

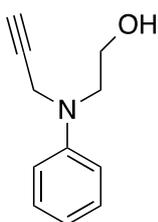
Supplementary information

Dye-doped silica nanoparticle probes for fluorescence lifetime imaging of reductive environment in living cells

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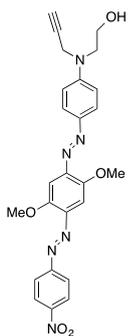
1. Synthesis and characterization

1.1. Synthesis and characterization of BHQ derivatives



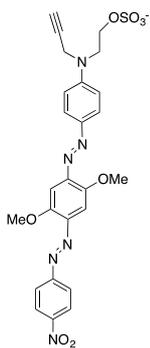
1: in a flamed 250 mL round bottom flask dried with a heat gun under a flow of Argon, to a solution of N-(2-Hydroxyethyl)aniline (5 g, 36.45 mmol) in Dimethyl sulfoxide (DMSO) (40 mL) was added Propargyl bromide (solution 80 wt. % in toluene) (4.8 mL, 43.10 mmol) and Sodium bicarbonate (6.13 g, 73 mmol) and the reaction mixture was stirred at room temperature for 15 h. The reaction mixture was then diluted with H₂O (40 mL) and extracted four times with Ethyl acetate (EtOAc). The combined organic phases were washed two times with H₂O and one time with Brine, dried over Sodium sulfate and concentrated *in vacuo* to give the title compound **1** as a yellow-brown oil (6.219 g, yield 98 %); $R_f = 0.61$ (SiO₂, EtOAc:Heptane (Hept) = 60/40).

- **Chemical formula:** C₁₁H₁₃NO;
- **Molecular Weight:** 175.2;
- **¹H-NMR** (400 MHz, CDCl₃, 25°C): $\delta = 7.31$ (2H, t, $J = 8.2$ Hz, ar. CH), 6.95 (2H, d, $J = 7.8$ Hz, ar. CH), 6.87 (1H, t, $J = 7.3$ Hz, ar. CH), 4.11 (2H, d, $J = 2.4$ Hz, -NCH₂-), 3.86 (2H, t, $J = 5.1$ Hz, -NCH₂CH₂-), 3.59 (2H, t, $J = 5.5$ Hz, -CH₂CH₂OH), 2.28 (1H, t, $J = 2.4$ Hz, -CCH), 2.05 (1H, s, -CH₂OH);
- **¹³C-NMR** (100 MHz, CDCl₃, 25°C): $\delta = 148.3, 129.4, 119.0, 114.8, 80.1, 72.6, 60.2, 54.0, 41.1$;
- **ESI-MS** m/z calculated for C₁₁H₁₃NO⁺ is 175.1; obs.: 176.1 (M+H⁺).



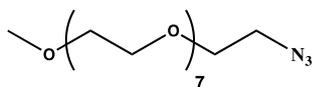
BHQ-OH: in a flamed 1 L round bottom flask dried with a heat gun under a flow of Argon, the compound **1** (5.077 g, 29 mmol) was solubilized with 100 mL of 1,4-Dioxane (Diox). Fast Black K salt (30% dye content, 20.4 g, 14.64 mmol) was solubilized in a mixture 1,4-Diox/H₂O (7/3, 400 mL) and added to the previous solution, and the reaction mixture was stirred at room temperature for 72 h. Diox was then removed *in vacuo*; a black solid was attached to the flask, and H₂O was discarded. The residue was purified by silica gel chromatography eluting with EtOAc/Hept (9:1-5:5) to give the compound **BHQ-OH** as a purple solid (1.417 g, yield 10 %); $R_f = 0.37$ (SiO₂, EtOAc:Hept = 60/40).

- **Chemical formula:** C₂₅H₂₄N₆O₅;
- **Molecular Weight:** 488.5;
- **¹H-NMR** (400 MHz, CDCl₃, 25°C): $\delta = 8.39$ (2H, d, $J = 8.9$ Hz, ar. CH), 8.06 (2H, d, $J = 8.8$ Hz, ar. CH), 7.99 (2H, d, $J = 8.9$ Hz, ar. CH), 7.51 (1H, s, ar. CH), 7.48 (1H, s, ar. CH), 6.99 (2H, d, $J = 9.5$ Hz, ar. CH), 4.25 (2H, s, -NCH₂C-), 4.10 (3H, s, -OCH₃), 4.06 (3H, s, -OCH₃), 3.95 (2H, t, $J = 5.1$ Hz, -NCH₂CH₂OH), 3.74 (2H, t, $J = 5.2$ Hz, -CH₂CH₂OH), 2.34 (1H, s, -CCH);
- **¹³C-NMR** (100 MHz, CDCl₃, 25°C): $\delta = 156.6, 153.7, 151.3, 151.0, 148.6, 146.6, 145.7, 142.6, 126.0, 124.9, 123.8, 113.1, 101.3, 100.3, 79.5, 73.0, 60.6, 57.0, 56.9, 53.9, 41.3$;
- **ESI-MS** m/z calculated for C₂₅H₂₄N₆O₅⁺ is 488.2; obs.: 489.2 (M+H⁺).



BHQ-SO₃: in a flamed 50 mL round bottom flask dried with a heat gun under a flow of Argon, to a solution of **BHQ-OH** (0.2 g, 0.41 mmol) in Dimethylformamide (DMF, 15 mL) was added Sulfur trioxide triethylamine complex (0.8 g, 4.4 mmol) and the reaction mixture was heated at 55°C for 18 h. After cooling down to room temperature, the reaction mixture was then concentrated *in vacuo* and the residue was purified by silica gel chromatography eluting with Dichloromethane (DCM)/Methanol (MeOH) (9:1-8:2) to give the compound **BHQ-SO₃** as a purple solid (0.202 g, yield 87 %); $R_f = 0.56$ (SiO₂, DCM:MeOH = 80/20).

- **Chemical formula:** C₂₅H₂₃N₆O₈S⁻;
- **Molecular Weight:** 567.5;
- **¹H-NMR** (400 MHz, CDCl₃, 25°C): $\delta = 8.38$ (2H, d, $J = 9.0$ Hz, ar. CH), 8.05 (2H, d, $J = 9.0$ Hz, ar. CH), 7.95 (2H, d, $J = 9.1$ Hz, ar. CH), 7.51 (1H, s, ar. CH), 7.46 (1H, s, ar. CH), 6.96 (2H, d, $J = 9.2$ Hz, ar. CH), 4.33 (2H, t, $J = 6.2$ Hz, -CH₂CH₂OS-), 4.25 (2H, d, $J = 2.0$ Hz, -NCH₂C-), 4.10 (3H, s, -OCH₃), 4.05 (3H, s, -OCH₃), 3.87 (2H, t, $J = 6.0$ Hz, -NCH₂CH₂OS-), 2.26 (1H, t, $J = 2.2$ Hz -CCH);
- **¹³C-NMR** (100 MHz, CDCl₃, 25°C): $\delta = 156.4, 153.5, 151.0, 148.4, 146.5, 145.1, 142.3, 132.3, 125.8, 124.7, 123.6, 112.7, 101.1, 100.2, 72.4, 64.8, 56.8, 50.5, 46.1$;
- **ESI-MS** m/z calculated for C₂₅H₂₃N₆O₈S⁺ is 567.1; obs.: 567.1.

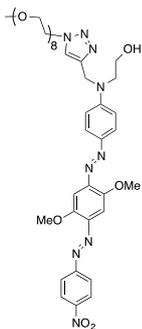


2: in a flamed 25 mL round bottom flask dried with a heat gun under a flow of Argon, to a solution of octaethylene glycol monomethyl ether (0.5 g, 1.3 mmol) in Chloroform (CHCl₃, 5 mL) was added Pyridine (Pyr, 0.105 mL, 1.3 mmol) and Thionyl chloride (0.142 mL, 1.95 mmol) and the reaction mixture was heated at 60°C for 1 h. After cooling down to room temperature, the reaction mixture was then concentrated *in vacuo* to give **Cl-PEG₈-OMe** as a yellow oil (0.445 g, yield 85 %) which was used for the next step without further purification.

- **Chemical formula:** C₁₇H₃₅ClO₈;
- **Molecular Weight:** 402.9;
- **¹H-NMR** (400 MHz, CDCl₃, 25°C): $\delta = 3.76$ (2H, t, $J = 5.8$ Hz, -CH₂Cl), 3.69-3.62 (28H, m, -OCH₂CH₂O-), 3.56-3.54 (2H, m, -CH₂CH₂Cl), 3.38 (3H, s, -OCH₃);
- **¹³C-NMR** (100 MHz, CDCl₃, 25°C): $\delta = 71.7, 71.1, 70.4, 70.3, 70.2, 58.8, 42.6$;
- **ESI-MS** m/z calculated for C₁₇H₃₅ClO₈⁺ is 402.2; obs.: 420.2 (M+NH₄⁺).

In a flamed 25 mL round bottom flask dried with a heat gun under a flow of Argon, to a solution of **Cl-PEG₈-OMe** (0.969 g, 2.4 mmol) in DMF (9.5 mL) was added sodium azide (0.313 g, 4.8 mmol) and the reaction mixture was heated at 80°C for 18 h. After cooling down to room temperature, the reaction mixture was then concentrated *in vacuo* and the obtained residue was redispersed with 5 mL of H₂O and extracted four times with DCM. The combined organic phases were dried over Sodium sulfate and concentrated *in vacuo* to give the compound **2** as a yellow oil (0.908 g, yield 93 %); $R_f = 0.51$ (SiO₂, DCM:MeOH = 95/5).

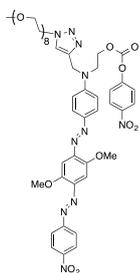
- **Chemical formula:** C₁₇H₃₅N₃O₈;
- **Molecular Weight:** 409.5;
- **¹H-NMR** (400 MHz, CDCl₃, 25°C): $\delta = 3.66$ -3.60 (28H, m, -OCH₂CH₂O-), 3.53-3.51 (2H, m, -CH₂CH₂N₃), 3.37-3.36 (2H, m, -CH₂N₃), 3.35 (3H, s, -OCH₃);
- **¹³C-NMR** (100 MHz, CDCl₃, 25°C): $\delta = 71.2, 70.6, 69.9, 69.8, 69.7, 69.3, 58.2, 49.9, 42.1$;
- **ESI-MS** m/z calculated for C₁₇H₃₅N₃O₈⁺ is 409.2; obs.: 427.3 (M+NH₄⁺).



3: in a flamed 250 mL round bottom flask dried with a heat gun under a flow of Argon, **BHQ-OH** (0.709 g, 1.45 mmol) and the compound **2** (0.714 g, 1.74 mmol) were solubilized with 65 mL of Diox. Copper(II) sulfate pentahydrate (0.181 g, 0.73 mmol) and Sodium ascorbate (0.201 mg, 1.02 mmol) were solubilized with 3 mL of H₂O and added to the previous solution, and the reaction mixture was stirred at room temperature for 30 min. The reaction mixture was then concentrated *in vacuo* and the residue was purified by silica gel chromatography eluting with DCM/MeOH (9:1) to give the compound **3** as a purple solid (0.975 g, yield 75 %); R_f = 0.24 (SiO₂, DCM:MeOH = 90/10).

- **Chemical formula:** C₄₂H₅₉N₉O₁₃;

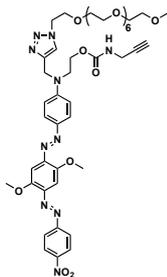
- **Molecular Weight:** 898.0;
- **¹H-NMR** (400 MHz, CDCl₃, 25°C): δ = 8.36 (2H, d, *J* = 9.0 Hz, ar. CH), 8.03 (2H, d, *J* = 9.1 Hz, ar. CH), 7.89 (2H, d, *J* = 9.0 Hz, ar. CH) - 7.87 (1H, s, -NCH) overlapped, 7.47 (1H, s, ar. CH), 7.43 (1H, s, ar. CH) 6.92 (2H, d, *J* = 7.4 Hz, ar. CH), 4.82 (2H, s, -NCH₂C-), 4.52 (2H, t, *J* = 5.0 Hz, -NCH₂CH₂O-), 4.06 (3H, s, -OCH₃), 4.02 (3H, s, -OCH₃), 3.95 (2H, t, *J* = 4.6 Hz, -NCH₂CH₂OH), 3.83 (2H, t, *J* = 4.8 Hz, -CH₂CH₂OH), 3.64-3.60 (30H, m, -OCH₂CH₂O-), 3.35 (3H, s, -CH₂OCH₃);
- **¹³C-NMR** (100 MHz, CDCl₃, 25°C): δ = 156.6, 153.7, 151.2, 151.1, 148.6, 146.8, 145.1, 145.0, 142.4, 126.0, 124.9, 123.7, 123.6, 112.4, 101.2, 100.3, 72.1, 71.5, 70.8, 70.7, 69.5, 60.7, 59.2, 57.0, 55.8, 50.7, 47.7, 42.9;
- **ESI-MS** m/z calculated for C₄₂H₅₉N₉O₁₃⁺ is 897.4; obs.: 898.4 (M+H⁺).



4: in a flamed 100 mL round bottom flask dried with a heat gun under a flow of Argon, **3** (0.975 g, 1.09 mmol), Pyr (0.264 mL, 3.27 mmol) and 4-Nitrophenyl chloroformate (0.659 g, 3.27 mmol) were solubilized with 36 mL of DCM and the reaction mixture was stirred at room temperature for 18 h. The reaction mixture was then concentrated *in vacuo* and the residue was purified by silica gel chromatography eluting with EtOAc/Acetone (AcO) (1:1) to give the compound **4** as a purple oil (0.819 g, yield 71 %); R_f = 0.22 (SiO₂, EtOAc:AcO = 50/50).

- **Chemical formula:** C₄₉H₆₂N₁₀O₁₇

- **Molecular Weight:** 1063.1
- **¹H-NMR** (400 MHz, CDCl₃, 25°C): δ = 8.39 (2H, d, *J* = 9.0 Hz, ar. CH), 8.27 (2H, d, *J* = 9.1 Hz, ar. CH), 8.06 (2H, d, *J* = 8.9 Hz, ar. CH), 7.97 (2H, d, *J* = 9.0 Hz, ar. CH), 7.72 (1H, s, -NCH), 7.51 (1H, s, ar. CH), 7.47 (1H, s, ar. CH), 7.33 (2H, d, *J* = 9.2 Hz, ar. CH), 6.98 (2H, d, *J* = 9.1 Hz, ar. CH), 4.85 (2H, s, -NCH₂CNNN-), 4.58 (2H, t, *J* = 5.7 Hz, -COOCH₂CH₂N-), 4.54 (2H, t, *J* = 4.9 Hz, -NCH₂CH₂O-), 4.10 (3H, s, -OCH₃), 4.06 (3H, s, -OCH₃), 4.00 (2H, t, *J* = 5.2 Hz, -NNNCH₂CH₂O-), 3.86 (2H, t, 5.2 Hz, -NNNCH₂CH₂O-) 3.65-3.53 (28H, m, -OCH₂CH₂O-), 3.37 (3H, s, -CH₂OCH₃);
- **¹³C-NMR** (100 MHz, CDCl₃, 25°C): δ = 158.0, 156.1, 153.4, 150.9, 151.0, 148.4, 148.1, 146.3, 145.7, 145.5, 144.4, 142.9, 126.2, 125.2, 125.1, 124.4, 123.1, 123.0, 122.3, 121.9, 110.4, 101.8, 101.2, 71.4, 70.6, 69.6, 66.3, 59.1, 56.8, 50.7, 49.4, 48.8, 42.9;
- **ESI-MS** m/z calculated for C₄₉H₆₂N₁₀O₁₇⁺ is 1062.4; obs.: 1063.4 (M+H⁺).

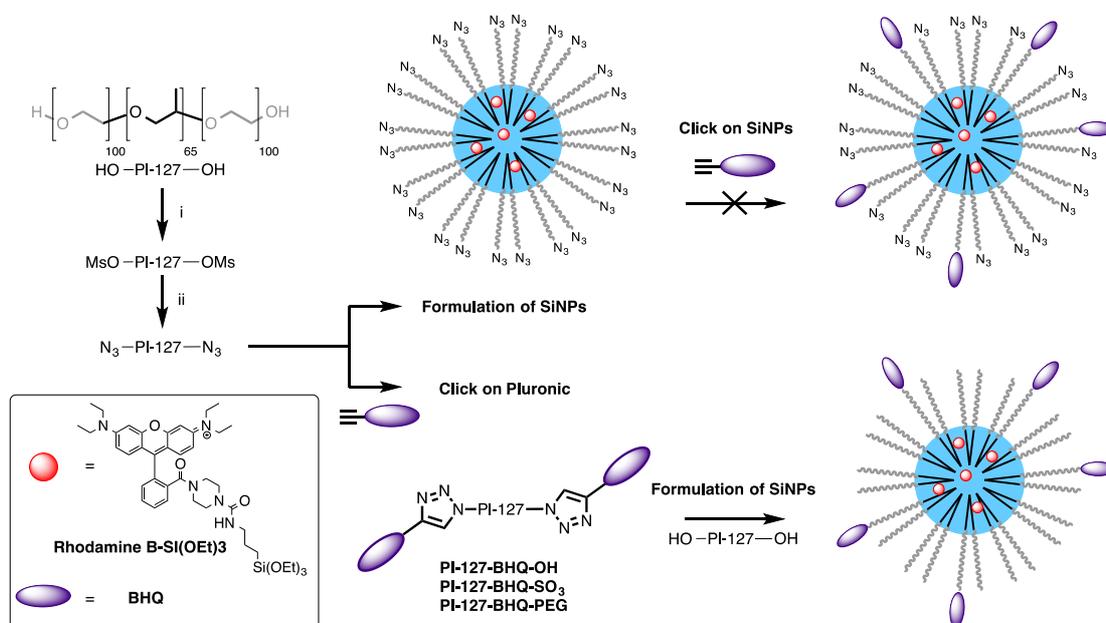


BHQ-PEG: in a flamed 25 mL round bottom flask dried with a heat gun under a flow of Argon, to a solution of **4** (0.1 g, 0.094 mmol) in DMF (5 mL) was added N,N-Diisopropylethylamine (DIEA, 0.065 mL, 0.376 mmol) and Propargylamine (0.024 mL, 0.376 mmol) and the reaction mixture was stirred at room temperature for 18 h. The reaction mixture was then diluted with H₂O (5 mL) and extracted three times with DCM. The combined organic phases were washed three times with a saturated solution of Sodium bicarbonate, dried over Sodium sulfate and concentrated *in vacuo* to give the compound **BHQ-PEG** as a purple oil (0.074 g, yield 81 %); $R_f = 0.49$ (SiO₂, EtOAc:AcO = 50/50).

- **Chemical formula:** C₄₆H₆₂N₁₀O₁₄;
- **Molecular Weight:** 979.1;
- **¹H-NMR** (400 MHz, CDCl₃, 25°C): $\delta = 8.39$ (2H, d, $J = 9.0$ Hz, ar. CH), 8.06 (2H, d, $J = 9.0$ Hz, ar. CH), 7.93 (2H, d, $J = 9.2$ Hz, ar. CH), 7.62 (1H, s, -NCH), 7.51 (1H, s, ar. CH), 7.45 (1H, s, ar. CH), 6.91 (2H, d, $J = 9.1$ Hz, ar. CH), 5.29 (1H, s, -COONH-), 4.81 (2H, s, -NCH₂CNN-), 4.52 (2H, t, $J = 4.8$ Hz, -COOCH₂CH₂N-), 4.36 (2H, t, $J = 5.2$ Hz, -NCH₂CH₂O-), 4.10 (3H, s, -OCH₃), 4.05 (3H, s, -OCH₃), 3.98 - 3.95 (2H, m, -NHCH₂C-), 3.86 (2H, t, $J = 4.8$ Hz, -NNCH₂CH₂O-), 3.65 - 3.53 (30H, m, -OCH₂CH₂O-) + (1H, s, CCH) overlapped, 3.38 (3H, s, -CH₂OCH₃);
- **¹³C-NMR** (100 MHz, CDCl₃, 25°C): $\delta = 156.4, 155.8, 153.5, 151.1, 150.9, 148.4, 146.4, 145.1, 144.5, 142.3, 126.0, 124.7, 123.5, 123.1, 112.2, 101.0, 100.1, 79.8, 71.9, 71.7, 71.4, 70.7, 70.4, 69.4, 62.4, 59.0, 56.8, 50.3, 47.3, 42.7, 29.7$;
- **ESI-MS** m/z calculated for C₄₆H₆₂N₁₀O₁₄⁺ is 978.4; obs.: 979.4 (M+H⁺).

1.2. Synthesis and characterization of F127 derivatives

1.2.1. Pluronic-F127 derivatives



Dimesyl derivative of Pluronic F127 (DMF127) was synthesized adapting a reported procedure¹.

In a flamed 100 mL round bottom flask dried with a heat gun under a flow of Argon, Pluronic F127 (6.3 g, 0.5 mmol) was solubilized in DCM (25 mL) and the reaction was cooled to 0°C. Next, Triethylamine (TEA, 140 μ L, 1 mmol) and methanesulfonyl chloride (78 μ L, 1 mmol) were added. The reaction mixture was stirred at 0°C for 3 h and then at room temperature for 15 h. The reaction mixture was concentrated *in vacuo*, then the residue was redispersed with H₂O and the purification was carried out by means of dialysis against H₂O (72 h) to give **DMF127** as a white solid (5.01 g, yield 80 %).

- ¹H-NMR (400 MHz, CDCl₃, 25°C): δ = 4.40-4.38 (4H, m, -SOCH₂CH₂-), 3.84-3.81 (4H, m, -SOCH₂CH₂-), 3.65-3.61 (-OCH₂CH₂O-) + 3.59-3.51 (m, -OCH₂CH-) + 3.43-3.38 (m, -CHCH₃) ~1000H, 3.09 (6H, s, CH₃SOO-), 1.16-1.13 (~195H, m, -CHCH₃);
- ¹³C-NMR (100 MHz, CDCl₃, 25°C): δ = 75.0, 74.9, 74.8, 72.9, 72.6, 72.3, 70.1, 68.9, 68.6, 45.5, 17.1, 16.9.

Diazide derivative of Pluronic F127 (DAF127) was synthesized adapting a reported procedure¹.

In a flamed 100 mL round bottom flask dried with a heat gun under a flow of Argon, **DMF127** (4.8 g, 0.38 mmol) and Sodium azide (74 mg, 1.1 mmol) were solubilized in Acetonitrile (MeCN, 24 mL) and the reaction was heated under reflux for 48h. Next, the solvent was removed under reduced pressure with a rotary evaporator and the obtained solid was redispersed with a 5% aqueous solution of Sodium bicarbonate, then saturated with NaCl and extracted five times with DCM. The combined organic phases were dried over Sodium sulfate, concentrated with the rotary evaporator and desiccated *in vacuo*, affording a white solid substance. The residue was redispersed with H₂O and purification was carried out by means of dialysis against H₂O (72 h) to give **DAF127** as a white solid (3.28 g, yield 69 %).

- ¹H-NMR (400 MHz, CDCl₃, 25°C): δ = 3.83-3.81 (4H, m, -N₃CH₂CH₂OCH₂-), 3.64-3.60 (-OCH₂CH₂O-) + 3.58-3.50 (m, -OCH₂CH-) + 3.42-3.38 (m, -CHCH₃ + -CH₂N₃) ~1000H, 1.15-1.13 (~195H, m, -CHCH₃);
- ¹³C-NMR (100 MHz, CDCl₃, 25°C): δ = 75.0, 74.8, 74.6, 72.9, 72.8, 72.5, 70.1, 50.1, 17.0, 16.9.

General procedure for the synthesis of PI-127-BHQs: in a flamed 20 mL round bottom, diazido pluronic F-127 **DAF127** (1 eq., 0.012 mmol) and **BHQ** (-OH, -SO₃ or -PEG, 4 eq.) were solubilized with 3 mL of Diox. Copper(II) sulfate pentahydrate (5 eq.) and Sodium ascorbate (7 eq.) were solubilized with 1 mL of H₂O and added to the previous solution, and the reaction mixture was stirred at room temperature for 8 h. The reaction mixture was concentrated *in vacuo*, then the residue was purified by means means of dialysis against H₂O (24 h) and Size-exclusion chromatography (Sephadex G-50) eluting with H₂O to obtain the desired compound.

PI-127-BHQ-OH (yield 45 %).

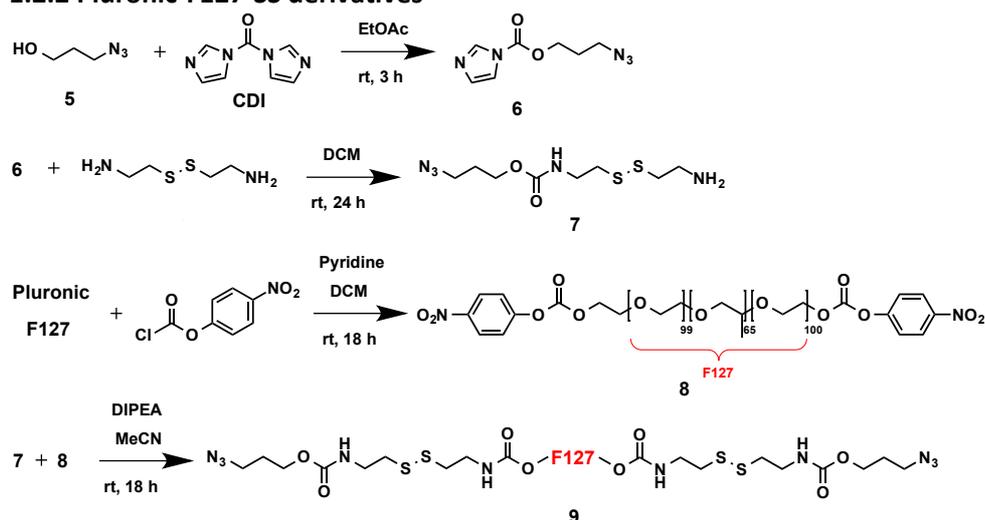
- ¹H-NMR (400 MHz, CDCl₃, 25°C): δ = 8.42-8.37 (4H, m, ar. CH), 8.10-8.04 (4H, m, ar. CH), 7.90-7.82 (4H, m, ar. CH), 7.53-7.43 (4H, m, ar. CH) + (2H, s, -NCHC-) overlapped, 6.86-6.77 (4H, m, ar. CH), 4.35-4.31 (4H, m, -NCCH₂N-), 4.09 (6H, s, -OCH₃-), 4.04 (6H, s, -OCH₃-), 3.83-3.81 (4H, m, -NCH₂CH₂OH) + (4H, m, -NCH₂CH₂OH) + (4H, m, -NNNCH₂CH₂OCH₂-) overlapped, 3.71-3.60 (m, -CH₂OCH₂-) + 3.58-3.50 (m, -OCH₂CH-) + 3.42-3.39 (m, -CHCH₃) ~1000H, 1.15-1.13 (~195H, m, -CHCH₃).
- ¹³C-NMR (100 MHz, CDCl₃, 25°C): δ = 153.4, 150.9, 148.5, 148.4, 147.6, 125.8, 124.7, 123.5, 118.8, 113.9, 112.8, 101.0, 75.5, 75.3, 75.1, 73.3, 72.9, 70.5, 68.4, 61.5, 56.7, 53.4, 29.6, 17.0.

PI-127-BHQ-SO₃ (yield 52 %).

- ¹H-NMR (400 MHz, CDCl₃, 25°C): δ = 8.35 (4H, d, *J* = 9.0 Hz, ar. CH), 8.07 (2H, s, -NCHC-), 8.03 (4H, d, *J* = 9.0 Hz, ar. CH), 7.84 (4H, d, *J* = 9.0 Hz, ar. CH), 7.47 (2H, s, ar. CH), 7.41 (2H, s, ar. CH), 6.90 (4H, d, *J* = 9.0 Hz, ar. CH), 4.50-4.47 (4H, m, -NCH₂C-), 4.07 (6H, s, -OCH₃-), 4.01 (6H, s, -OCH₃-), 3.81-3.79 (4H, m, -NCH₂CH₂OS-) + (4H, m, -NCH₂CH₂OS-) + (4H, m, -NNNCH₂CH₂OCH₂-) overlapped, 3.67-3.58 (m, -CH₂OCH₂-) + 3.56-3.48 (m, -OCH₂CH-) + 3.41-3.36 (m, -CHCH₃) + 3.36 (8H, m, -OCNHCH₂-) ~1000H, 1.13-1.11 (~195H, m, -CHCH₃);
- ¹³C-NMR (100 MHz, CDCl₃, 25°C): δ = 156.9, 155.3, 151.1, 147.3, 144.9, 128.1, 125.8, 124.7, 123.5, 122.5, 113.8, 112.4, 100.1, 80.5, 75.5, 75.3, 75.1, 72.9, 72.8, 72.4, 70.5, 68.4, 61.5, 56.8, 17.4, 17.3.

PI-127-BHQ-PEG (yield 52 %). Well resolved NMR spectra could not have been obtained. Despite this, the resulting SiNP gave good results with small size DLS ~25 nm and PDI ≤ 0.05.

1.2.2 Pluronic-F127-SS derivatives



Scheme S2. Schematization of the synthesis of the Pluronic-F127-SS derivatives.

5 was synthesized following a reported procedure.²

6 was synthesized following a reported procedure.²

7 was synthesized adapting reported procedures.^{3,4} Cystamine dihydrochloride was neutralized by 4 M NaOH and extracted three times with DCM to yield Cystamine. In a flamed 50 mL round bottom flask dried with a heat gun under a flow of Argon, to a solution of Cystamine (1.267 g, 8.3 mmol) in DCM (20 mL) was added 3-Azidopropyl 1H-imidazole-1-carboxylate (6) (1.350 g, 6.9 mmol) and the reaction mixture was stirred at room temperature for 24 h. The reaction mixture was concentrated *in vacuo*, and the residue was treated with NaH₂PO₄ (50 mL, 1.0 M) and extracted three times with Diethyl ether (Et₂O). The reaction mixture was then concentrated *in vacuo* to give the compound 7 as a yellow solid (0.412 g, yield 22 %).

- **Chemical formula:** C₈H₁₇N₅O₂S₂;
- **Molecular Weight:** 279.4;

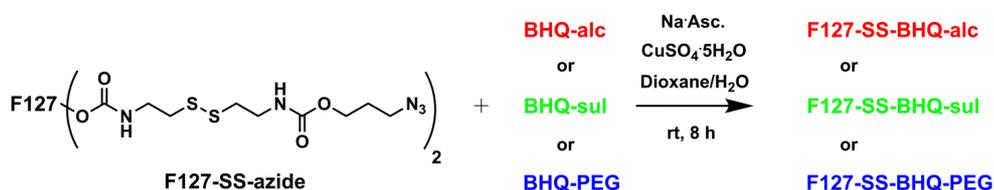
- **¹H-NMR** (400 MHz, CDCl₃, 25°C): δ = 5.76 (1H, s, -NHCO-), 4.14 (2H, t, *J* = 6.1 Hz, -OCH₂CH₂-), 3.52-3.47 (2H, m, CH₂N₃), 3.36 (2H, t, *J* = 6.5 Hz, -OCNHCH₂-), 3.01 (2H, t, *J* = 6.2 Hz, -CH₂NH₂), 2.78-2.74 (4H, m, -CH₂SSCH₂-), 1.90-1.84 (2H, m, -CH₂CH₂CH₂-);
- **¹³C-NMR** (100 MHz, CDCl₃, 25°C): δ = 156.4, 61.4, 47.7, 41.1, 39.7, 39.5, 37.5, 28.1;
- **ESI-MS** *m/z* calculated for C₈H₁₇N₅O₂S₂⁺ is 279.1; obs.: 280.1 (M+H⁺).

8: in a flamed 100 mL round bottom flask dried with a heat gun under a flow of Argon, **Pluronic F127** (6.3 g, 0.5 mmol), Pyridine (0.242 mL, 3 mmol) and 4-Nitrophenyl chloroformate (**6**) (0.605 g, 3 mmol) were solubilized with 30 mL of DCM and the reaction mixture was stirred at room temperature for 18 h. The reaction mixture was then concentrated *in vacuo*, then the residue was redispersed with HCl 0.1 M and extracted five times with DCM. The combined organic phases were dried over Sodium sulfate, concentrated *in vacuo* to give the compound **8** as a white solid (5.85 g, yield 91 %).

- **¹H-NMR** (400 MHz, CDCl₃, 25°C): δ = 8.25 (2H, d, *J* = 9.1 Hz, ar. CH), 8.06 (2H, d, *J* = 9.1 Hz, ar. CH), 7.36 (2H, d, *J* = 9.2 Hz, ar. CH), 6.88 (2H, d, *J* = 9.1 Hz, ar. CH), 4.42-4.39 (4H, m, -CH₂COO-), 3.79-3.77 (4H, m, -CH₂CH₂COO-), 3.63-3.59 (-OCH₂CH₂O-) + 3.57-3.49 (m, -OCH₂CH-) + 3.41-3.39 (m, -CHCH₃) ~1000H, 1.12-1.09 (~195H, m, -CHCH₃);
- **¹³C-NMR** (100 MHz, CDCl₃, 25°C): δ = 155.3, 152.2, 145.1, 138.9, 126.0, 125.0, 121.6, 116.1, 75.3, 75.1, 74.9, 73.1, 72.6, 70.3, 68.4, 68.1, 17.2, 17.1.

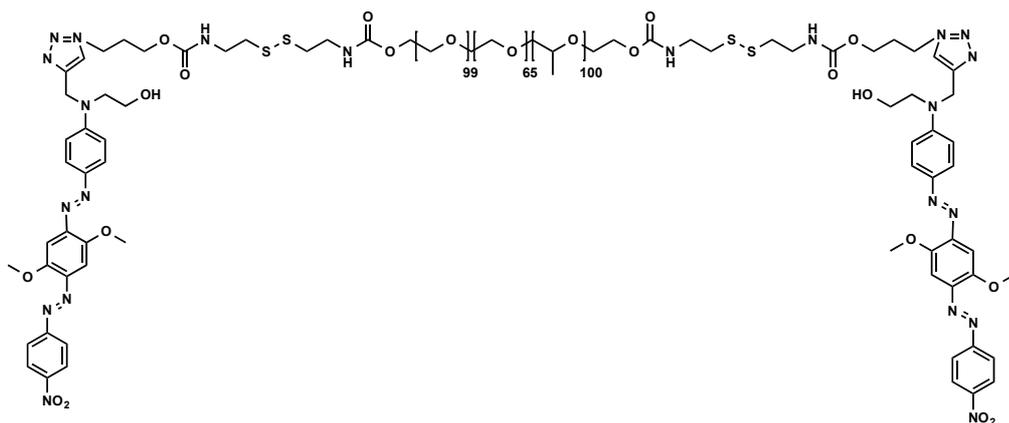
9: in a flamed 100 mL round bottom flask dried with a heat gun under a flow of Argon, compound (**8**) (1.75 g, 0.135 mmol), DIEA (0.092 mL, 0.54 mmol) and compound (**7**) (0.151 g, 0.54 mmol) were solubilized with 20 mL of MeCN and the reaction mixture was stirred at room temperature for 18 h. The reaction mixture was concentrated *in vacuo*, then the residue was redispersed with H₂O and purification was carried out by means of dialysis against water (72 h) to give the compound **9** as a yellow solid (1.075 g, yield 61 %).

- **¹H-NMR** (400 MHz, CDCl₃, 25°C): δ = 4.27-4.13 (8H, m, -CH₂OOCNH-), 3.80 (4H, t, *J* = 4.3 Hz, -NHCOOCH₂CH₂O-), 3.66-3.61 (m, -OCH₂CH₂O-) + 3.56-3.49 (m, -OCH₂CH-) + (4H, m, CH₂N₃) + 3.40-3.37 (m, -CHCH₃) + (8H, m, -OCNHCH₂-) ~1000H, 2.80 (8H, t, *J* = 6.0 Hz, -CH₂SSCH₂-), 1.92-1.86 (4H, m, -CH₂CH₂CH₂N₃), 1.13-1.11 (~195H, m, -CHCH₃).
- **¹³C-NMR** (100 MHz, CDCl₃, 25°C): δ = 156.3, 156.2, 75.4, 75.2, 75.0, 73.2, 72.8, 72.7, 70.4, 69.4, 68.5, 68.4, 61.7, 48.1, 39.7, 28.4, 17.3, 17.2.



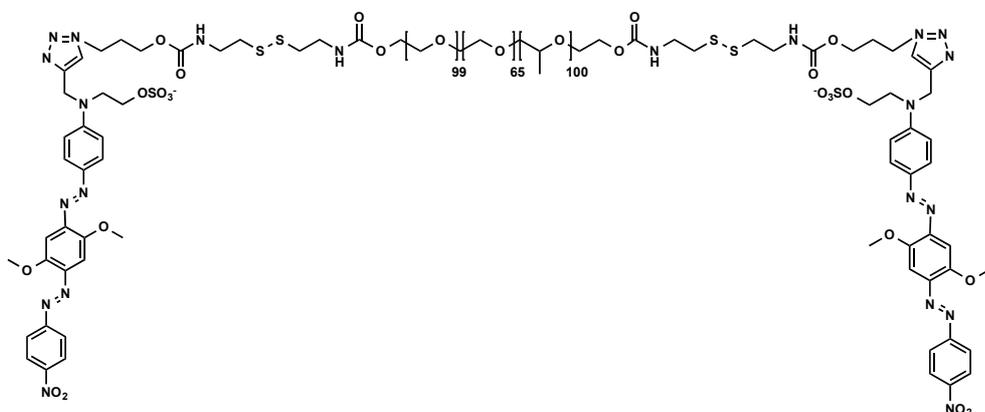
Scheme S3. Schematization of the general procedure for Pluronic **PI-127-SS-BHQ** derivatives.

General procedure: in a flamed 20 mL round bottom flask, **9** (1 eq., 0.012 mmol) and **BHQ** (-OH, -SO₃⁻ or -PEG, 4 eq.) were solubilized with 3 mL of Diox. Copper(II) sulfate pentahydrate (5 eq.) and Sodium ascorbate (7 eq.) were solubilized with 1 mL of H₂O and added to the previous solution, and the reaction mixture was stirred at room temperature for 8 h. The reaction mixture was concentrated *in vacuo*, then the residue was purified by means of size-exclusion chromatography (LH-20) eluting with DCM/MeOH (1:1) to obtain the desired compound (**PI-127-SS-BHQ-OH**, **PI-127-SS-BHQ-SO₃** or **PI-127-SS-BHQ-PEG**).



PI-127-SS-BHQ-OH (yield: 68 %).

- **¹H-NMR** (400 MHz, CDCl₃, 25°C): δ = 8.36 (4H, d, *J* = 8.8 Hz, ar. CH), 8.04 (4H, d, *J* = 8.8 Hz, ar. CH), 7.87 (4H, d, *J* = 8.8 Hz, ar. CH), 7.61 (2H, s, -CCH-), 7.48 (2H, s, ar. CH), 7.42 (2H, s, ar. CH), 6.82 (4H, d, *J* = 9.0 Hz, ar. CH), 4.81 (4H, s, -NCH₂C-), 4.43 (4H, t, *J* = 6.3 Hz, -NCH₂CH₂CH₂-), 4.22-4.18 (8H, m, -CH₂OOCNH-), 4.07 (6H, s, -OCH₃-), 4.03 (6H, s, -OCH₃-), 3.96-3.94 (4H, m, -NCH₂CH₂OH), 3.82-3.80 (4H, m, -NCH₂CH₂OH) + (4H, m, -NHCOOCH₂CH₂O-) overlapped, 3.64-3.62 (m, -CH₂OCH₂-) + 3.57-3.49 (m, -OCH₂CH-) + 3.41-3.39 (m, -CHCH₃) + 3.36 (8H, m, -OCNHCH₂-) ~1000H, 2.80 (8H, t, *J* = 6.3 Hz, -CH₂SSCH₂-), 2.17-2.16 (4H, m, -NCH₂CH₂CH₂O-), 1.14-1.12 (~195H, m, -CHCH₃);
- **¹³C-NMR** (100 MHz, CDCl₃, 25°C): δ = 164.4, 156.3, 151.8, 146.6, 143.3, 125.7, 124.6, 123.4, 112.0, 75.4, 75.2, 75.0, 73.2, 72.8, 72.7, 70.4, 69.4, 68.4, 63.9, 63.5, 61.5, 56.7, 47.1, 39.7, 38.0, 29.5, 17.3, 17.2.



PI-127-SS-BHQ-SO₃ (yield: 68 %).

- **¹H-NMR** (400 MHz, CDCl₃, 25°C): δ = 8.36 (4H, d, *J* = 8.4 Hz, ar. CH), 8.04 (4H, d, *J* = 8.8 Hz, ar. CH), 7.86 (4H, d, *J* = 7.6 Hz, ar. CH), 7.53-7.43 (2H, s, -CCH-) + (2H, s, ar. CH) + (2H, s, ar. CH) overlapped, 6.93 (4H, d, *J* = 8.4 Hz, ar. CH), 4.84 (4H, s, -NCH₂C-), 4.45-4.40 (4H, m, -CH₂OS-), 4.35-4.30 (4H, m, -NCH₂CH₂CH₂-), 4.24-4.19 (8H, m, -CH₂OOCNH-), 4.08 (6H, s, -OCH₃-), 4.03 (6H, s, -OCH₃-), 3.96-3.91 (4H, m, -NCH₂CH₂OS), 3.83-3.79 (4H, m, -NHCOOCH₂CH₂O-), 3.65-3.62 (m, -CH₂OCH₂-) + 3.58-3.49 (m, -OCH₂CH-) + 3.42-3.37 (m, -CHCH₃) + 3.37 (8H, m, -OCNHCH₂-) ~1000H, 2.89-2.79 (8H, m, -CH₂SSCH₂-), 2.18-2.16 (4H, m, -NCH₂CH₂CH₂O-) 1.15-1.12 (~195H, m, -CHCH₃).
- **¹³C-NMR** (100 MHz, CDCl₃, 25°C): δ = 156.4, 156.3, 124.6, 123.4, 75.4, 75.2, 75.0, 73.2, 72.8, 72.7, 70.4, 68.4, 68.3, 63.5, 61.4, 56.7, 40.7, 40.0, 29.6, 17.3, 17.2.

2. Spectroscopic studies

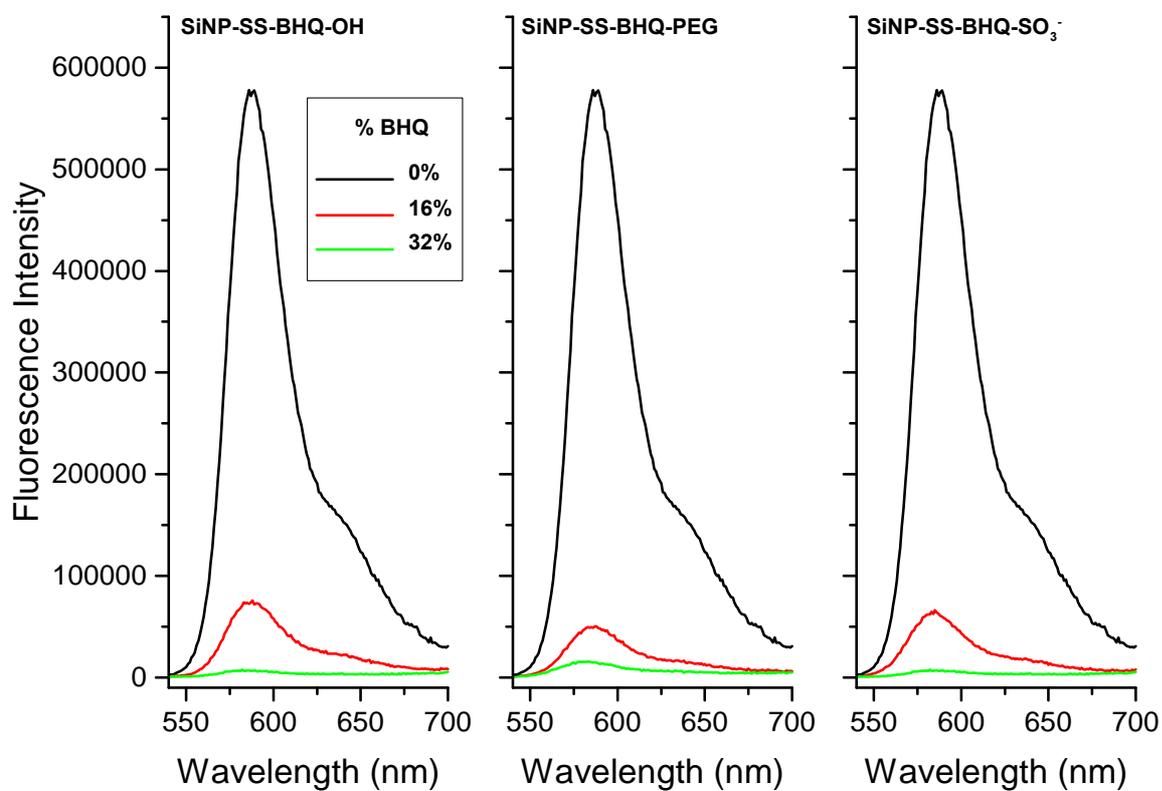


Figure S1. Emission spectra of the reductive SiNPs (200 nm) with various amount of BHQ (black: 0%, red: 16% and green: 32%) Excitation wavelength was 530 nm.

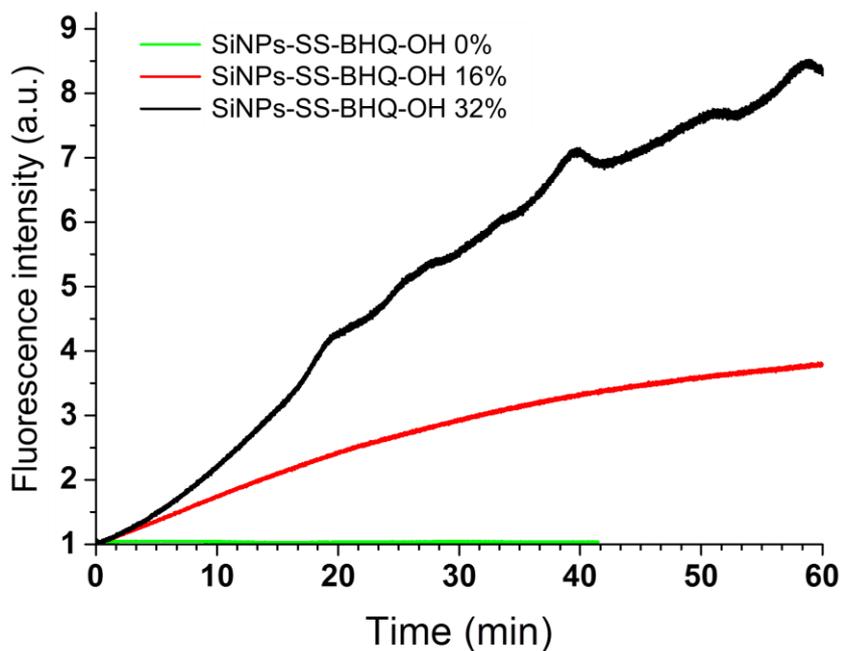


Figure S2. Fluorescence increase over 1 hour (monitored at 580 nm, excitation at 530 nm) of BHQ-OH SiNPs (20 nM) in the presence of 40 mM glutathion in phosphate buffer 20 mM pH 7.4. Excitation and emission wavelengths were 530 nm and 580 nm, respectively.

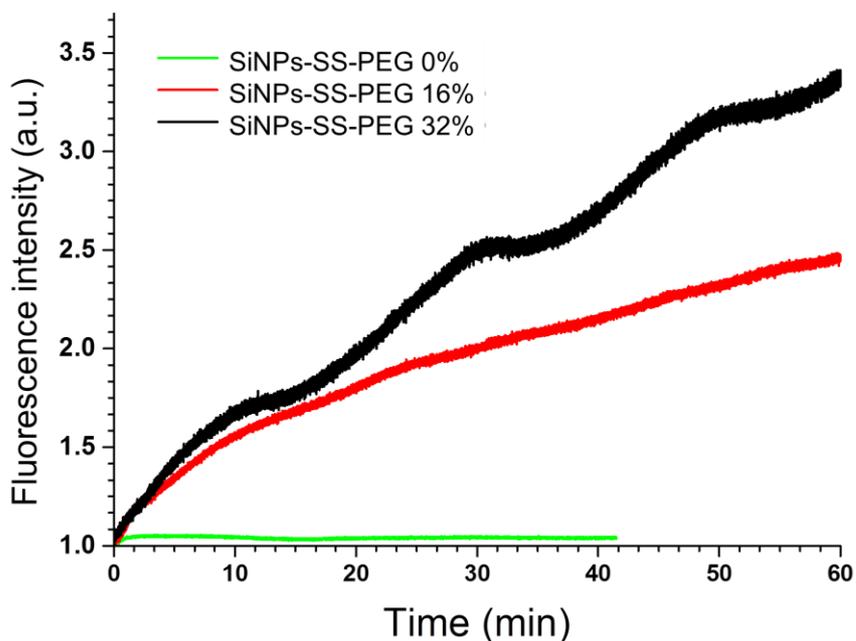


Figure S3. Fluorescence increase over 1 hour (monitored at 580 nm, excitation at 530 nm) of BHQ-PEG SiNPs (20 nM) in the presence of 20 mM TCEP in phosphate buffer 20 mM pH 7.4. Excitation and emission wavelengths were 530 nm and 580 nm, respectively.

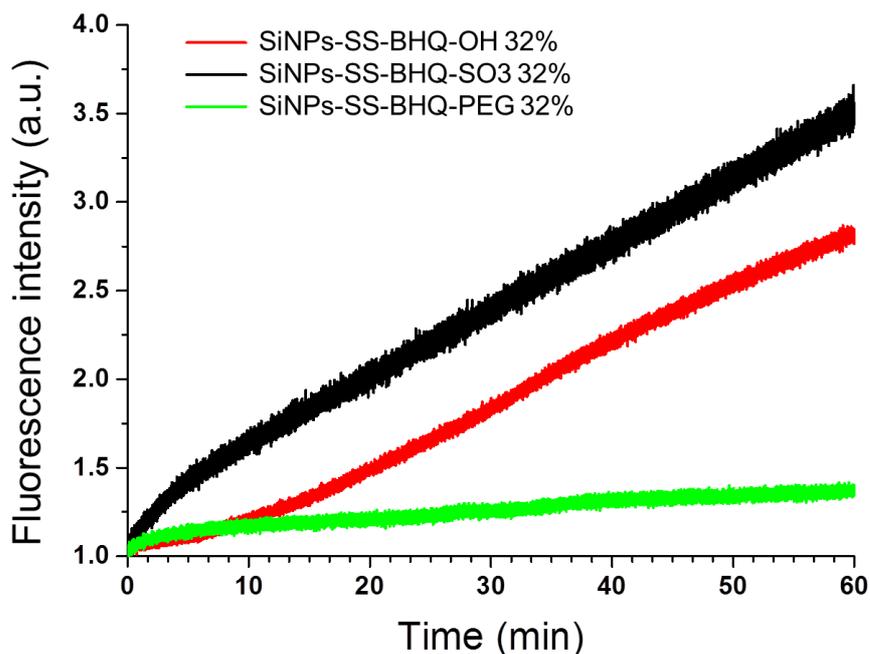


Figure S4. Fluorescence increase over 1 hour (monitored at 580 nm, excitation at 530 nm) of SiNPs bearing 32% BHQ-OH (red), BHQ-SO₃ (black) and BHQ-PEG (green) (20 nM) in the presence of 40 mM glutathion in phosphate buffer 20 mM pH 7.4. Excitation and emission wavelengths were 530 nm and 580 nm, respectively.

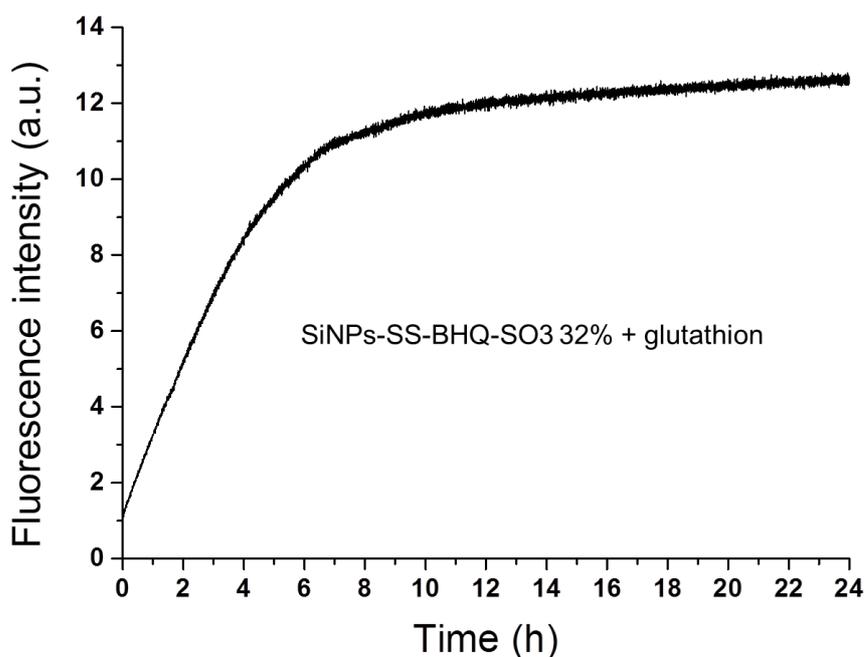


Figure S5. Fluorescence increase over 24 hours (monitored at 580 nm, excitation at 530 nm) of SiNPs bearing 32% reducible BHQ-SO₃ (20 nM) in the presence of 40 mM glutathion in phosphate buffer 20 mM pH 7.4. Excitation and emission wavelengths were 530 nm and 580 nm, respectively.

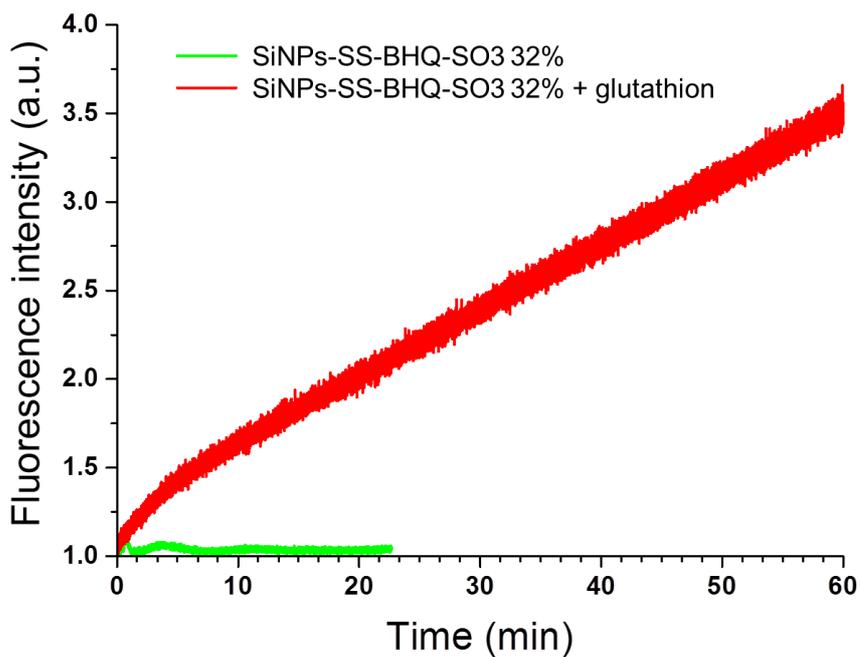


Figure S6. Fluorescence increase over 1 hour (monitored at 580 nm, excitation at 530 nm) of SiNPs bearing 32% reducible-BHQ-SO3 (20 nM) in absence of glutathion (green) and in the presence of 40 mM glutathion (red) in phosphate buffer 20 mM pH 7.4. Excitation and emission wavelengths were 530 nm and 580 nm, respectively.

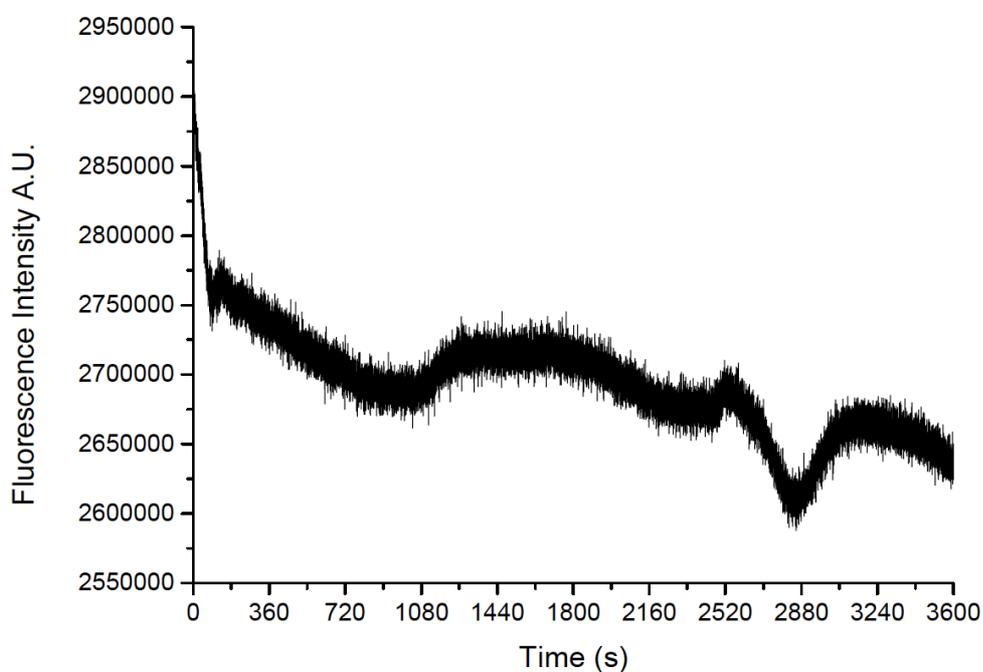


Figure S7. Fluorescence intensity over 1 hour (monitored at 580 nm, excitation at 530 nm) of BHQ-free SiNPs (20 nM) in the presence of 40 mM glutathion in phosphate buffer 20 mM pH 7.4. Excitation and emission wavelengths were 530 nm and 580 nm, respectively.

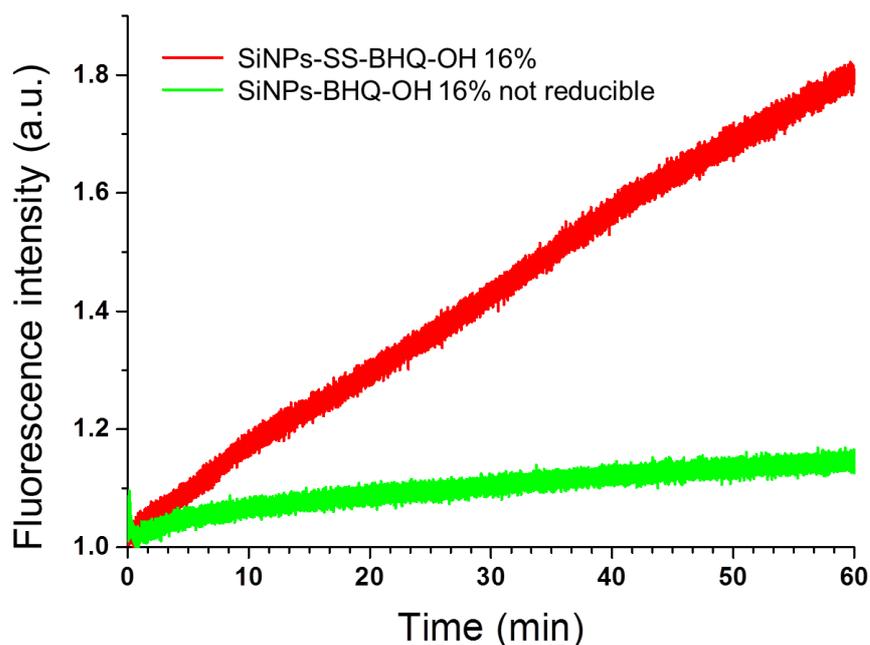


Figure S8. Fluorescence increase over 1 hour (monitored at 580 nm, excitation at 530 nm) of SiNPs bearing 16% reducible-BHQ-OH (red) and 16% non reducible BHQ-OH (green) (20 nM) in the presence of 40 mM glutathione in phosphate buffer 20 mM pH 7.4. Excitation and emission wavelengths were 530 nm and 580 nm, respectively.

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