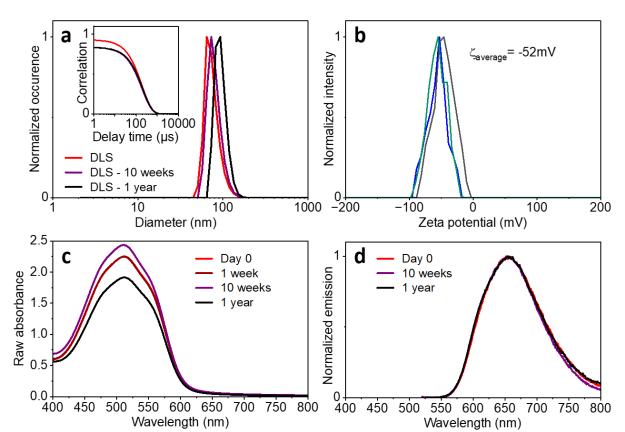
3D Real-Time Single Particle Tracking using two-photon fluorescence from bright dye-based organic nanoparticles

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Supporting Information

Figure S1. (a) Normalized size distributions (DLS) of freshly prepared (red), 10-week old (purple) and 1 year old (black) Nano2Track-840/650 nanoparticles in water. The associated mean hydrated diameters are presented table S1. Inset: Autocorrelation functions. (b) Zeta potential distributions measured on freshly prepared Nano2Track-840/650 nanoparticles in water (3 distinct measurements). (c) Raw absorption spectra of Nano2Track-840/650 nanoparticles in water: freshly prepared (red), after one week (dark red), after 10 weeks (purple) and after one year (black) (The increase of absorbance after 10 weeks can be attributed to evaporation of water, the decrease in absorption after one year is due to partial setting of the nanoparticles). (d) Normalized emission spectra of Nano2Track-840/650 nanoparticles: freshly prepared (red), after 10 weeks (purple) and after one year (black). The associated fluorescence quantum yields are presented table S1.

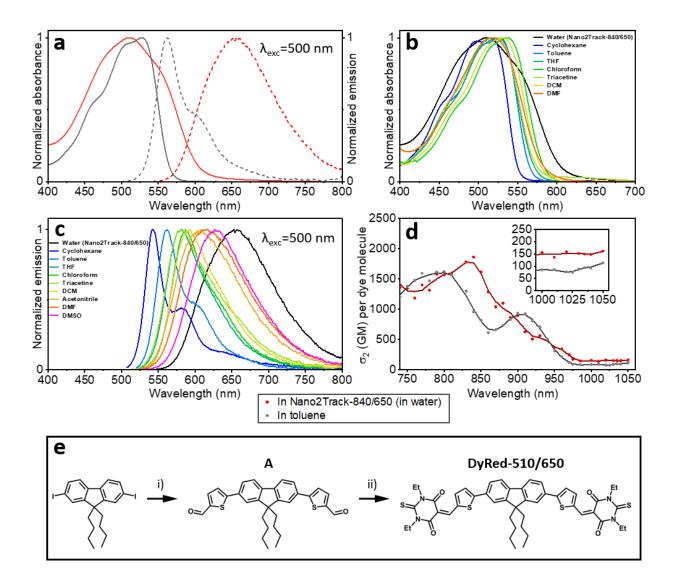


Figure S2. (a) Normalized absorption and emission spectra (excitation at 500 nm) of Nano2Track-840/650 nanoparticles in water (red) and DyRed-510/650 in toluene (grey). (b) Normalized absorption spectra of DyRed-510/650 in various organic solvents. Normalized absorption of Nano2Track-840/650 nanoparticles in water in black for comparison. (c) Normalized emission spectra of DyRed-510/650 in various organic solvents, upon excitation at 500 nm. Normalized emission of Nano2Track-840/650 nanoparticles in water in black for comparison. (d) Two-photon absorption cross-section for one DyRed-510/650 molecule, measured as dFONs molecular subunit of Nano2Track-840/650 in water (red) or in solution in in toluene (grey). (e) Synthesis of DyRed-510/650. i) 2,7-diiodo-9,9-dibutyl-9*H*-fluorene, 5-formyl-2-thienylboronic acid, K_2CO_3 , [PdCl₂(dppf)], toluene/MeOH, reflux, 3 days (53% of A). ii) A, 1,3-diethyl-2-thiobarbituric acid, β -alanine, EtOH, reflux, 24 h (38% of DyRed-510/650).

	Age of the	$Ø_{\rm DLS}^{\rm a}$	PDI ^{b)}	A(λ _{max}) ^{c)}	Φ _F ^{d)}
	solution	[nm]			
	0 days	70±4	0.12±0.06	2.25	0.02
	10 weeks	75±5	0.14±0.04	2.44	0.02
	1 year	89±4	0.10±0.07	1.91	0.03

Table S1. Summary of Nano2Track-840/650 nanoparticle solution properties over time.

^{a)} Mean hydrodynamic diameter calculated from 5 DLS measurements. Error: standard deviation of those 5 measurements. ^{b)}Polydispersity index (PDI) from 5 DLS measurements. Error: standard deviation of those 5 measurements. ^{c)} Absorbance value at 510 nm, measured in 1cm quartz cuvettes. ^{d)}Fluorescence quantum yield.