. Pathmamanoharan, Magnetic silica dispersions: preparation and stability of surface-modified silica particles with a magnetic core, *Langmuir*, 1994, **10** (1), 92-99.
2. J. Kozio and E. Knobloch, The solvent effect on the fluorescence and light absorption of riboflavin and lumiflavin, *Biochim. Biophys. Acta*, 1965, **102** (1), 289-300.
3. Y. Jiang, J. V. Hunt and S. P. Wolff, Ferrous oxidation in the presence of xylenol orange for detection of lipid hydroperoxides in low density lipoproteins, *Anal. Biochem.*, 1992, **202** (2), 384-389.
4. R. L. Levine, J. A. Williams, E. P. Stadtman, E. Shacter and P. Lester, in *Met. Enzymol.*, Academic Press, 1994, pp. 346-357.
5. B. Fink, K. Laude, L. McCann, A. Doughan, D. G. Harrison and S. Dikalov, Detection of intracellular superoxide formation in endothelial cells and intact tissues using dihydroethidium and an HPLC-based assay, *Am. J. Physiol. Cell Physiol.*, 2004, **287** (4), C895-C902.
6. H. M. Peshavariya, G. J. Dusting and S. Selemidis, Analysis of dihydroethidium fluorescence for the detection of intracellular and extracellular superoxide produced by NADPH oxidase, *Free Rad. Res.*, 2007, **41** (6), 699-712.