Electronic Supplementary Information for:

One-Pot Synthesized Organosilica Nanospheres for Multiplexed Fluorescent Nanobarcoding and Subcellular Tracing

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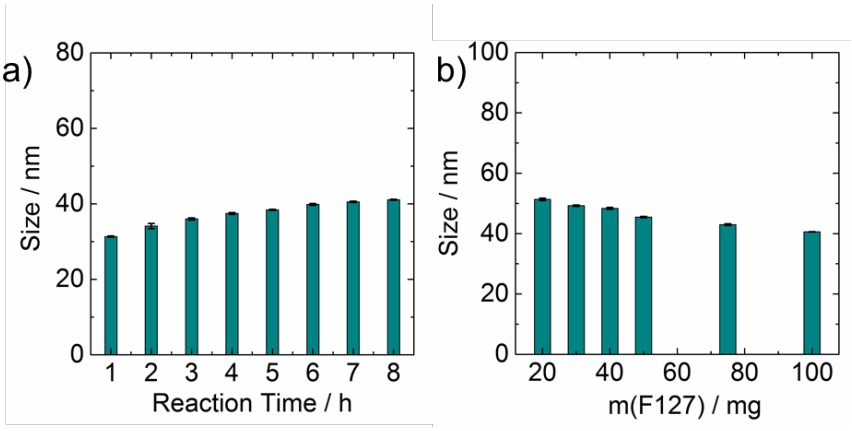

Figure S1. a) Hydrodynamic size of the organosilica nanospheres utilizing F127 as template for different reaction time. b) Hydrodynamic size of the organosilica nanospheres utilizing different amount of F127 as template.

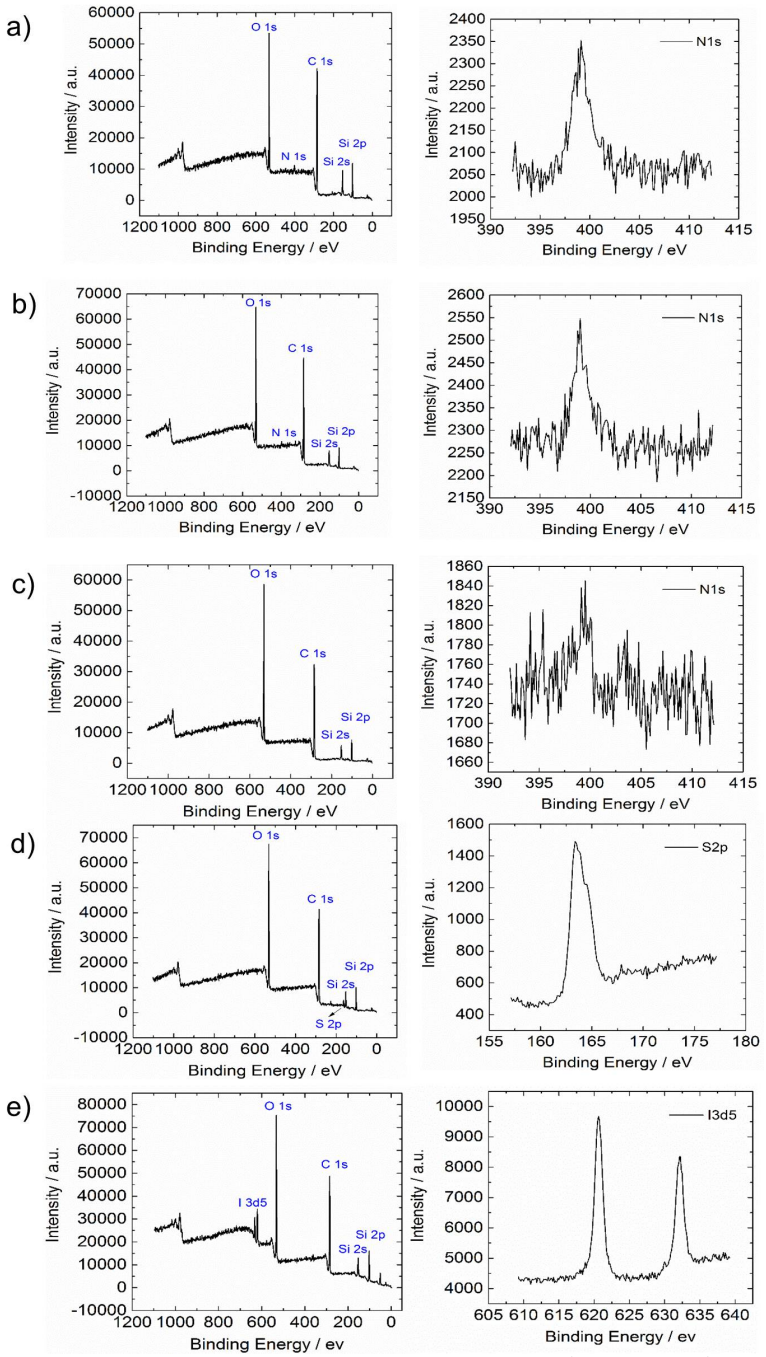

Figure S2. XPS spectra of the organosilica nanospheres containing a) -C_{11}-N_3, b) -NH_2, c) -PEG-N_3, d) -SH, and e) -I groups.

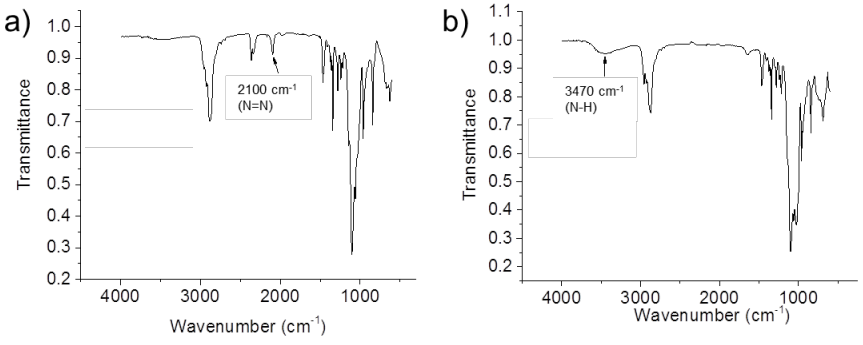

Figure S3. FT-IR spectra of the organosilica nanospheres containing amine, thiol, and azide groups.

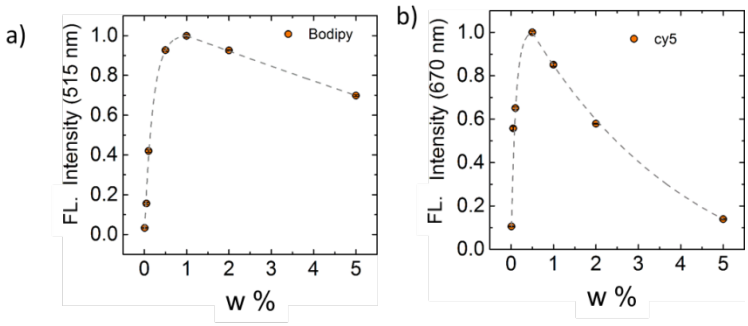

Figure S4. Fluorescence intensity of the organosilica nanospheres loaded with different amount of a) BODIPY, and b) Cy5.

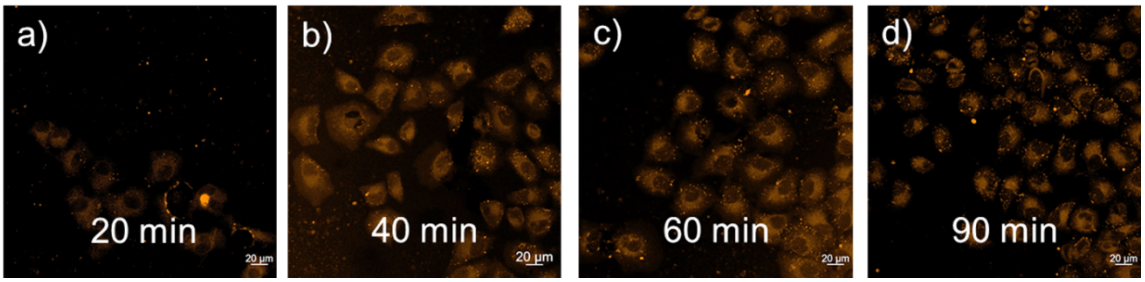

Figure S5. CLSM images of HeLa cells after incubated with organosilica nanospheres modified with Rhodamine for different time. Scale bar: 20 μm.

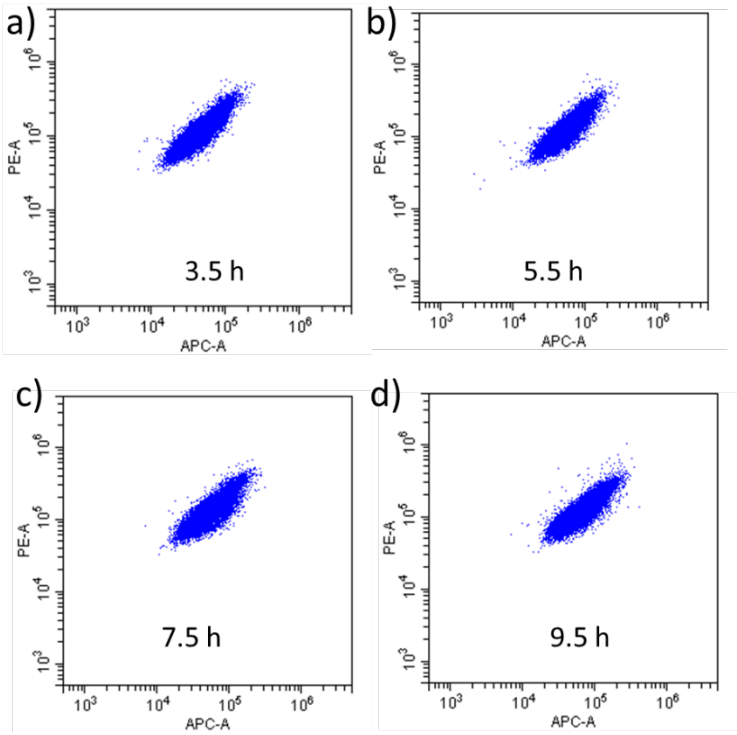

Figure S6. Flow cytometry of the fluorescent NBs in HeLa cells with different incubation time. The NBs were coded with Cy5 and RhITC.

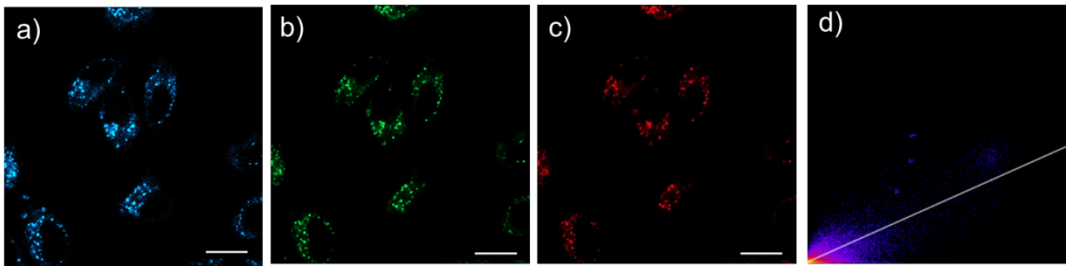

Figure S7. CLSM images of HeLa cells incubated with the organosilica nanospheres modified with MEQ and BODIPY as well as stained with LysoTracker Deep Red. a) Blue channel for MEQ. b) Green channel for BODIPY. c) Red channel for LysoTracker Deep Red. d) Co-localization of green channel and red channel. Scale bar: 20 μm.

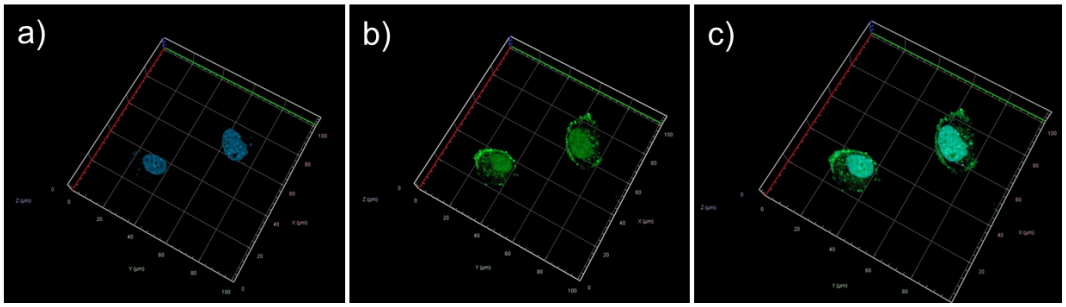

Figure S8. 3D-stacking CLSM images of HeLa cells incubated with FITC-modified nanospheres and Hoechst-33342. a) Blue channel for Hoechst-33342. b) Green channel for FITC. c) Overlay of a) and b)

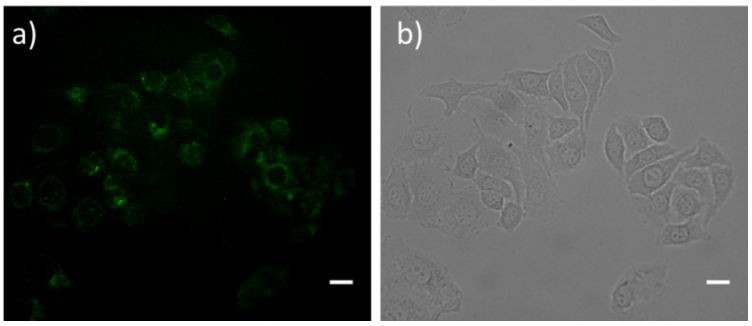

Figure S9. a) Fluorescence imaging of HeLa cells directly incubated with the dye FITC (1 μM in the culture medium for 3 h). b) Bright field image. Scale bar: 20 μm.

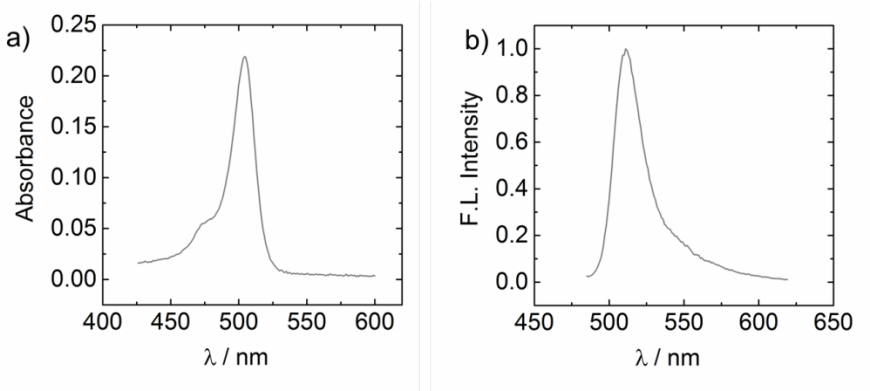

Figure S10. The absorbance and fluorescence spectra of BODIPY modified organosilica nanospheres.

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Table S1. Size distribution and PDI of the organosilica nanoparticles prepared with different templates and organosilanes

Template	Organosilanes	Size / nm	PDI
F127	TEOS	382.7±22.91	0.69± 0.063
F127	Si-propyl	52.44±0.03	0.01±0.001
F127	Si-phenethyl	38.53± 0.10	0.005±0.004
F127	Si-propyl, Si-PEG-N_3	51.68±0.06	0.03±0.005
F127	Si-propyl, Si-NH_2	49.35±0.42	0.04±0.003
F127	Si-propyl, Si-SH	52.38±0.19	0.04±0.014
F127	Si-propyl, Si-I	38.61±0.25	0.08±0.009
F127	Si-propyl, Si-C_{11}-N_3	41.08±0.62	0.15±0.012
F127	Si-propyl, Si-epoxy	55.45±0.22	0.048±0.016
F127	Si-propyl, Si-PEG-$N_{3,}$ Si-SH	51.28±0.24	0.05±0.006
Triton-X	Si-propyl	15.61±0.32	0.19±0.0073
Tween-20	Si-propyl	15.47±0.12	0.11±0.007
PEG	Si-propyl	202.8±2.62	0.16±0.008
CTAB	Si-propyl	22.83±10.17	0.38±0.151
SDS	Si-propyl	129.8±0.53	0.11±0.012

Table S2. Size distribution and PDI of organosilica nanospheres before and after 5 months storage.

Nanospheres	Size / nm	PDI
Newly prepared	52.38±0.19	0.04±0.014
After 5 months storage	52.81±0.35	0.03±0.007