

Fine Tuning of the Photoacoustic Generation Efficiency by Aggregation-Caused Quenching and Excitation Energy Transfer in Bodipy-Labeled Polylactide Nanoparticles

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Supplementary Information

Materials and methods

Bodipy and polymer synthesis

Bodipy, polylactide-Bodipy (PLA-Bodipy), polylactide-b-polyethylene glycol (PLA-PEG₅₀₀₀) were obtained as described in [40]. Briefly, **Bodipy** was chemically grafted on a polylactide polymer by a ring opening polymerization of a DL-Lactide catalyzed by stannous octoate and initiated by **Bodipy** alcohol function. Polymer characteristics were the followings: PLA-Bodipy ($M_n = 14500 \text{ g.mol}^{-1}$, $\bar{D} = 1.60 \pm 0.05$, dye labelling efficiency 97.5%) and PLA-PEG₅₀₀₀ (17100 g.mol^{-1} , $\bar{D} = 1.20 \pm 0.05$).

Nanoparticles synthesis

Nanoparticles were formulated with the solvent emulsion-evaporation technique described in [40]. Different NP batches were obtained by changing the mass percentage of PLA-Bodipy compared to PLA-PEG₅₀₀₀: **NP-2.5%**, **NP-5%**, **NP-10%**, **NP-25%** and **NP-50%**.

Bodipy reprecipitation experiments

Solutions were prepared from a stock solution of **Bodipy** in 100% THF. Different volume percentages of water/THF solutions were obtained. To yield the same Bodipy concentration for each water fraction, two dilutions from the stock solution were carried out. A first dilution in THF and a second one in water. The dilution factors were adjusted to obtain the same final concentration $C_{\text{Bodipy}} = 9.0 \times 10^{-7} \text{ mol.L}^{-1}$ and a water volume fraction from 0% to 70% compared with THF.

Solvatochromism

A stock solution of **Bodipy**, S_0 , was prepared for each solvent in a 20 mL volumetric flask by dissolution of 3-5 mg of **Bodipy**. Five different concentrations were obtained for each solvent, by successive dilution from the stock solution (successive dilution factors x20, x2, x5, x2, x2). For methanol, ethanol, and cyclohexane, **Bodipy** was not soluble in S_0 . To favor the solvation, the stock solution was prepared with 5% volume of chloroform. Absorption spectra were obtained with the same experimental protocol as described above. The **Bodipy** extinction coefficient was obtained for each solvent, from the slope of the curve $A_{757\text{ nm}} = f(C_{\text{Bodipy}})$. Solutions with an absorbance value lower than 0.1 at the maximum were selected to measure the fluorescence spectra. For methanol, ethanol, and cyclohexane, solutions without chloroform were studied. The fluorescence spectra were obtained by the same method as described above. The emission spectra were measured with an excitation wavelength in the vibronic shoulder. The absolute fluorescence quantum yield was determined for each solvent with the Horiba integrating K-sphere. The fluorescence spectra and the excitation spectra were compared to a diffusive blank colloidal silica solution (Ludox[®] HS-40). The Fluorescence lifetime experiments were conducted as described above, excitation at 781 nm with Horiba Scientific NanoLED-785L (950 V, 781 nm, <200 ps, serial number 16601), emission detected at the maximum. For cyclohexane the excitation was done at 667 nm with the Horiba Nanoled-670L (950 V, 667 nm, <200ps, serial number 14482). Toluene, chloroform and DCM fluorescence decays were fitted with a mono exponential function. A biexponential function was used for cyclohexane, dioxane and DMSO and a triexponential one for EtOH and MeOH. For biexponential and triexponential, average lifetime was estimated: $\bar{\tau} = \frac{\sum_i B_i \tau_i^2}{\sum_i B_i \tau_i}$ with B_i the pre-exponential factors and τ_i the associated lifetimes.

Transient absorption spectroscopy

Transient absorption spectroscopy experiments were performed with a home-built setup [46]. A Nd-YAG laser (4 ns, 10 Hz, 8 mW) with an optical parametric oscillator was used as a pump at 680 nm. A ns pulse white light continuum laser (LEUKOS) (20Hz) was used as a probe. The probe beam was separated into two different pathways, one used as a reference to correct the pulse-to-pulse energy fluctuation (detection with a Princeton Instrument CCD (ICCD) detector PIMAX 4) and the other to probe the sample. The probing arm after passing the sample is coupled into a round to linear optical fiber bundle before being analyzed by a spectrograph SPEX 270M (Jobin-Yvon). Detection of the dispersed white light is performed by an intensified CCD (ICCD) detector PIMAX 4 (Princeton Instrument). The measured differential absorbance is defined as: $\Delta A(\lambda, t) = -\log \left[\frac{I_{probe}^{on}}{I_{ref}^{on}} \right] + \log \left[\frac{I_{probe}^{off}}{I_{ref}^{off}} \right]$ with I_{probe}^{on} and I_{ref}^{on} the probe and reference signal when the pump laser is on, and I_{probe}^{off} and I_{ref}^{off} the probe and reference signal when the pump is off. Spectra were obtained in 10 mm path

length quartz cuvettes with a volume of 1 mL. All the solutions were purged with argon and the absorbance values, at the band maximum, were fixed at 0.4 for all the solutions. Dilution factors for NPs were: x2, x10.7, x15.4 for **NP-5%**, **NP-25%** and **NP-50%**, respectively. For Bodipy in DCM the concentration was $C_{\text{Bodipy}} = 5.0 \times 10^{-6} \text{ mol.L}^{-1}$. For all samples, absorbance spectra were checked before and after the purge. To compare Bodipy NPs and Bodipy solution in DCM, the transient absorption spectra were observed at the same fixed delay between pump and probe: $0 \mu\text{s}$.

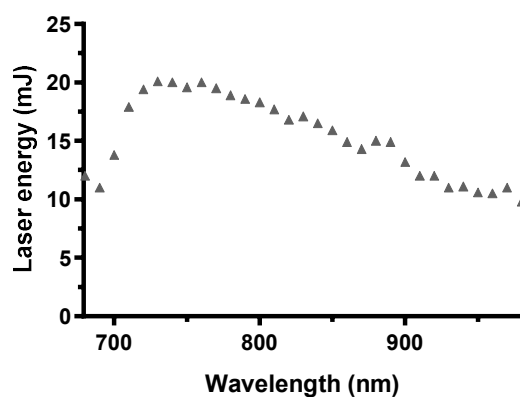


Figure S1. Laser energy at the output of the optical fiber as a function of the wavelength

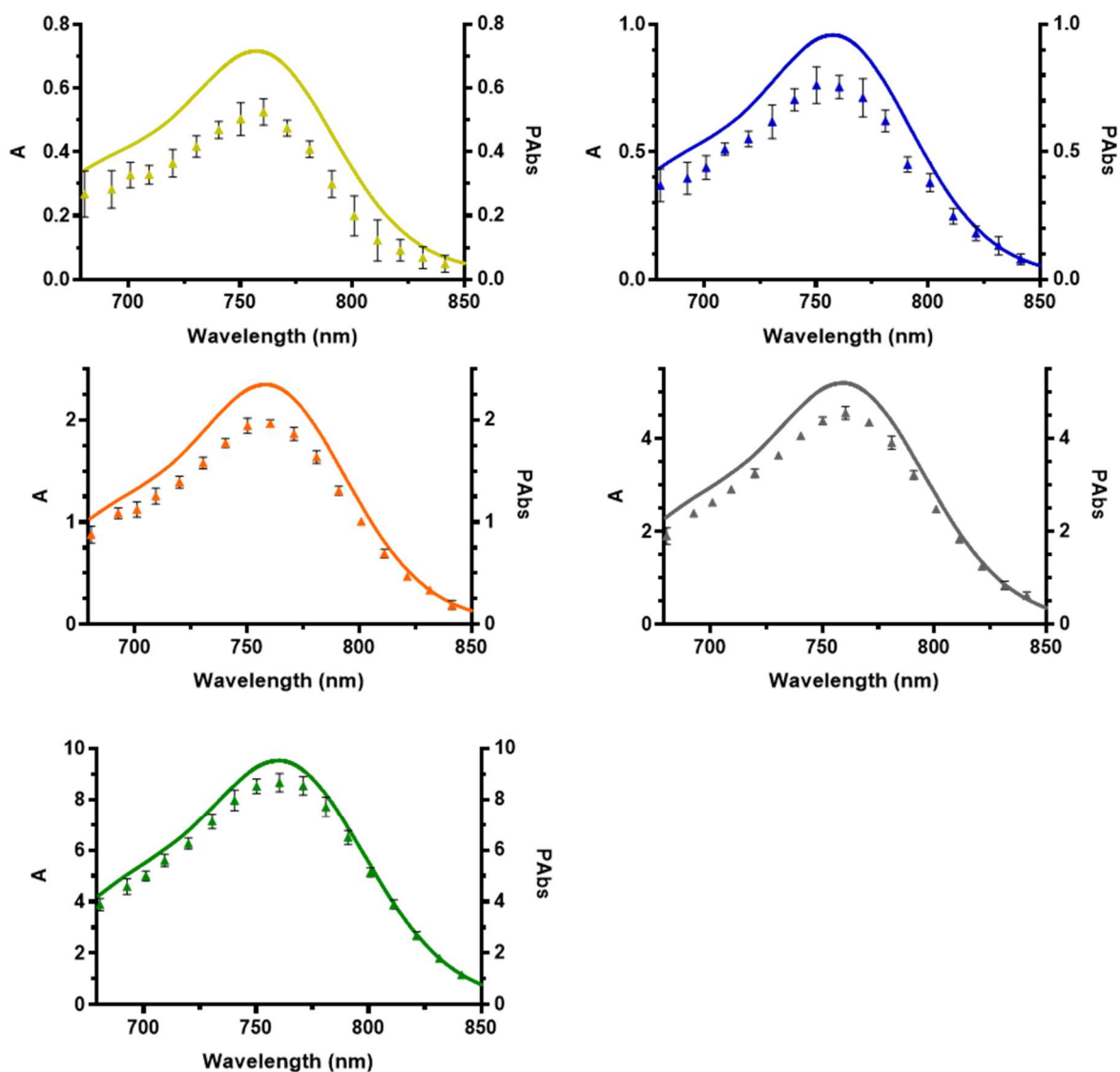


Figure S2. Absorption spectra (continuous line) and photoacoustic excitation spectra (triangle) for each NP's batch (green NP-50%), (grey NP-25%), (orange NP-10%), (blue NP-5%), (yellow NP-2.5%). Laser fluence $0.98 \text{ mJ}\cdot\text{cm}^{-2}$. Absorbance values were corrected from dilution factor: no dilution for NP-2.5% and NP-5%; three times dilution for NP-10%; five times dilution for NP-25% and ten times dilution for NP-50%. PABs values were corrected from dilution factor: no dilution for NP-2.5%, NP-5% and NP-10%; two times dilution for NP-25% and three times dilution for NP-50%. The error bars for the PES corresponds to the median value \pm MAD.

	NP-5%	NP-10%	NP-25%	NP-50%
$B_{\text{PAB}} (\times 10^{-7}) \text{ (L}\cdot\text{mol}^{-1}\cdot\text{cm}^{-1})$	1.2	2.5	7.9	22

Table S1. NP photoacoustic brightness $B_{\text{NP}}^{\text{PA}} = \epsilon_{\text{NP}} \cdot \text{GPAE} = \epsilon_{\text{PLA-BY}} \cdot N_{\text{PLA-BY}} \cdot \text{GPAE}$. GPAE calculated with experiments with laser fluence of $0.98 \text{ mJ}\cdot\text{cm}^{-2}$ (figure 2). $N_{\text{PLA-BY}}$ were determined in our previous work ([40]).

	NP-2.5%	NP-5%	NP-10%	NP-25%	NP-50%
R_0 (nm)	3.5	3.4	3.4	3.4	3.4
Φ_F/Φ_F^0 (%) [‡]	125 ± 7	97 ± 7	62 ± 4	25 ± 2	12 ± 1
Φ_F/Φ_F^0 (%) [‡]	107 ± 11	75 ± 6	49 ± 6	22 ± 1	11 ± 2

Table S2. NP photophysical properties. ‡ NP QY relative to Bodipy in DCM, excitation at 670 nm. † NP QY relative to Bodipy in DCM, excitation at 540 nm. R_0 represents the Förster radius in nm for each NP batch.

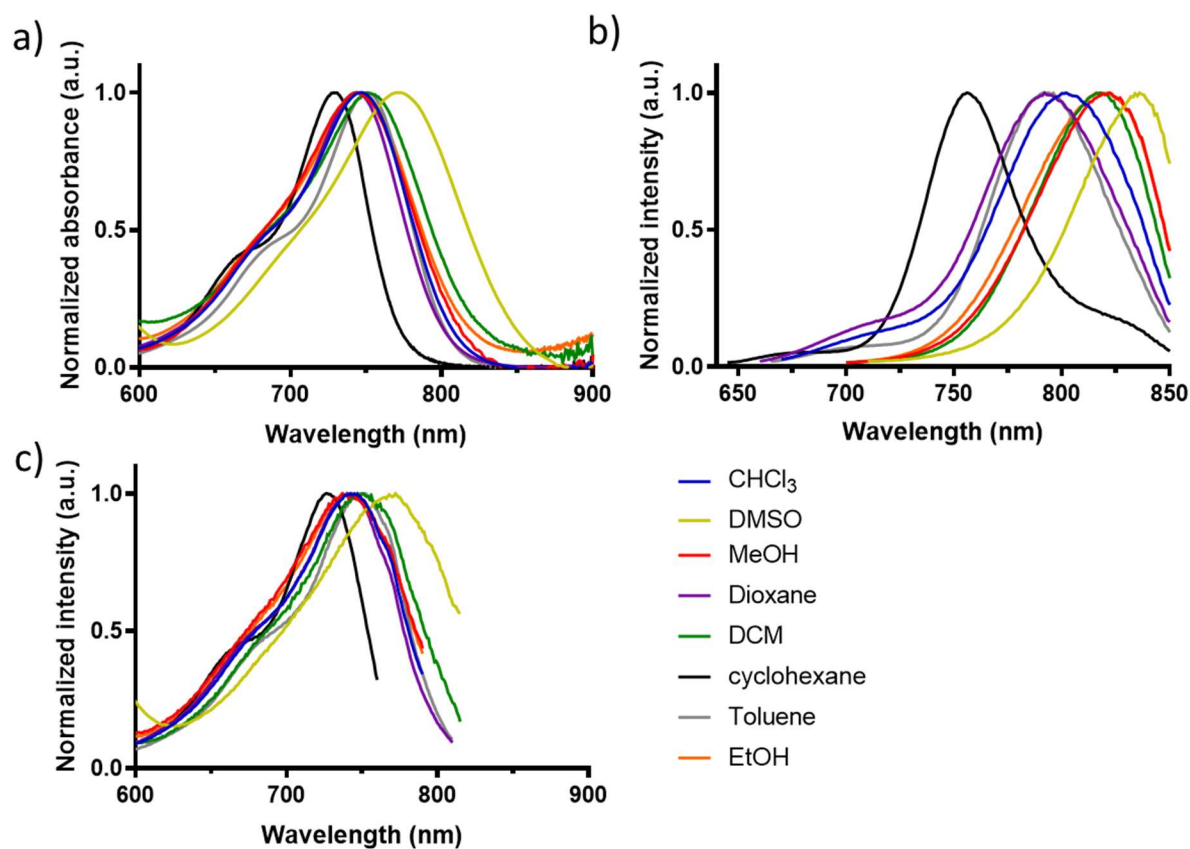


Figure S3. Solvatochromism experiments. (a) Normalized absorption spectra. (b) Normalized emission spectra. (c) Normalized excitation spectra.

solvent	$\lambda_{\text{abs,max}}$ (nm)	$\lambda_{\text{fluo,max}}$ (nm)	Stokes shift (cm^{-1})	ϵ ($\text{L}\cdot\text{mol}^{-1}\cdot\text{cm}^{-1}$)	QY	τ (ns)	ET(30) ()
Cyclohexane	728.5	756	499	$7.9 \times 10^{4\#}$	0.18	2.3*	30.9
Toluene	750	794	739	7.6×10^4	0.05	1.6	33.9
Dioxane	744	792	815	7.8×10^4	0.006	1.4*	36
CHCl_3	747	802	918	7.0×10^4	0.07	1.5	39.1
DCM	757	818	985	8.0×10^4	0.01	1.0	40.7
DMSO	772	836	992	6.3×10^4	<0.001	0.42*	45.1
EtOH	746	816	1150	$7.1 \times 10^{4\#}$	0.003	0.60 ¥	51.9
MeOH	743	822	1294	$6.9 \times 10^{4\#}$	0.009	0.40 ¥	55.4

Table S3. Solvatochromism experiments. * τ average for a biexponential fit. ¥ τ average for a triexponential fit. QY were determined with an integrated sphere. Polarity coefficient ET(30) based on literature values [54]. # Absorption coefficient obtained with successive dilutions from a solution 95/5 (v/v) solvent/chloroform.

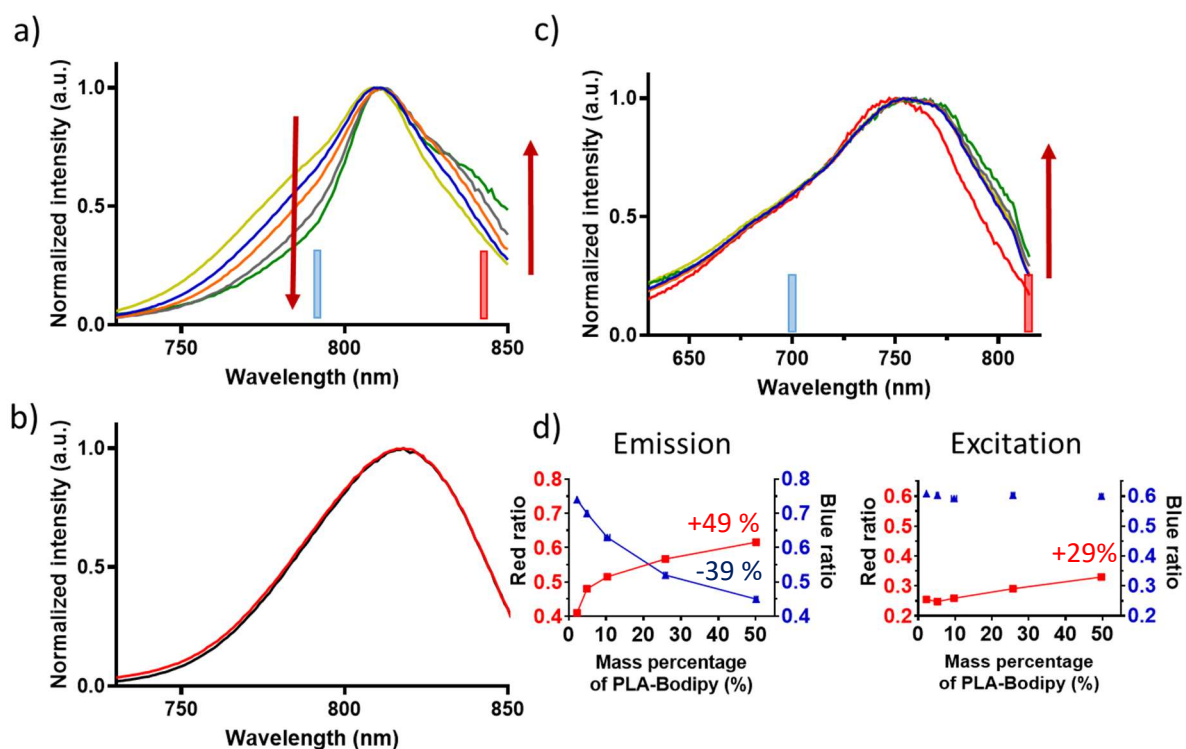


Figure S4. (a) Emission spectra of NPs (excitation at 540 nm), slit width 9.5 nm and $A < 0.1$. (Yellow, Bodipy NP-2.5%), (blue, Bodipy NP-5%), (orange, Bodipy NP-10%), (grey, Bodipy NP-25%), (green, Bodipy NP-50%). (b) Emission spectra of Bodipy in DCM, $C = 1.5 \times 10^{-6}$ M, $A < 0.1$. Black, excitation at 540 nm, slit width 9.5 nm. Red, excitation 670 nm, slit width 10 nm. (c) Excitation spectra of NPs emission at 845 nm, slit width 15 nm and $A < 0.1$. (Yellow, Bodipy NP-2.5%), (blue, Bodipy NP-5%), (orange, Bodipy NP-10%), (grey, Bodipy NP-25%), (green, Bodipy NP-50%), (red, Bodipy DCM). (d) Evolution of the red ratio ($I_{840\text{nm}}/I_{\text{max}}$) and the blue ratio ($I_{793\text{nm}}/I_{\text{max}}$) as a function of mass percentage of PLA-Bodipy for Emission spectra. Evolution of the red ratio ($I_{815\text{nm}}/I_{\text{max}}$) and the blue ratio ($I_{700\text{nm}}/I_{\text{max}}$) as a function of the mass percentage of PLA-Bodipy for the Excitation spectra.

NP	EXCITATION 670 nm				EXCITATION 540 nm		
	$\lambda_{\text{abs,max}}$ (nm)	$\lambda_{\text{fluo,max}}$ (nm)	σ (cm^{-1})	FWHM (cm^{-1})	$\lambda_{\text{fluo,max}}$ (nm)	σ (cm^{-1})	FWHM (cm^{-1})
NP-2.5%	756	804	790	1187	810	882	927
NP-5%	757	812	895	1119	810	864	873
NP-10%	757	818	985	1028	812	895	818
NP-25%	759	824	1039	930	813	875	764
NP-50%	761	825	1019	907	812	825	786

Table S4: Spectroscopic properties of NP emission spectra with two different excitation 540 nm and 670 nm. FWHM: Full Width at Half Maximum. σ : Stokes shift. Estimated error for FWHM: ± 15 nm.

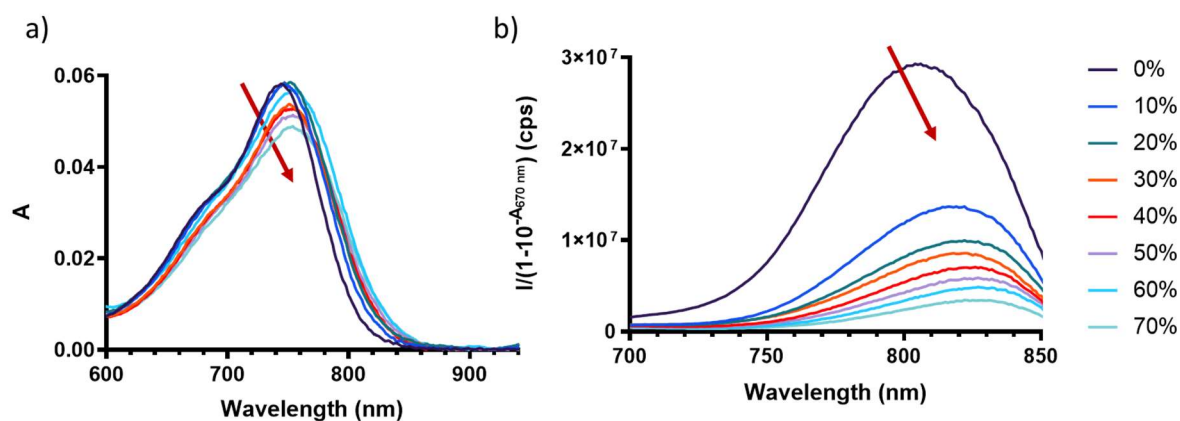


Figure S5. (a) Absorption spectra of Bodipy with different volume percentage of water/THF solution. For each solution $C = 9.0 \times 10^{-7}$ M and the water percentages were indicated from 0% to 70%. (b) Emission spectra of Bodipy, excitation at 670 nm, slit width 10 nm and $A < 0.1$, in different volume percentage of water/THF solution. For each solution $C = 9.0 \times 10^{-7}$ M and the water percentage was indicated from 0% to 70%.

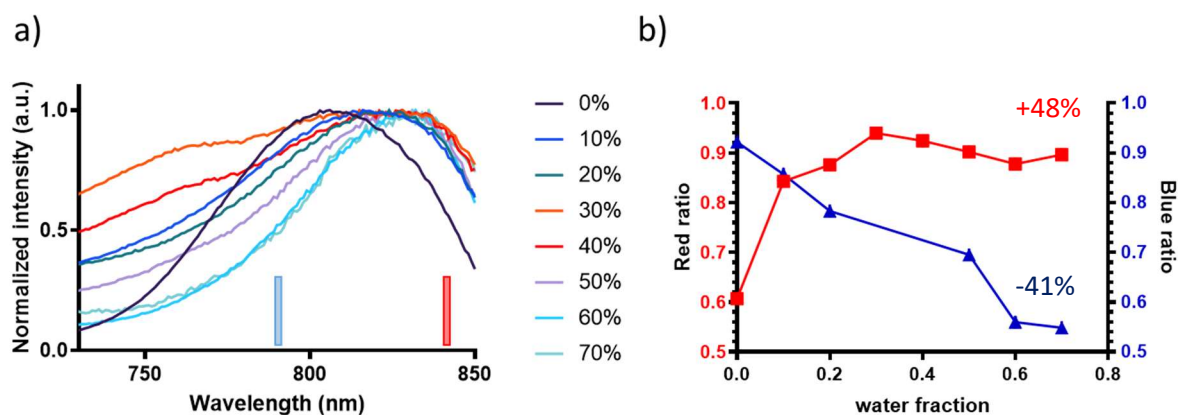


Figure S6. (a) Emission spectra of Bodipy excitation at 540 nm, slit width 9 nm and $A < 0.1$, in different volume percentage of water/THF solution. For each solutions $C = 9.0 \times 10^{-7}$ M and the water percentage was indicated from 0% to 70%. (b) Evolution of the red ratio ($I_{840\text{nm}}/I_{\text{max}}$) and the blue ratio ($I_{793\text{nm}}/I_{\text{max}}$) of the fluorescence emission spectra, as a function of the water fraction compared to THF.

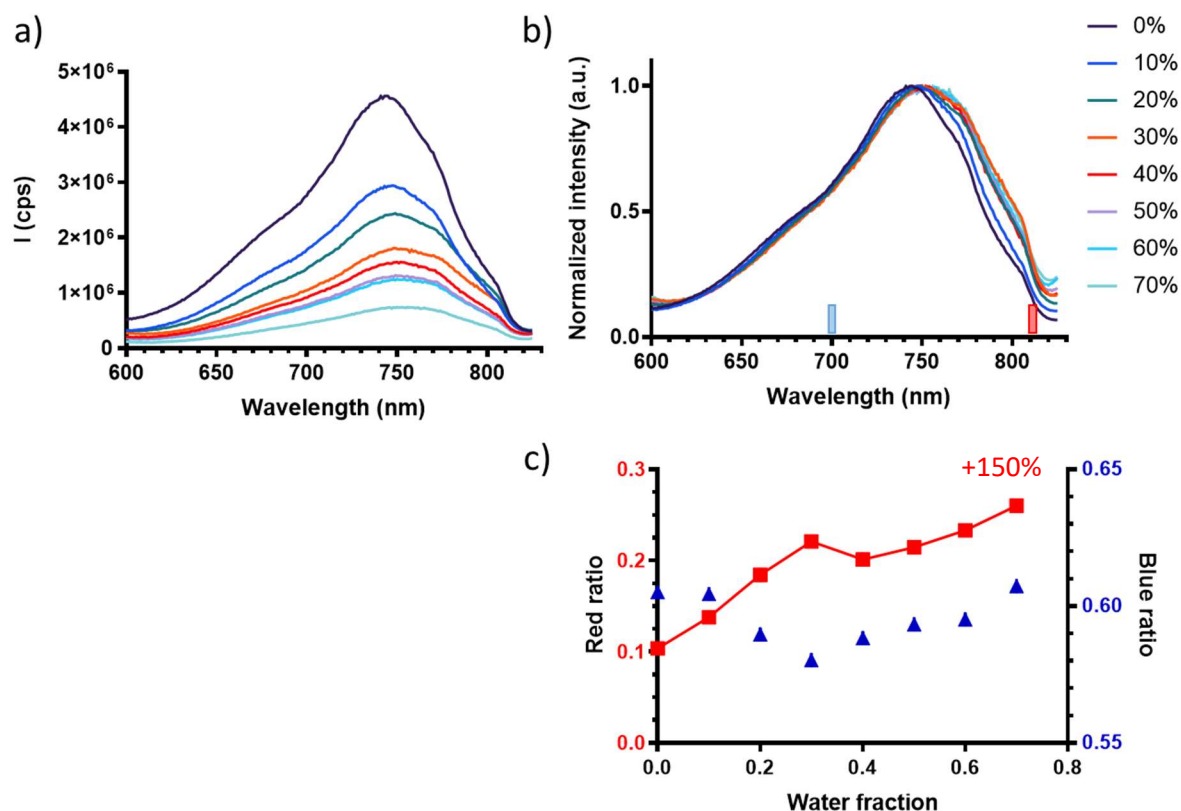


Figure S7. (a) Fluorescence excitation spectra of Bodipy emission at 845 nm, slit width 17 nm and $A < 0.1$, in different volume percentage of water/THF solution. For each solution $C = 9.0 \times 10^{-7}$ M and

the water percentage was indicated from 0% to 90%. (b) Normalized fluorescence excitation spectra of Bodipy emission at 845 nm, slit width 17 nm and $A < 0.1$, in different volume percentage of water/THF solution. For each solution $C = 9.0 \times 10^{-7}$ M and the water percentages was indicated from 0% to 90%. (c) Evolution of the red ratio ($I_{815\text{nm}}/I_{\text{max}}$) and the blue ratio ($I_{700\text{nm}}/I_{\text{max}}$) of the fluorescence excitation spectra as a function of the water fraction compared to THF.

	NP-2.5%	NP-5%	NP-10%	NP-25%	NP-50%
$\bar{\tau}(\text{ns})$ [‡]	1.02	0.89	0.61	0.35	0.23
$k_r (\text{s}^{-1})$ [@]	1.4×10^8	1.3×10^8	1.3×10^8	1.3×10^8	1.3×10^8
$k_{nr} (\text{s}^{-1})$	8.4×10^8	9.9×10^8	1.5×10^9	2.8×10^9	4.2×10^9

Table S5. Spectroscopic parameters of NP batches. [‡] Biexponential fit, excitation at 781 nm, emission detected at 820 nm, T1 value fixed at 1.09 ns, $\bar{\tau} = \frac{B_1 T_1^2 + B_2 T_2^2}{B_1 T_1 + B_2 T_2}$. [@] k_r calculated with Strickler-Berg equation.

	NP-5%	NP-25%	NP-50%
T_g	30.4°C	32°C	33.4°C

Table S6. NP's glass temperature measured by differential scanning calorimetry.

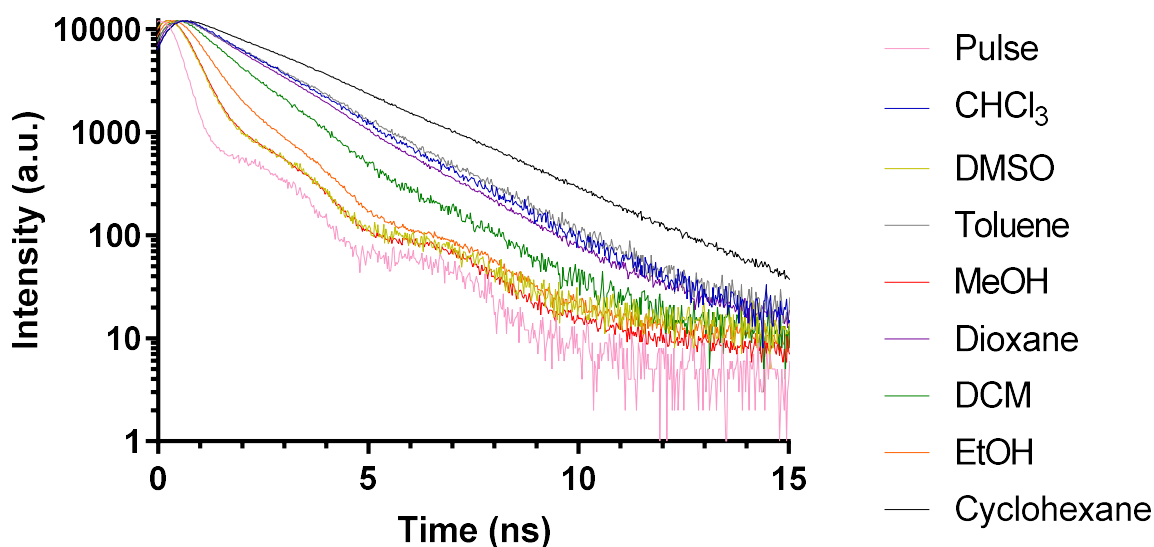


Figure S8. Solvatochromism experiments. Fluorescence lifetime of Bodipy in different solvents, emission detection at the band maximum, excitation at 667 nm for cyclohexane and 781 nm for all the other solvents.

Distribution function of the stretched exponential

The stretched exponential function is defined as:

$$y = B_1 e^{\left(\frac{-t}{T_1}\right)^\beta} = \int_0^\infty H(k) \times \exp(-kt) dt$$

With $0 \leq \beta \leq 1$ and $1/k$ and T_1 lifetimes values. $H(k)$, the distribution function is obtained thanks to the Laplace transformation of y . As no analytical results exist (except for $\beta = 0.5$), the $H(k)$ function can be estimated for $\beta > 0.5$ [43]:

$$H(k) = T_1 \frac{B}{(kT_1)^{\frac{1-\beta}{2}}} (1 + C(kT_1)^\delta) \times \exp\left(-\frac{(1-\beta) \times \beta^{\frac{\beta}{1-\beta}}}{(kT_1)^{\frac{\beta}{1-\beta}}}\right)$$

With $\delta = \frac{\beta(\beta-0.5)}{1-\beta}$, and C and B parameter whose values change with β .

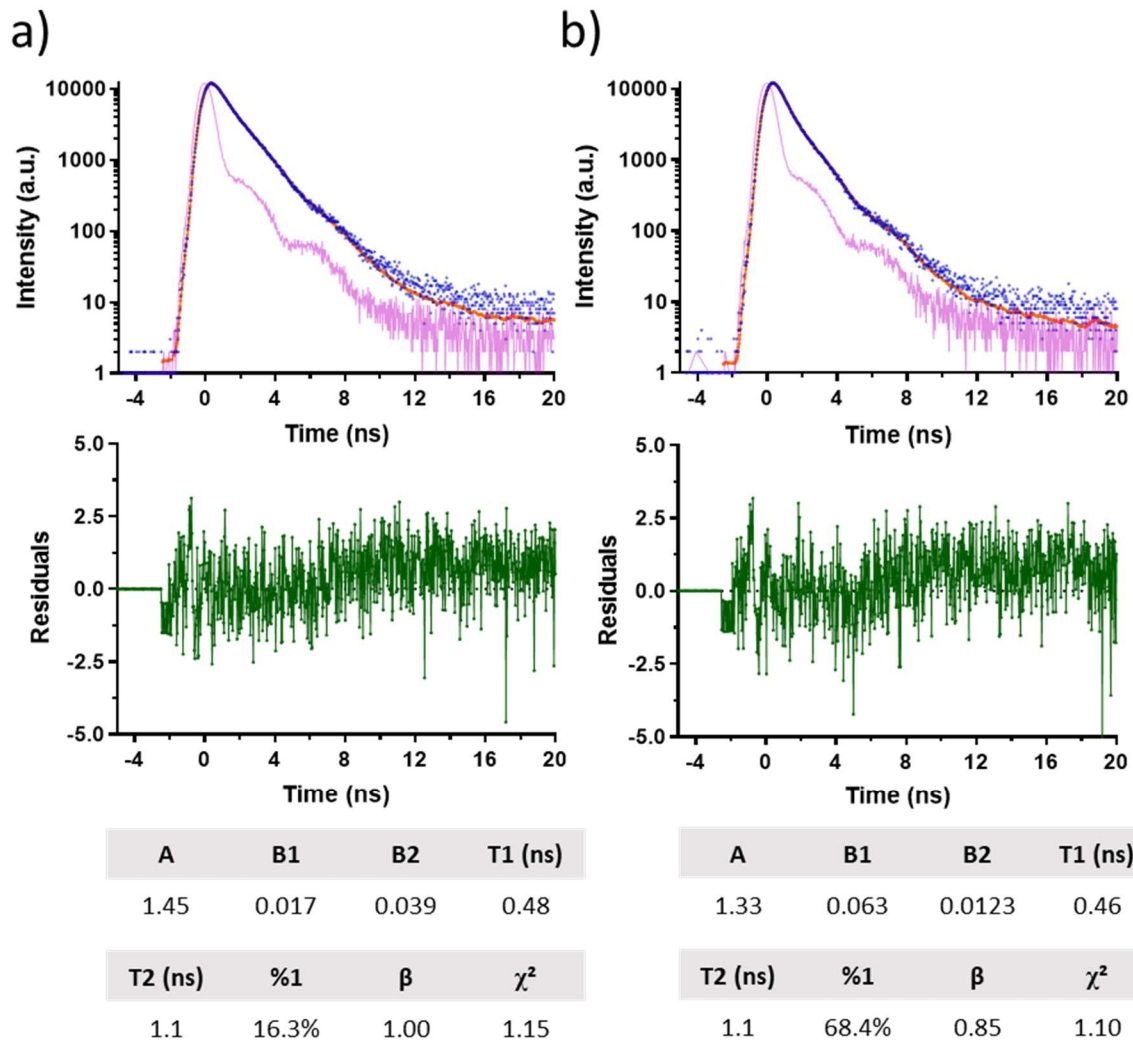


Figure S9. Fluorescence decays of the NP suspensions. Excitation at 781 nm with a pulsed diode <200 ps, emission at 820 nm, (blue, fluorescence decay), (red, Mathematical fit), (purple, instrument response function), (Green, residuals). The mathematical fit function is a sum of a Kohlrausch function and a monoexponential function: $y = A + B_1 e^{\left(-\frac{t}{T_1}\right)^\beta} + B_2 e^{-\frac{t}{T_2}}$. (a) NP5%, (b) NP10%. The fitted parameters were indicated below each graph. $\%1 = \frac{B_1 T_1}{B_1 T_1 + B_2 T_2}$

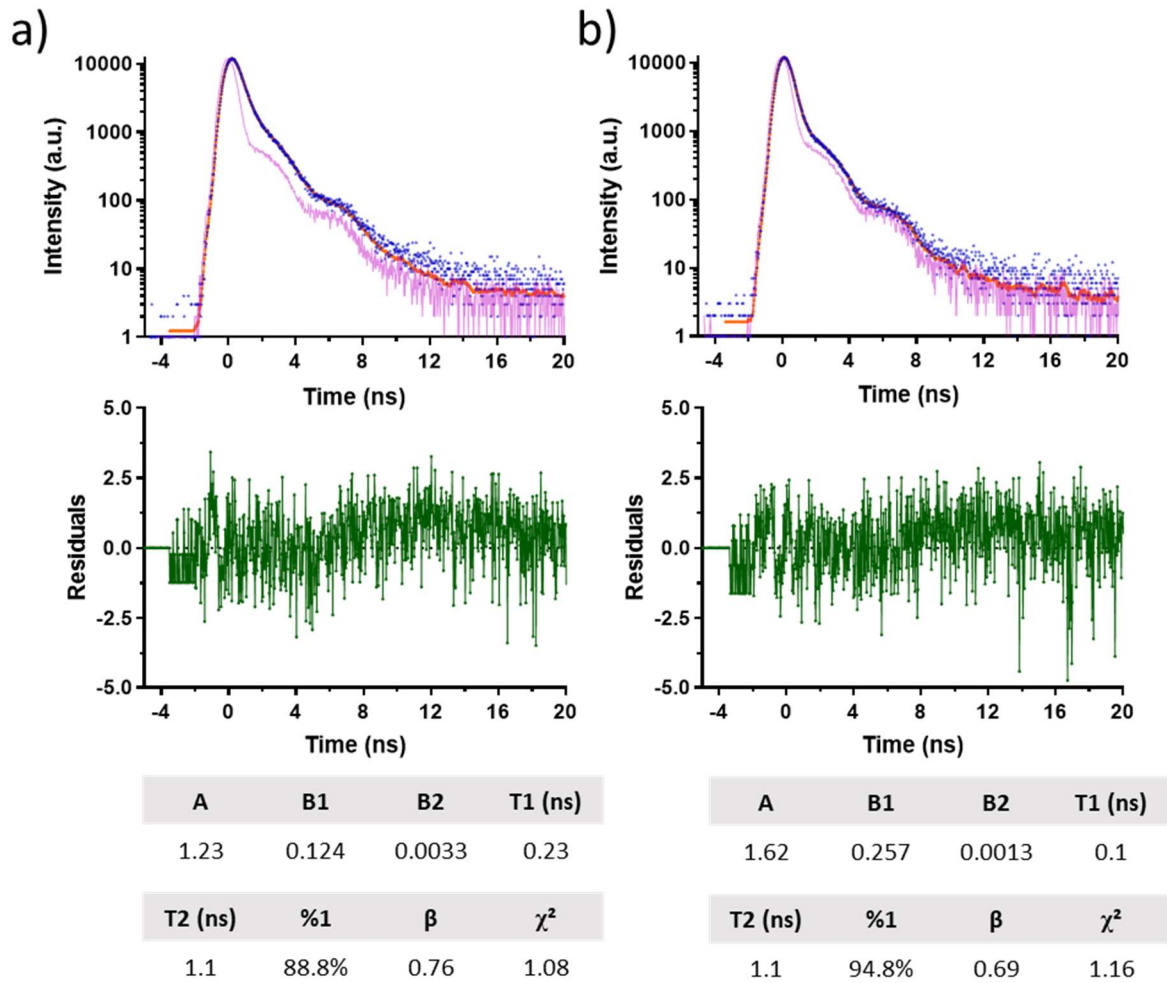


Figure S10. Fluorescence decays of the NP suspensions. Excitation at 781 nm with a pulsed diode <200 ps, emission at 820 nm, (blue, fluorescence decay), (red, Mathematical fit), (purple, instrument response function), (Green, residuals). The mathematical fit function is a sum of a Kohlrausch function and a monoexponential function: $y = A + B_1 e^{\left(\frac{-t}{T_1}\right)^\beta} + B_2 e^{-\frac{t}{T_2}}$. (a) NP25%, (b) NP50%. The fitted parameters were indicated below each graph. $\%1 = \frac{B_1 T_1}{B_1 T_1 + B_2 T_2}$

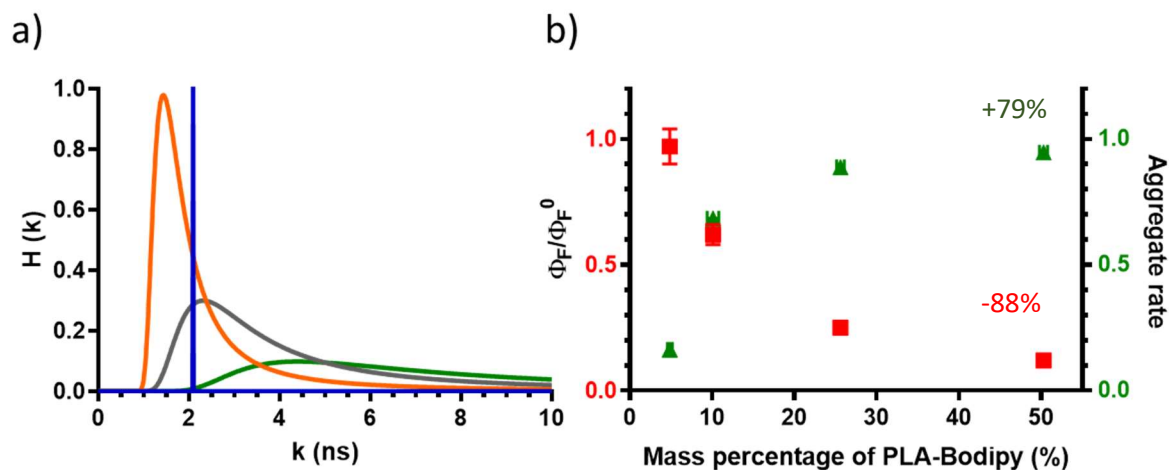


Figure S11. (a) Lifetime distribution $H(k)$ for the Kohlrausch function used for the fluorescence decay, (blue, Bodipy NP-5%), (orange, Bodipy NP-10%), (grey, Bodipy NP-25%), (green, Bodipy NP-50%). Fluorescence quantum yield (red squares) evolution and aggregates' proportion (green triangles) evolution as a function of the mass percentage of PLA-Bodipy. (b) Luminescence quantum yield (Φ_F) relative to Bodipy, 298 K ($\Phi_F^0 = 0.007$). Mean values and standard deviation were obtained for: (Bodipy NP-5%, $n=4$ batches); (Bodipy NP-10%, $n=3$ batches); (Bodipy NP-25%, $n=4$ batches); (Bodipy NP-50%, $n=3$ batches). Aggregate rate (%1) comes from the Kohlrausch fit of the fluorescence decay.

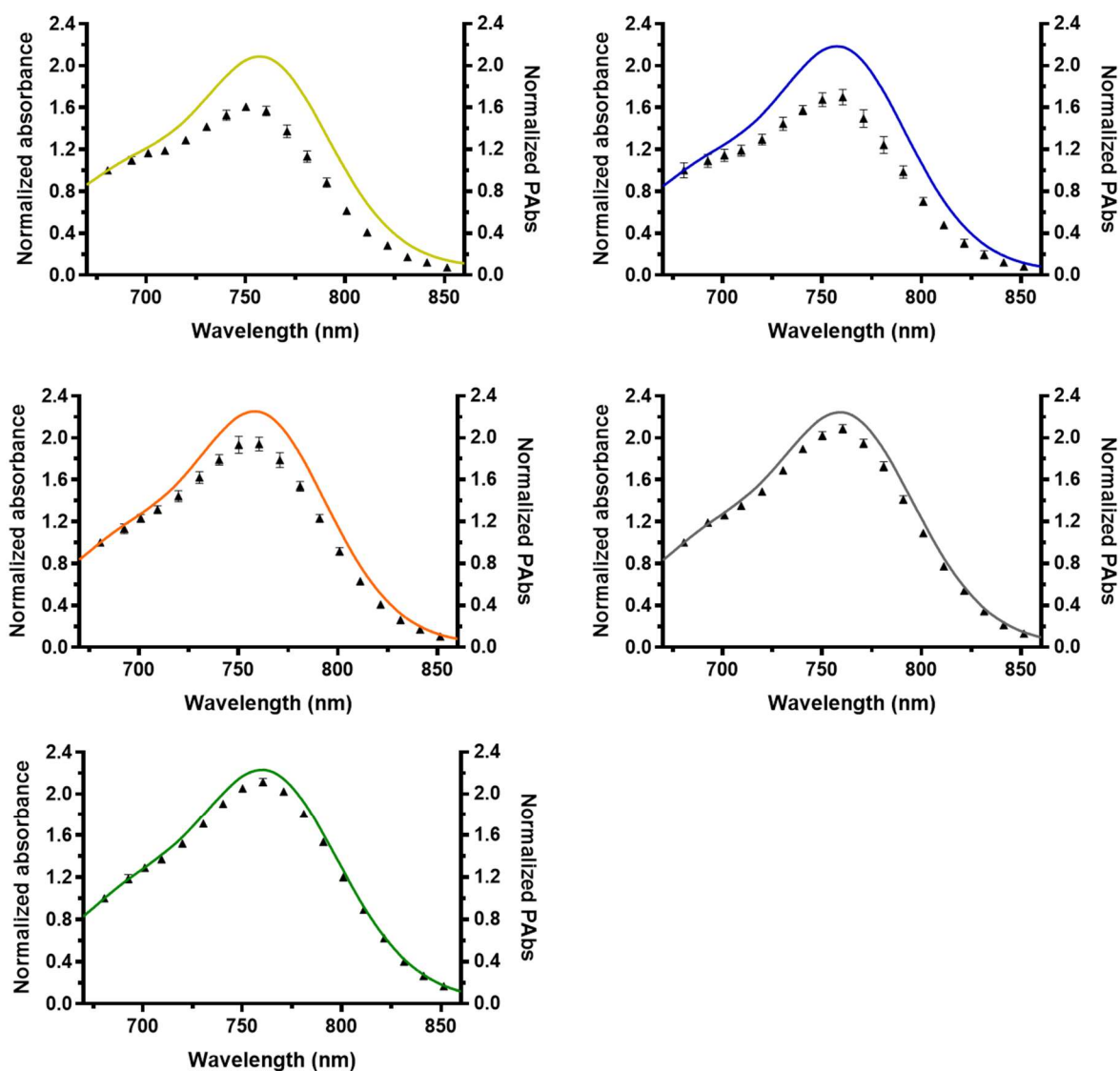


Figure S12. Normalized absorption spectra (continuous line) at 680.6 nm and normalized photoacoustic excitation spectra (dark triangle) at 680.6 nm for each NP batch (green NP-50%), (grey NP-25%), (orange NP-10%), (blue NP-5%), (yellow NP-2.5%). Laser fluence 3.5 mJ.cm^{-2} .

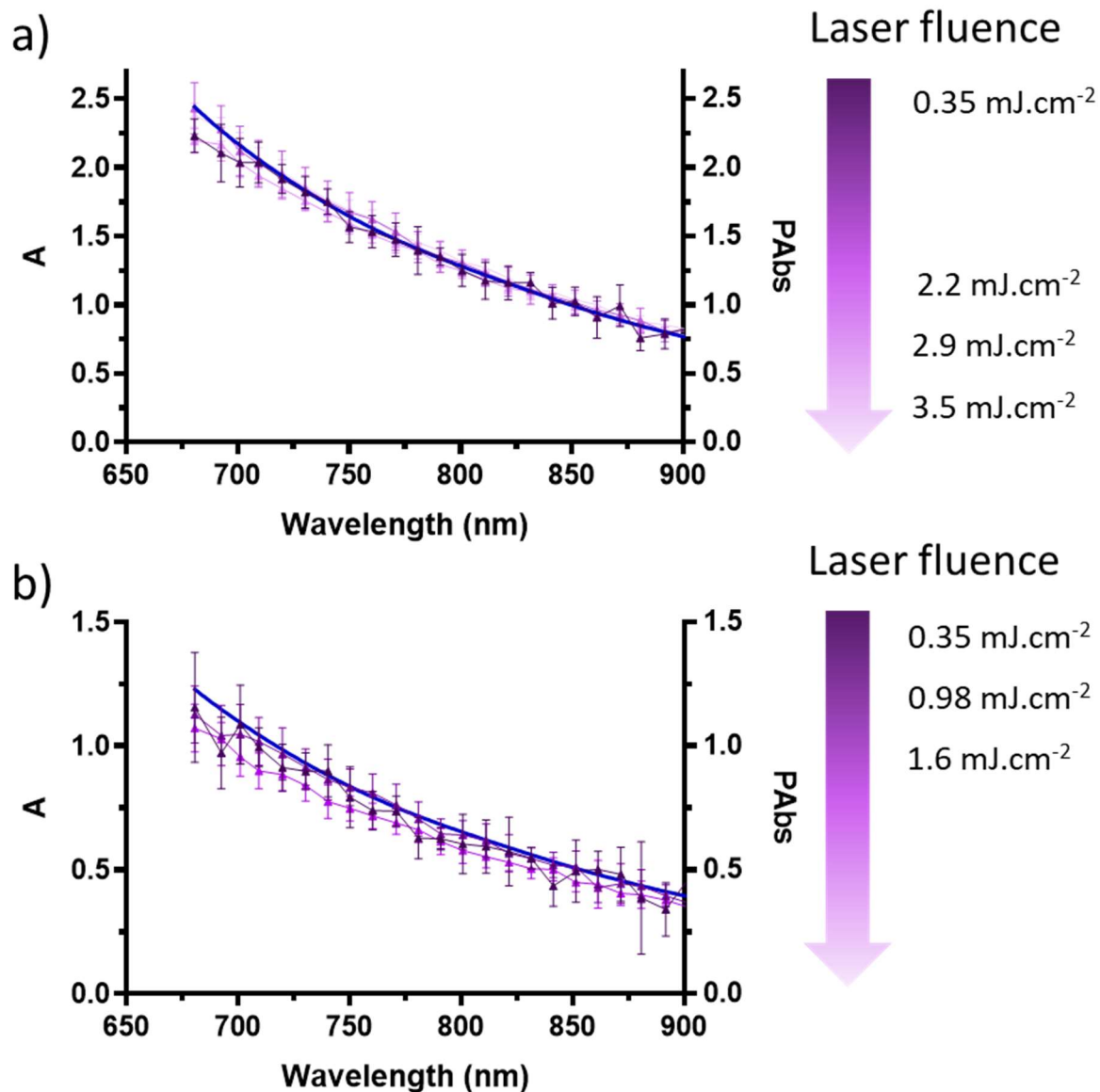


Figure S13. (a) PES of nigrosine solution measured with 0.35 mJ.cm⁻², 2.2 mJ.cm⁻², 2.9 mJ.cm⁻², 3.5 mJ.cm⁻² laser fluences and absorption spectra of the nigrosine solution (blue line). $C_{\text{Nigrosin}} = 9.5 \times 10^{-4}$ mol.L⁻¹ in water. (b) PES of nigrosine solution measured with 0.35 mJ.cm⁻², 0.98 mJ.cm⁻², 1.6 mJ.cm⁻² laser fluences and absorption spectra of the nigrosine solution (blue line). $C_{\text{Nigrosin}} = 4.84 \times 10^{-4}$ mol.L⁻¹ in water. The error bars for the PES corresponds to the median value \pm MAD.

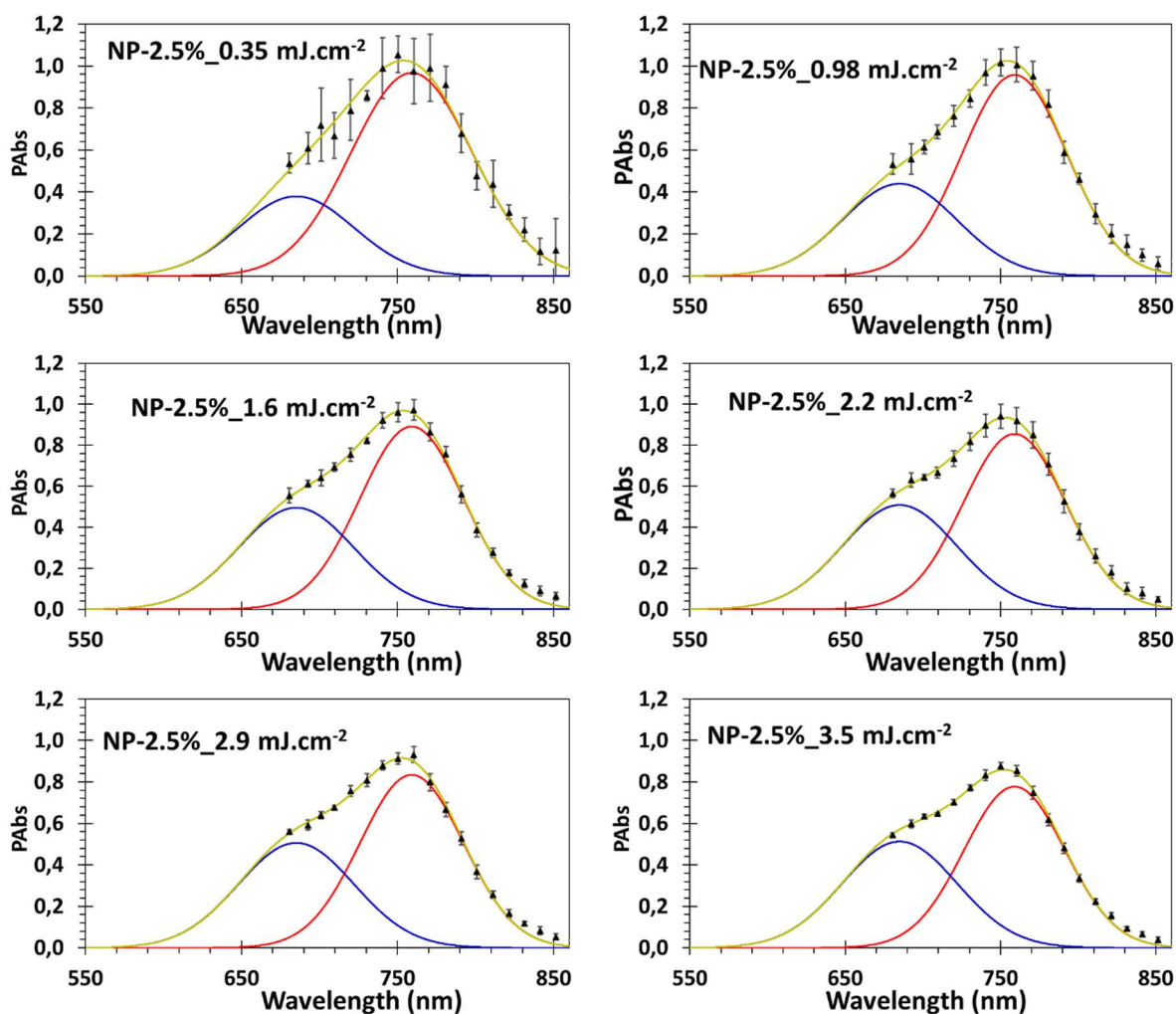


Figure S14. Photoacoustic excitation spectra of NP-2.5% (dark triangles) for every laser fluence: 0.35 mJ.cm⁻², 0.98 mJ.cm⁻² and 1.6 mJ.cm⁻², 2.2 mJ.cm⁻², 2.9 mJ.cm⁻², 3.5 mJ.cm⁻². The error bars for the PES corresponds to the median value \pm MAD. The corresponding laser fluence is indicated on each graph. For each fluence, the total fit curve (yellow) composed of two Gaussian functions (in red and blue) were plotted.

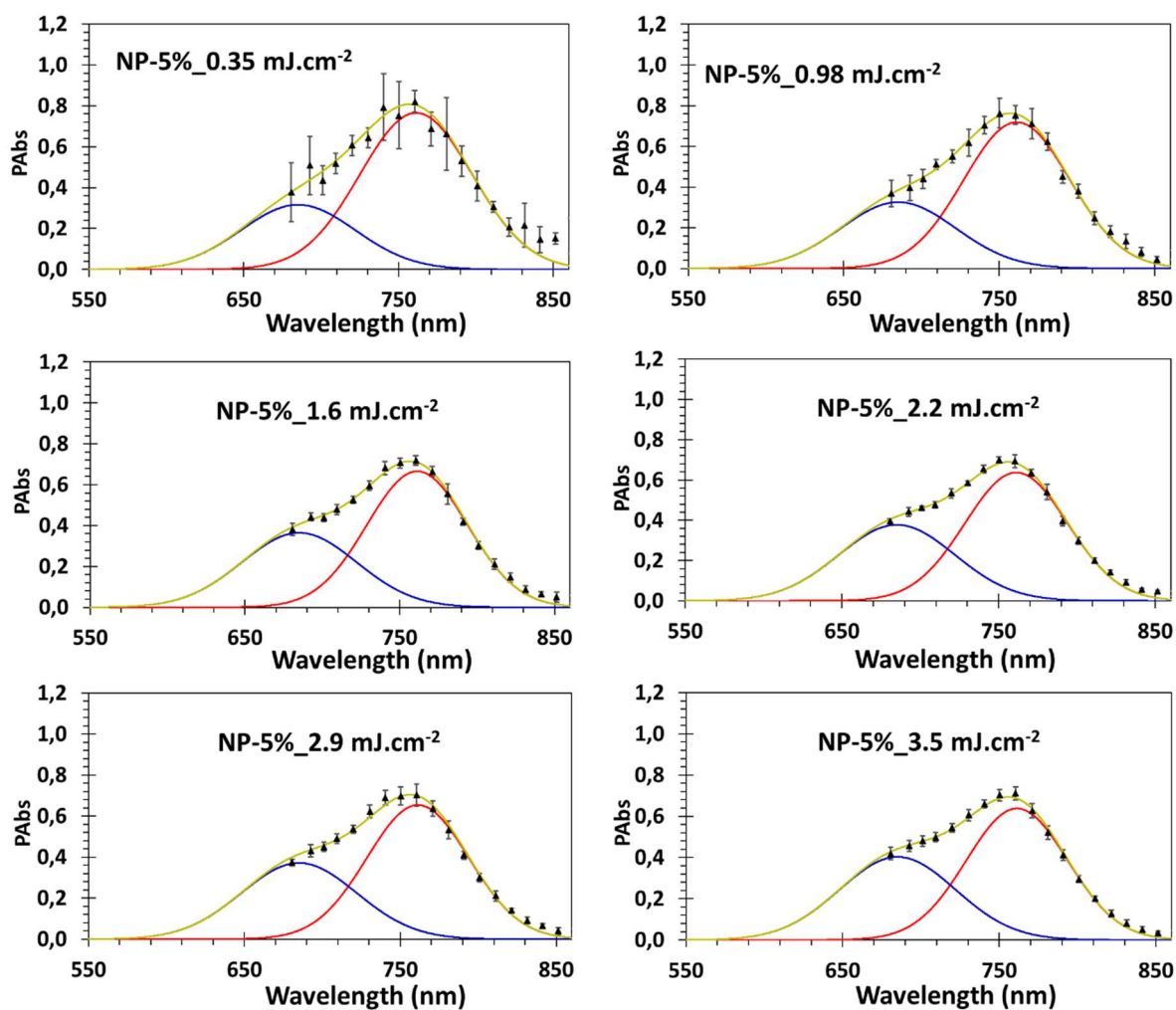


Figure S15. Photoacoustic excitation spectra of NP-5% (dark triangles) for every laser fluence: 0.35 mJ.cm⁻², 0.98 mJ.cm⁻² and 1.6 mJ.cm⁻², 2.2 mJ.cm⁻², 2.9 mJ.cm⁻², 3.5 mJ.cm⁻². The error bars for the PES corresponds to the median value \pm MAD. The corresponding laser fluence is indicated on each graph. For each fluence, the total fit curve (yellow) composed of two Gaussian functions (in red and blue) were plotted.

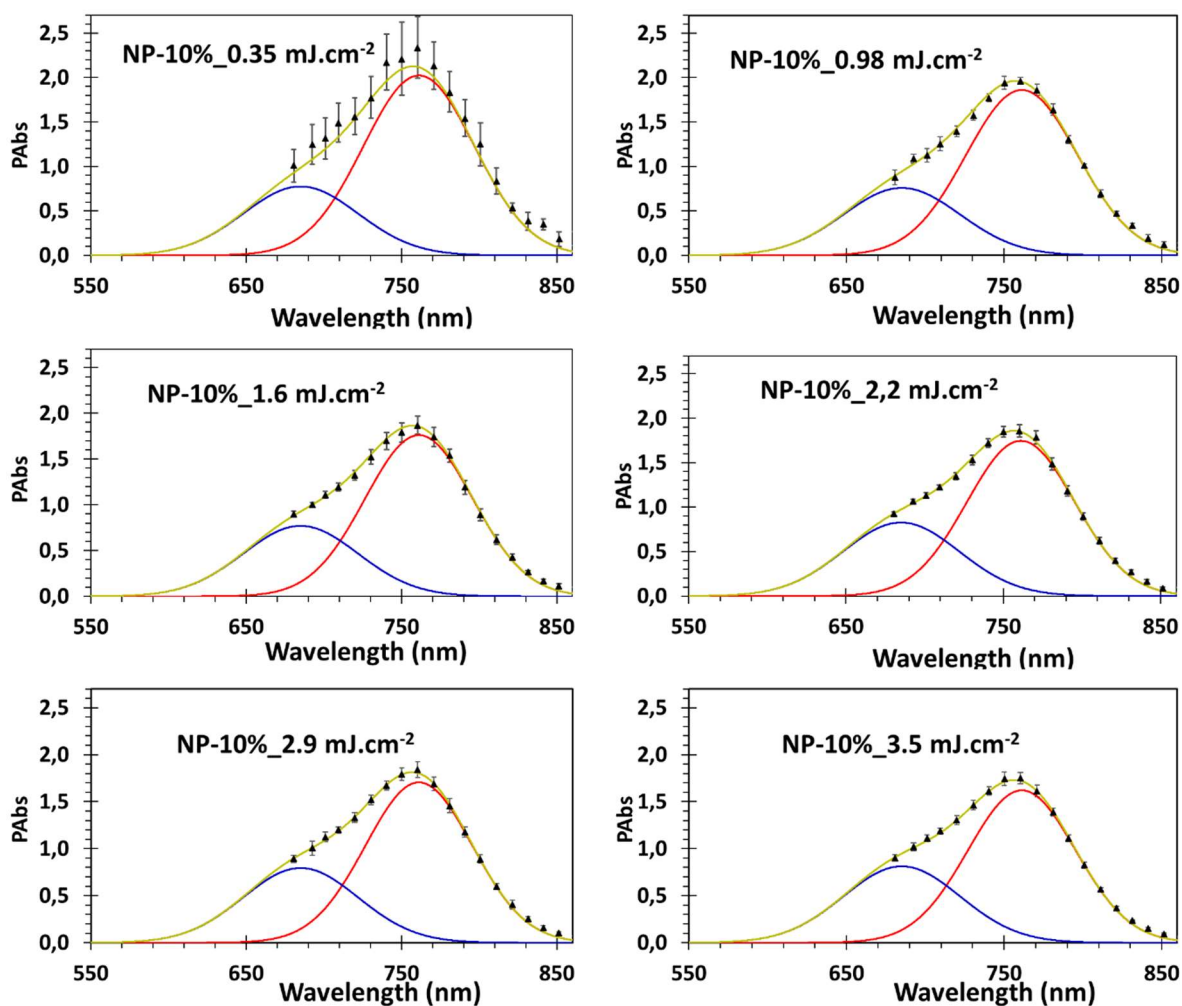


Figure S16. Photoacoustic excitation spectra of NP-10% (dark triangles) for every laser fluence: 0.35 mJ.cm⁻², 0.98 mJ.cm⁻² and 1.6 mJ.cm⁻², 2.2 mJ.cm⁻², 2.9 mJ.cm⁻², 3.5 mJ.cm⁻². The error bars for the PES corresponds to the median value \pm MAD. The corresponding laser fluence is indicated on each graph. For each fluence, the total fit curve (yellow) composed of two Gaussian functions (in red and blue) were plotted.

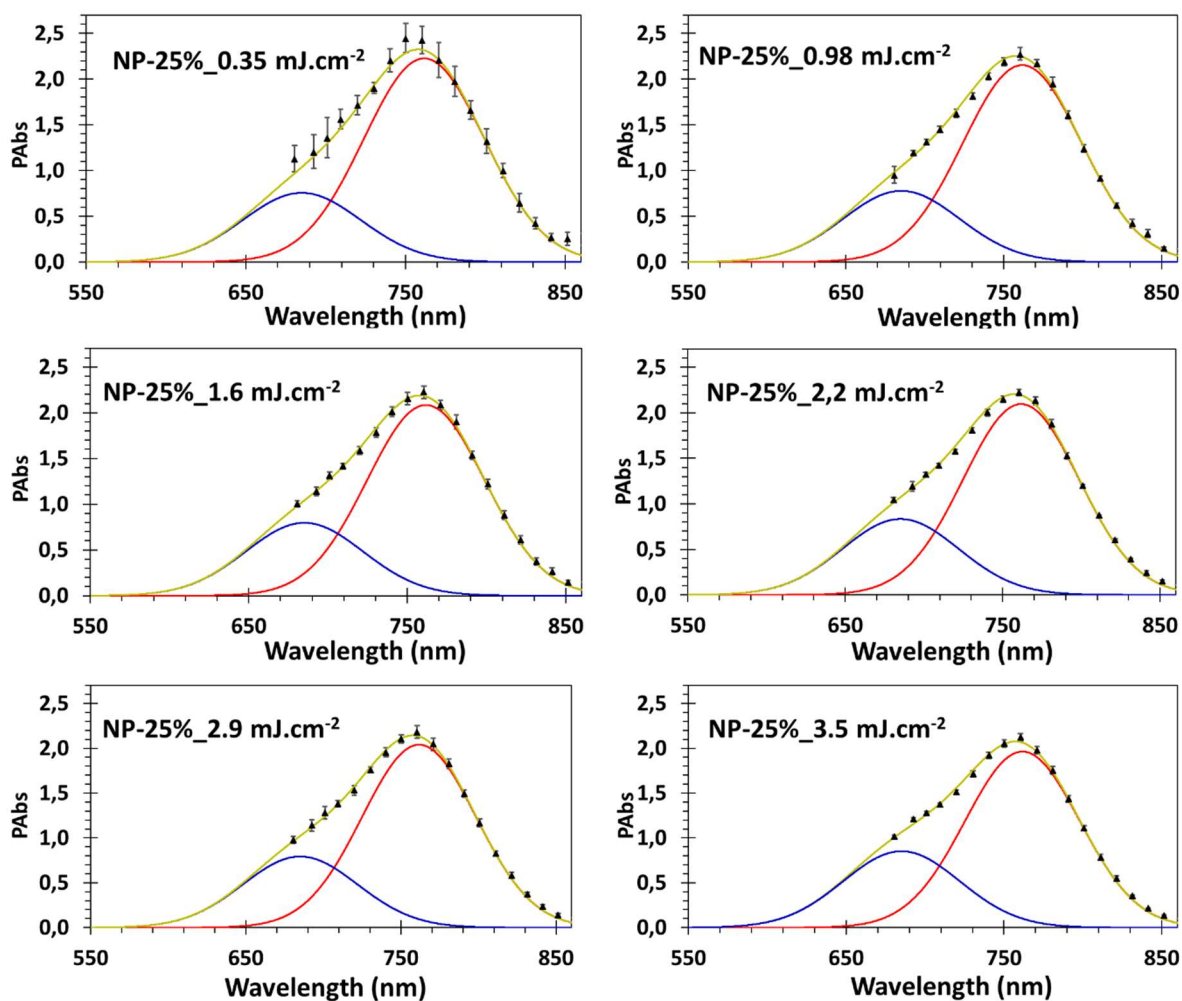


Figure S17. Photoacoustic excitation spectra of NP-25% (dark triangles) for every laser fluence: 0.35 mJ.cm⁻², 0.98 mJ.cm⁻² and 1.6 mJ.cm⁻², 2.2 mJ.cm⁻², 2.9 mJ.cm⁻², 3.5 mJ.cm⁻². The error bars for the PES corresponds to the median value \pm MAD. The corresponding laser fluence is indicated on each graph. For each fluence, the total fit curve (yellow) composed of two Gaussian functions (in red and blue) were plotted. NP-25% were diluted two times.

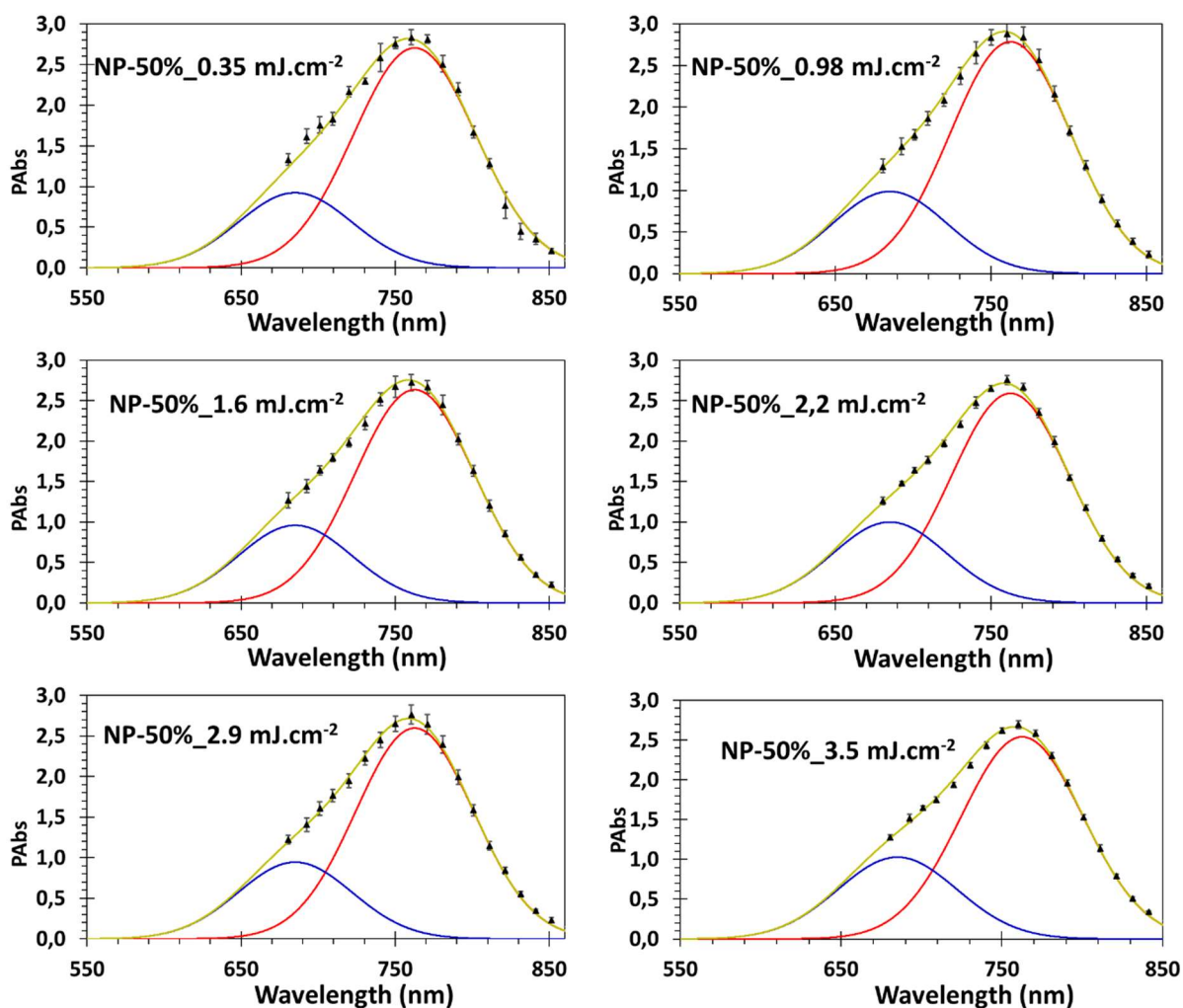


Figure S18. Photoacoustic excitation spectra of NP-50% (dark triangles) for every laser fluence: 0.35 mJ.cm⁻², 0.98 mJ.cm⁻² and 1.6 mJ.cm⁻², 2.2 mJ.cm⁻², 2.9 mJ.cm⁻², 3.5 mJ.cm⁻². The error bars for the PES corresponds to the median value \pm MAD. The corresponding laser fluence is indicated on each graph. For each fluence, the total fit curve (yellow) composed of two Gaussian functions (in red and blue) were plotted. NP-25% were diluted three times.

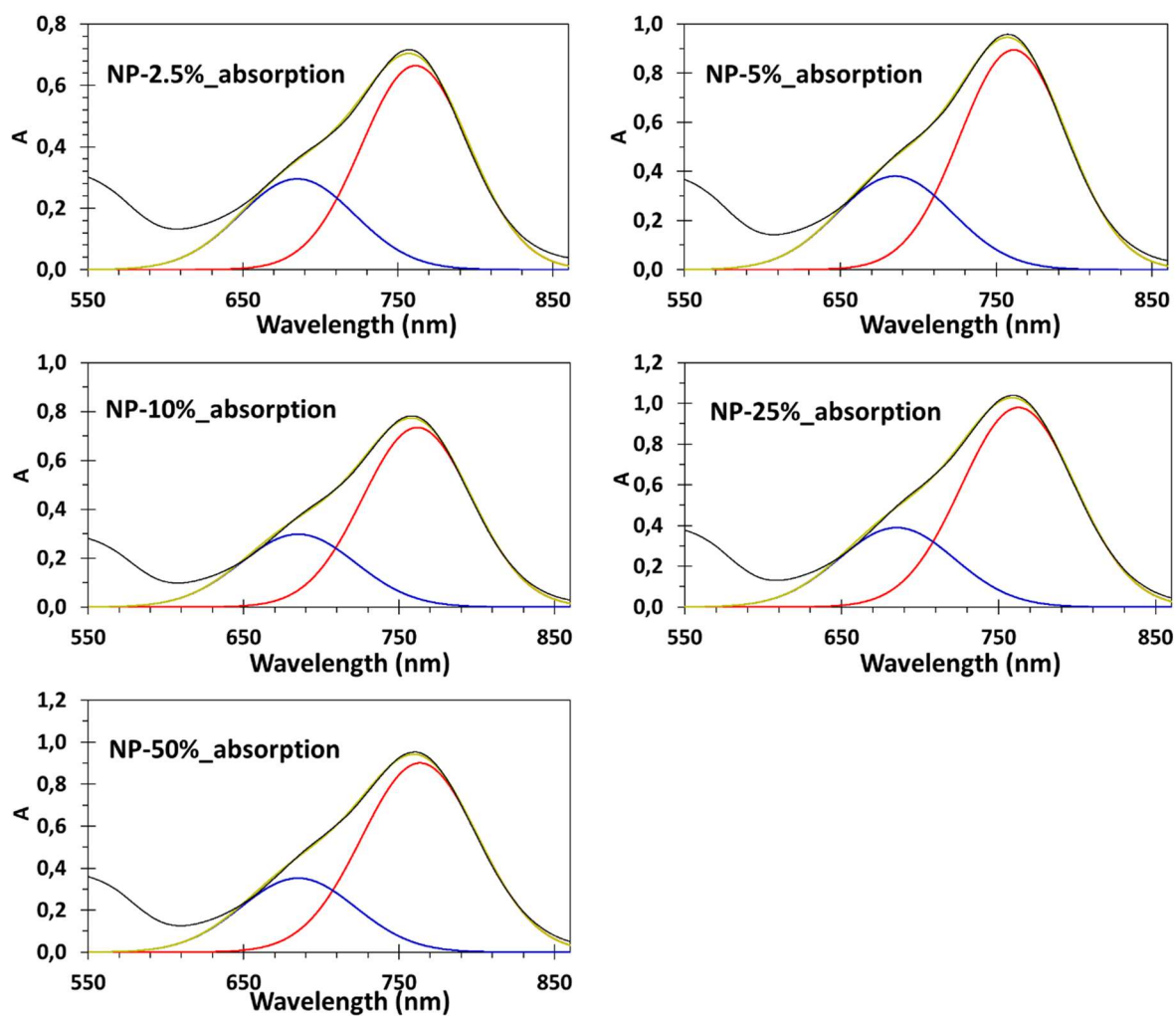


Figure S19. Absorption spectra of the different NP batches (dark curve). The corresponding NP batch is indicated on each graph. For each NP absorption, the total fit curve (yellow) composed of two Gaussian functions (in red and blue) were plotted. NP-10% was diluted three times, NP-25% was diluted five times and NP-50% was diluted ten times.

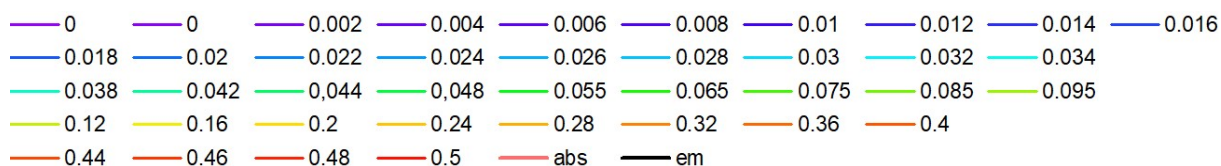
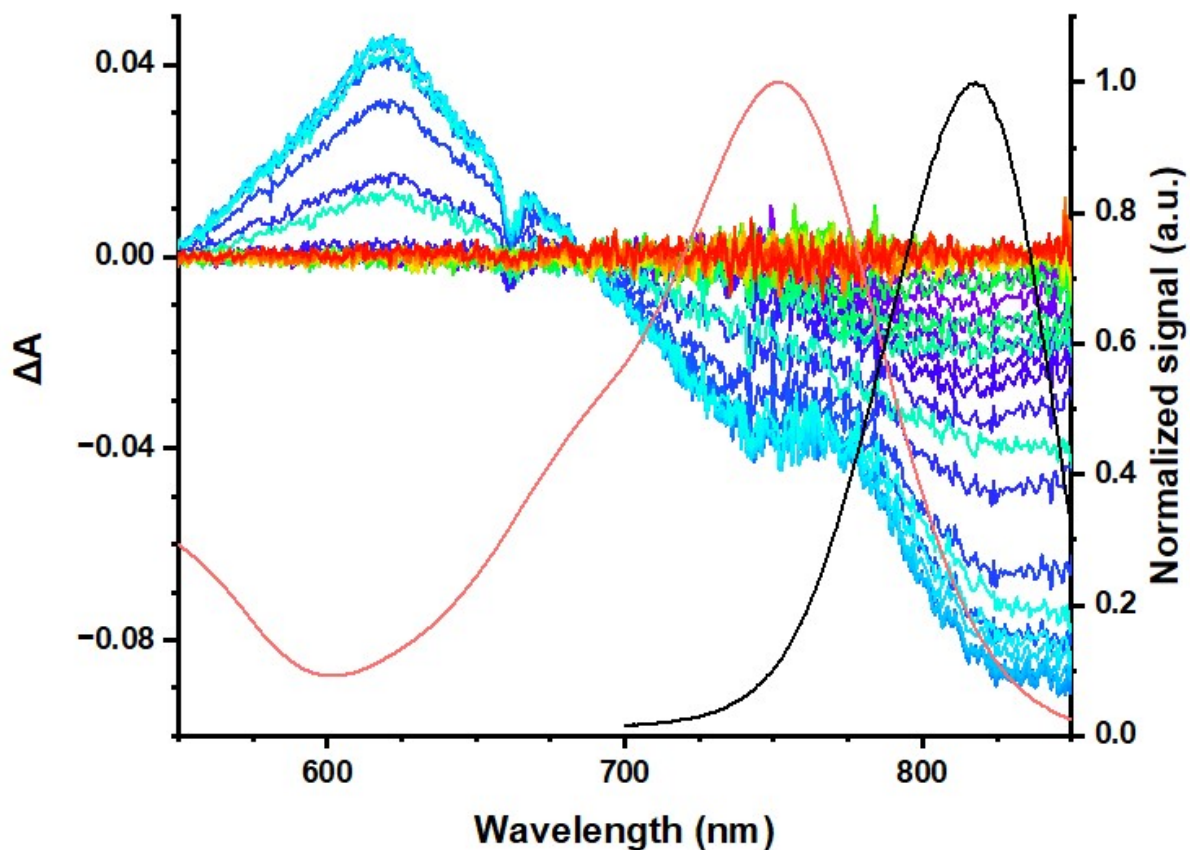


Figure S20. Transient absorption spectra of Bodipy in DCM for different time delays between pump and probe. Pump at 680 nm. Normalized Absorption spectra of Bodipy (salmon pink). Normalized fluorescence emission spectra of Bodipy (dark). Delays between pump and probe are indicated below in μs .

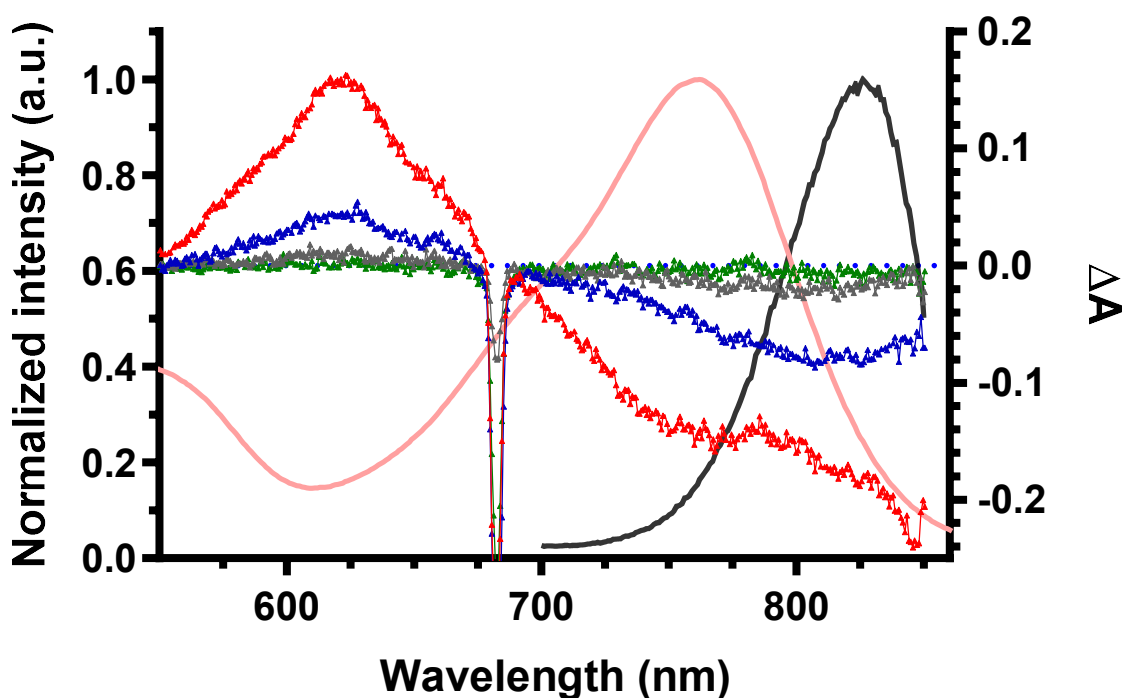


Figure S21. Transient absorption spectra of Bodipy in DCM (red), of NP5% (blue), of NP25% (grey), of NP50% (green). Pump at 680 nm and 0 μ s delay between pump and probe. Normalized Absorption spectra of NP50% (pink). Normalized fluorescence emission spectra of NP50% (light dark), excitation at 670nm. For all sample, the maximum absorption value was around 0.4.

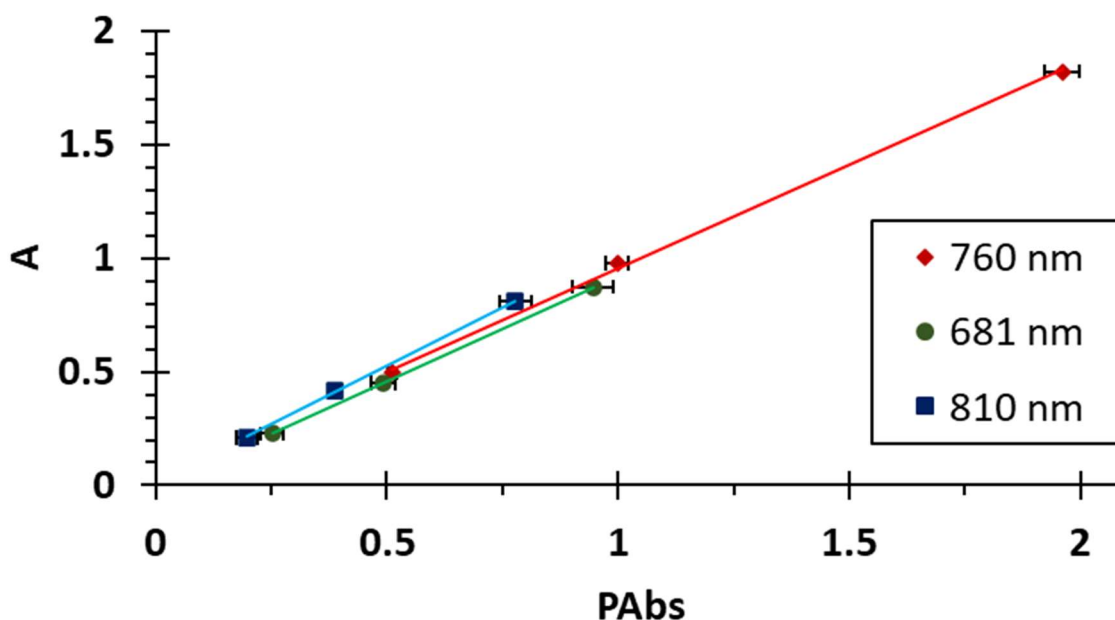


Figure S22: Absorption of the NP suspension as a function of the Photoacoustic absorbance (Laser fluence 3.5 mJ.cm^{-2}) for NP-50% at three NP concentration : dilution 5 times ($C_{\text{PLA-Bodipy}} = 0.5 \text{ mg.mL}^{-1}$); dilution 10 times ($C_{\text{PLA-Bodipy}} = 0.25 \text{ mg.mL}^{-1}$) and dilution 20 times ($C_{\text{PLA-Bodipy}} = 0.124 \text{ mg.mL}^{-1}$). Linear fit in continuous line. Red curve at 760 nm (fit $A = 0.910 \times \text{PAbs} + 0.045$; $R^2 = 0.999$).

Green curve at 681 nm (fit $A = 0.930 \times PAbs - 0.006$; $R^2 = 1.000$). Blue curve at 810 nm (fit $A = 1.025 \times PAbs + 0.014$; $R^2 = 0.999$).