Electronic Supplementary information: Quantitative photoacoustic spectral transformations in theranostic Solid Lipid Nanoparticles labelled with increasing concentrations of a Photoacoustic NIR BODIPY

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1. Material and methods

1.1. Synthesis of BODIPY-aniline palmitate.

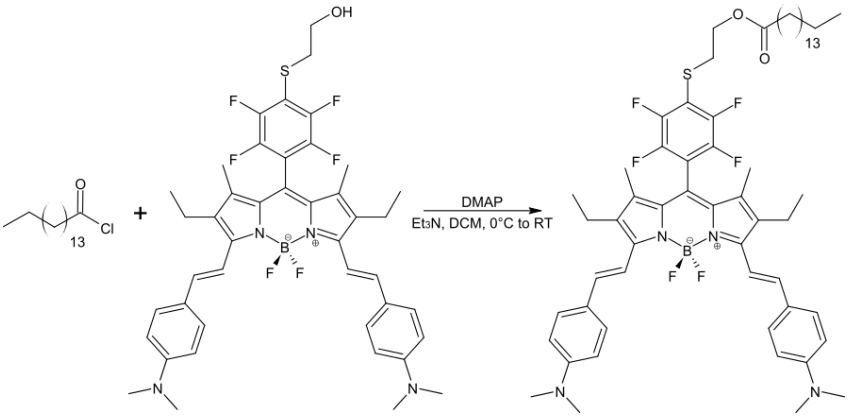

Fig. S1: Reaction scheme (esterification) to form BODIPY-aniline-palmitate (BY-aniline-Palm)

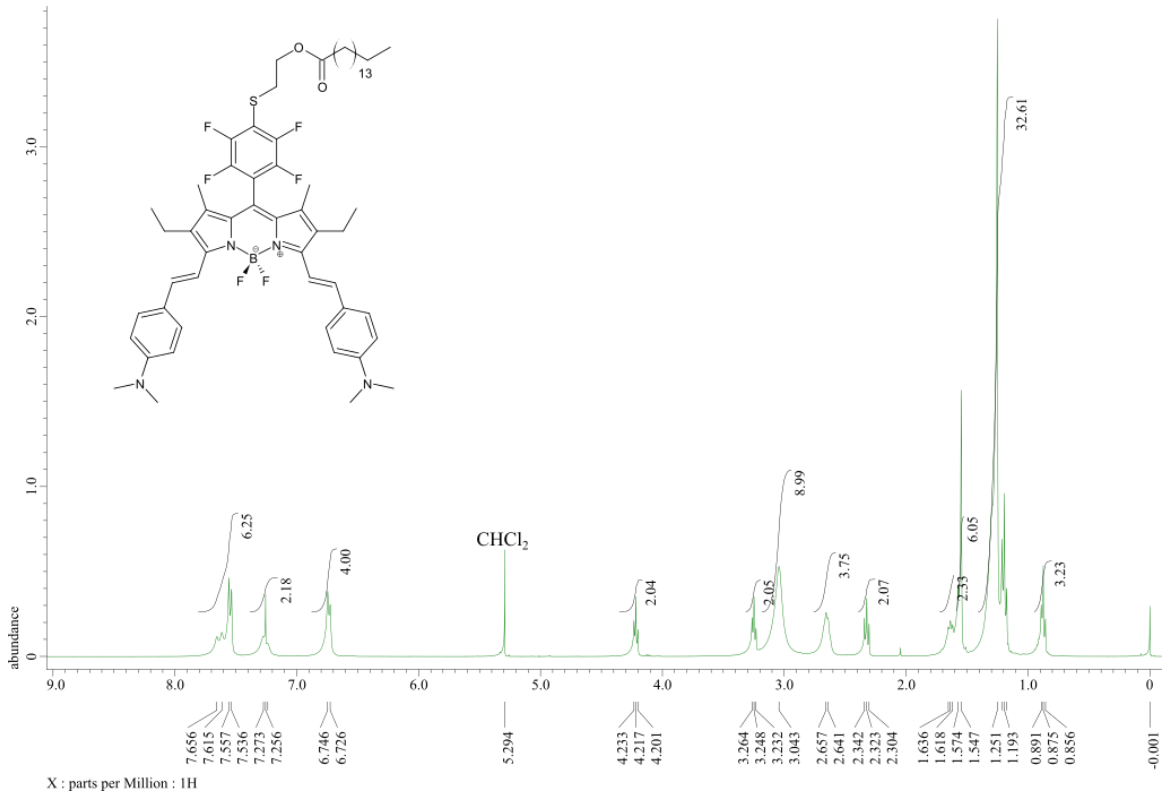

Fig. S2: ^{1}H NMR of BY-aniline-Palm

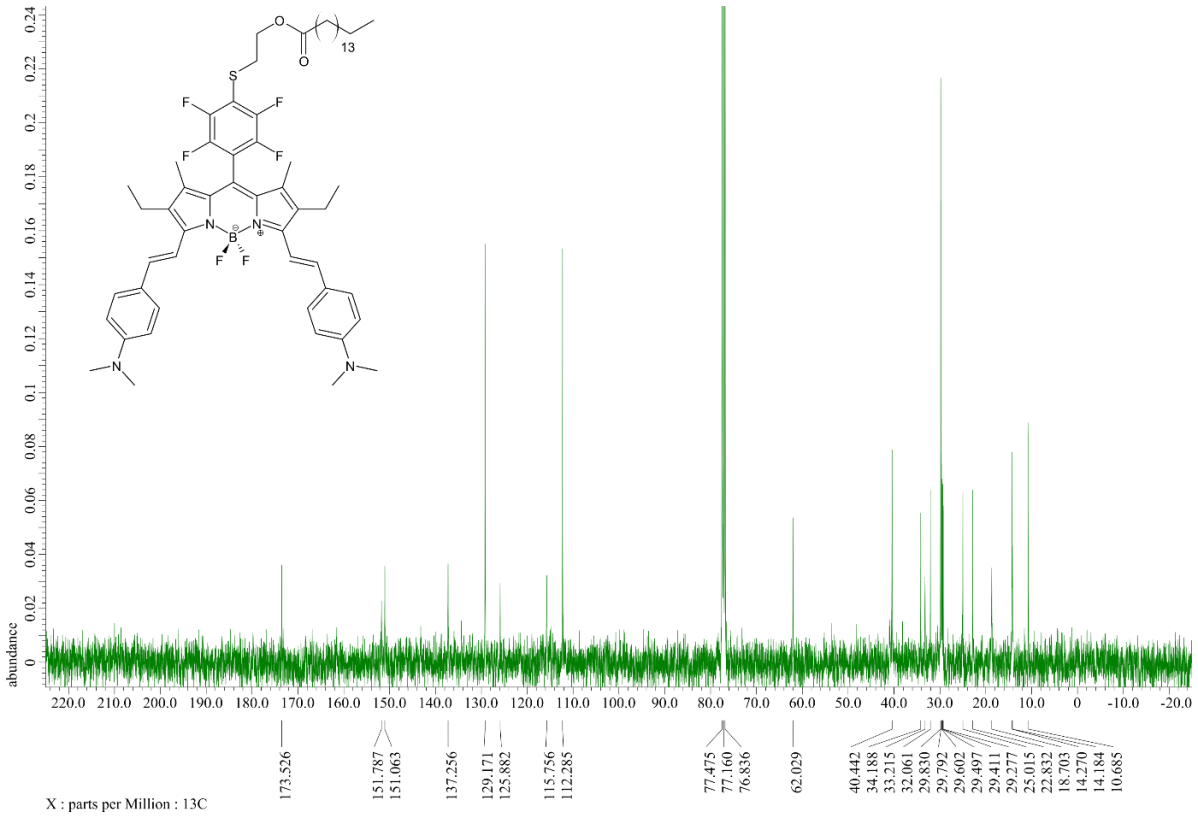

Fig. S3: ^{13}C NMR of BY-aniline-Palm

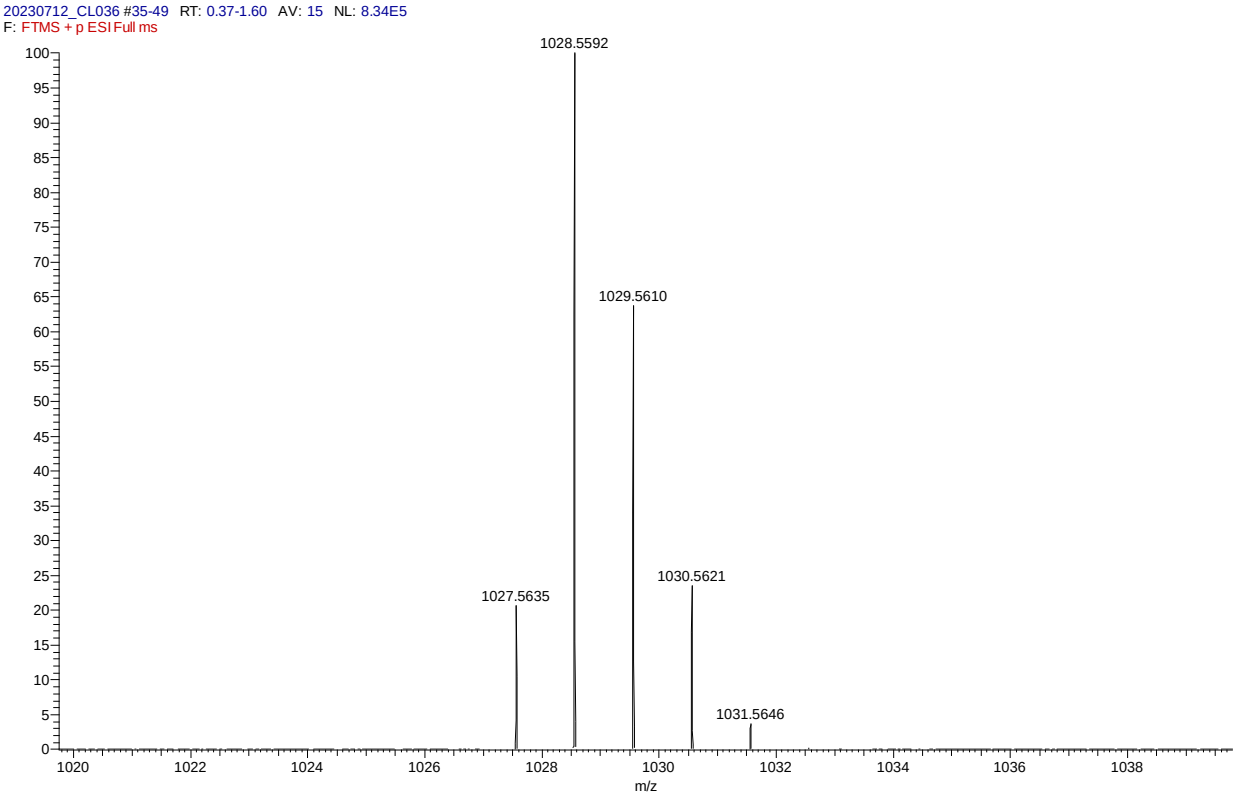

Fig. S4: HRMS (ESI) spectrum of BY-aniline-Palm

1.2. Nanoparticle Formulation.

Table S1 – BY-aniline-Palm (or BY-aniline -PLA) concentrations in SLN and PLA NPs with dilution factor to reach the same concentration.

	SLN-1%	*SLN-2%*	*SLN-6%*	*SLN-12%*	*SLN-25%*	*SLN-50%*	*SLN-75%*	*SLN-100%*	BY-aniline-PLA-47%
BY-aniline-Palm (or BY-aniline -PLA) concentration (mg.mL^{-1})	0.0810	0.161	0.472	0.910	1.76	3.10	4.15	5.00	2.35
BY-aniline concentration (mM)	7.87.10^{-2}	1.57.10^{-1}	4.58.10^{-1}	8.84.10^{-1}	1.71	3.01	4.04	4.86	1.57.10^{-1}
Dilution factor (to 1.73.10^{-2} mM)	4.5	9.0	26	51	100	169	230	280	9.0

1.3. Photophysical Measurements

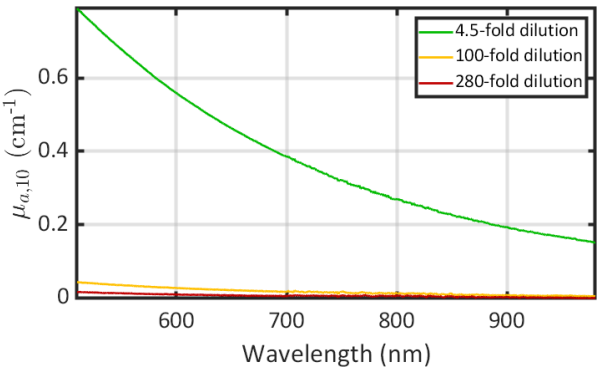

Fig. S5: Unloaded particles (SLN-0%) attenuation spectra used as blank for SLNs. The spectrum was measured for each needed dilution factor. Here 3 representative dilutions corresponding to SLN-1%, SLN-25% and SLN-100%, respectively.

1.4. Photoacoustic Spectra Measurements.

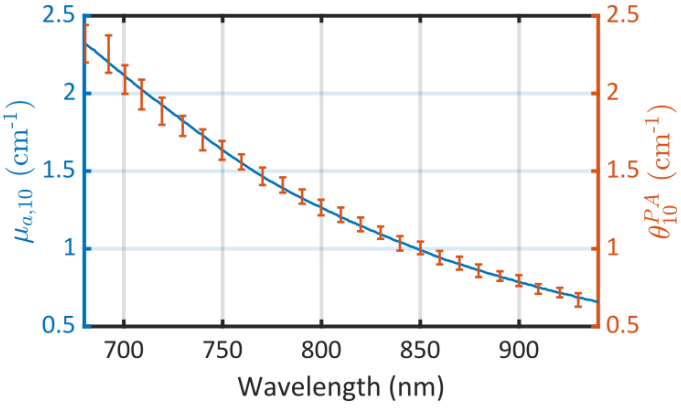

Fig. S6: Absorption and PA spectra of the solution of nigrosin with a PGE equal to 1.

2. Results

2.1. ζ Potential

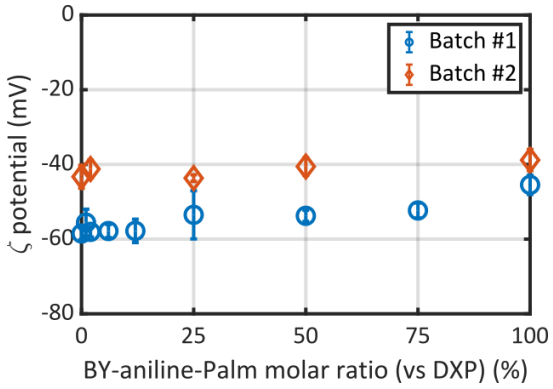

Fig. S7: Evolution of the Zeta potential as a function of the BY-aniline-Palm molar ratio (vs DXP). Markers are blue circle for Batch #1 and orange diamonds for Batch #2. Measurements were done with 20-fold dilution in NaCl 1 mM and error bars are standard deviations on 3 measurements.

2.2. Transmission Electron Microscopy

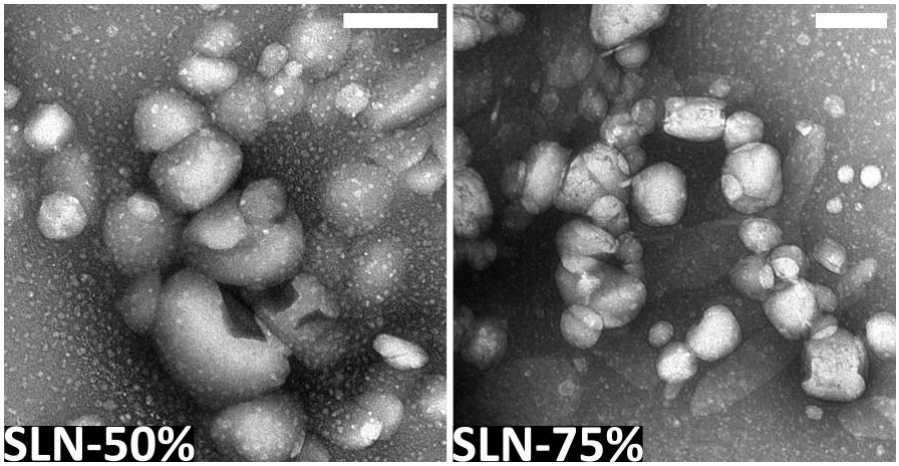

Fig. S8: TEM images of SLN-50% and SLN-75%, obtained with negative staining (uranyl acetate 2% w/w) and acquired with a JEOL JEM-1400 microscope at an acceleration voltage of 120 kV. Magnifications for SLN-50% and SLN-75% are 15,000 and 20,000, respectively. White scale bars on the top right corner represent 200 nm.

2.3. Absorption coefficient per mole of particles

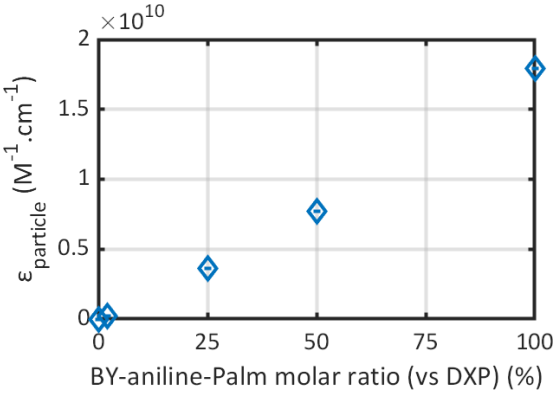

Fig. S9: Evolution of the absorption coefficient per mole of particles evaluated at the maximum absorption wavelength ($\varepsilon_{particle}$) as a function of the BY-aniline-Palm molar ratio (vs DXP) and determined on Batch #2.

2.4. Fluorescence, absorption and photoacoustic spectra

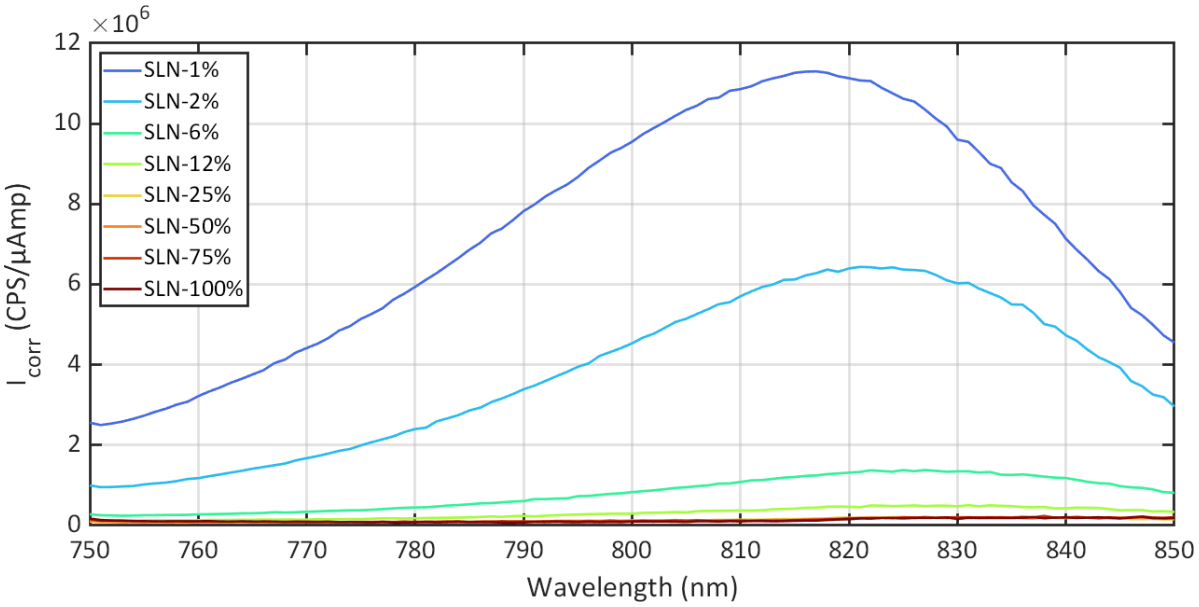

Fig. S10: Fluorescence spectrum (λ_{ex} = 730 nm, slits width 10 nm and A < 0.1) of each type of SLN. The amplitude was corrected by the absorbance at 730 nm.

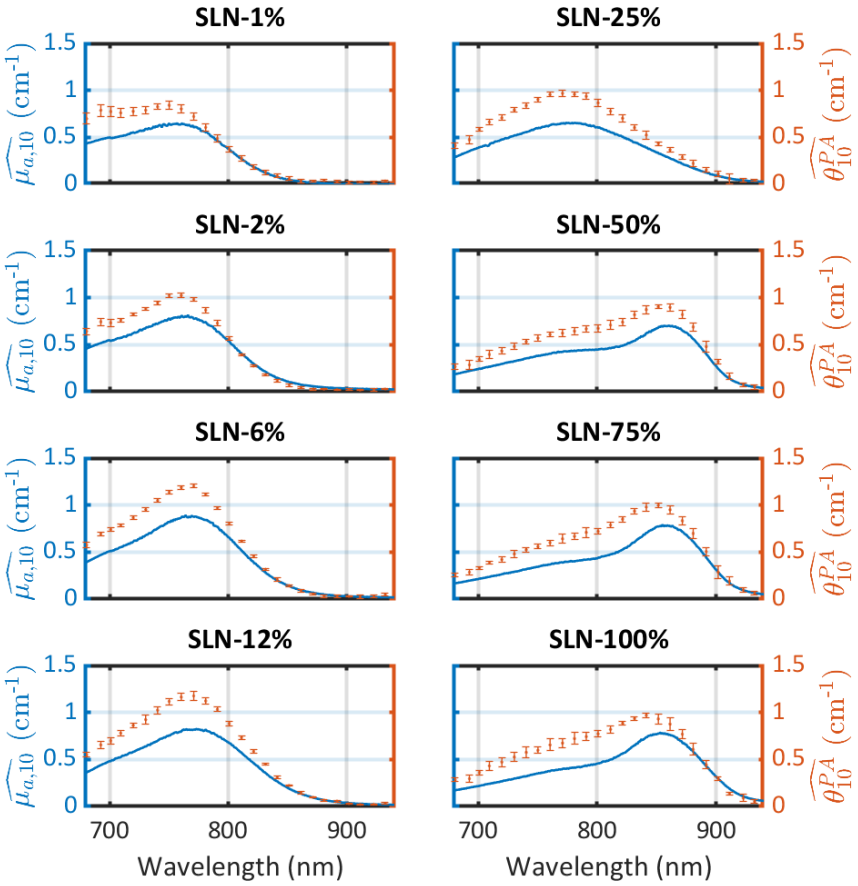

Fig. S11: Superposition of the absorption (blue) and PA (orange) spectra for each SLN (Batch #1).

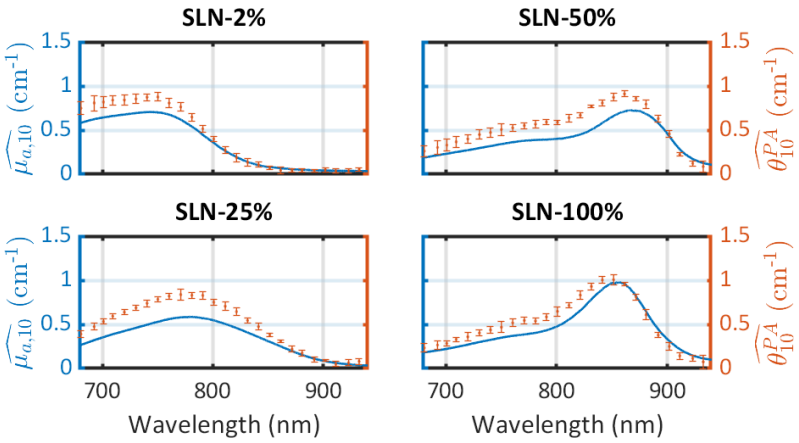

Fig. S12: Superposition of the absorption (blue) and PA (orange) spectra for each SLN (Batch #2).

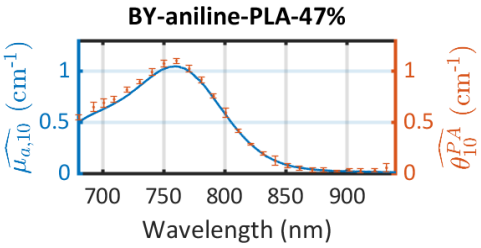

Fig. S13: Superimposition of the absorption (blue) and PA (orange) spectra for BY-aniline-PLA-47% NPs.

2.5. Spectra modelling

Table S2 – Parameters determined for the Gaussian decomposition of the absorption spectra and the PA spectra of Batch #1. a_i (cm^{-1}) is the amplitude of the gaussian curve, λ_i (nm) is the central wavelength and ω_i (nm) is proportional to the full width half maximum ($FWHM_i = 2\sqrt{2\ln 2}\cdot\omega_i$). Band photoacoustic efficiencies (BPAE) were defined as in the Material and Methods section as the ratio between the PA band area and the corresponding absorption band area. As the widths ω_i are set equal for PA and absorption, BPAE corresponds to an amplitude ratio.

			SLN-1%	SLN-2%	SLN-6%	SLN-12%	SLN-25%	SLN-50%	SLN-75%	SLN-100%
Absorption	**Gaussian #1**	a_1						0.51	0.56	0.53
		λ_1						865	864	863
		ω_1						26.5	26.5	26.5
	Gaussian #2	a_2	0.57	0.73	0.85	0.80	0.63	0.42	0.40	0.43
		λ_2	765	769	770	773	783	789	798	799
		ω_2	37.4	39.8	41.8	46.5	59.5	58.6	59.5	59.5
	Gaussian #3	a_3	0.4	0.4	0.31	0.26	0.15	0.11	0.11	0.12
		λ_3	685	685	685	685	685	685	685	685
		ω_3	41.0	41.6	38.0	38.0	45.2	49.4	52.8	46.2
PA	**Gaussian #1**	a_1'						0.60	0.59	0.51
		λ_1'						863	860	858
		ω_1						26.5	26.5	26.5
	Gaussian #2	a_2'	0.66	0.89	1.13	1.12	0.94	0.65	0.67	0.72
		λ_2'	762	764	767	769	778	791	799	797
		ω_2	37.4	39.8	41.8	46.5	59.5	58.6	59.5	59.5
	Gaussian #3	a_3'	0.64	0.53	0.44	0.35	0.18	0.16	0.17	0.18
		λ_3'	685	685	685	685	685	685	685	685
		ω_3	41.0	41.6	38.0	38.0	45.2	49.4	52.8	46.2
BPAE	**Gaussian #1**	$\frac{a_1'}{a_1}$						1.18	1.06	0.96
	Gaussian #2	$\frac{a_2'}{a_2}$	1.16	1.21	1.33	1.41	1.49	1.54	1.67	1.70
	Gaussian #3	$\frac{a_3'}{a_3}$	1.61	1.32	1.41	1.37	1.19	1.41	1.52	1.55

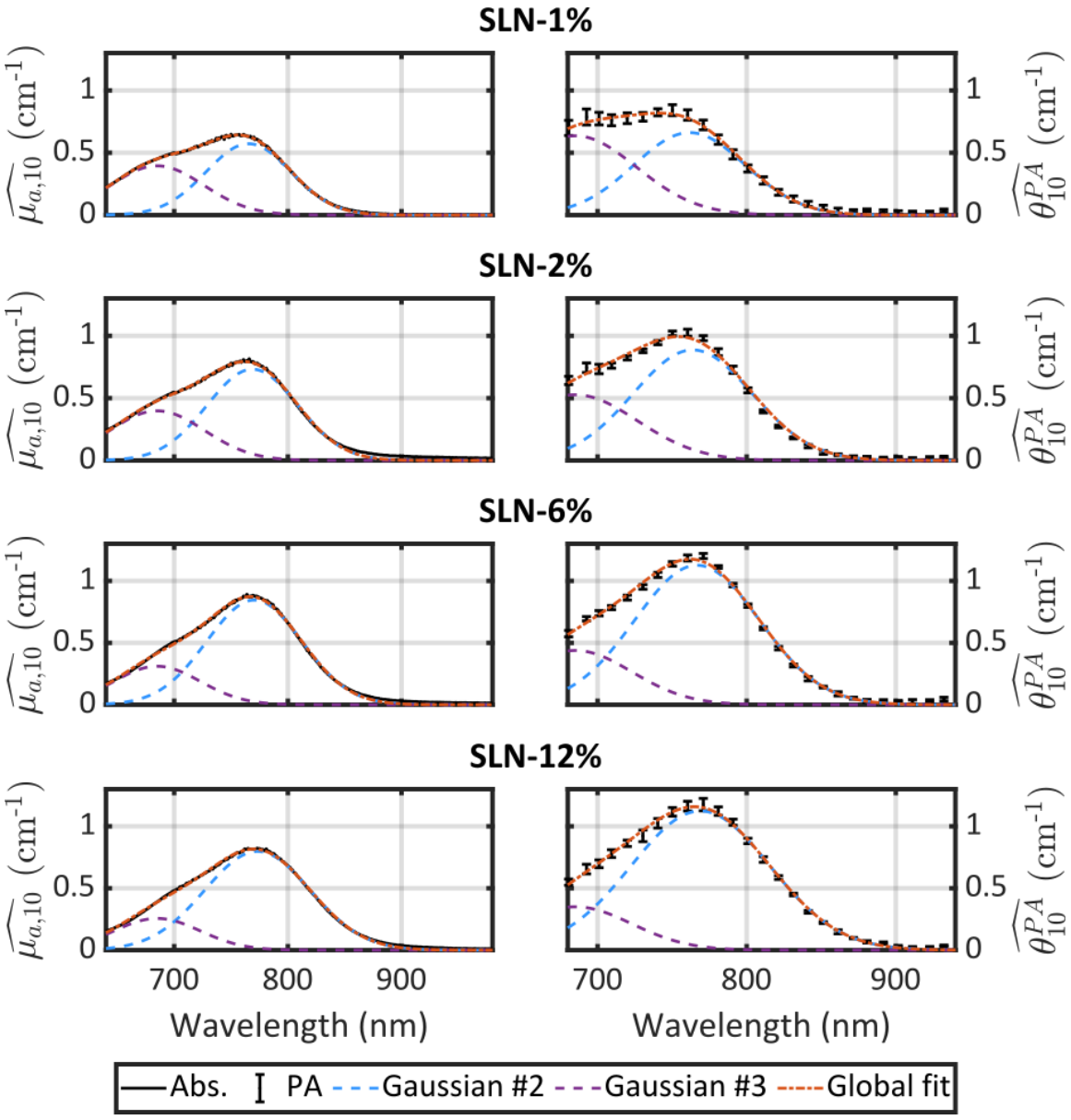

Fig. S14: Gaussian decomposition of absorption and PA spectra for SLN of Batch #1 with BY-aniline-Palm percentage below 25% (excluded).

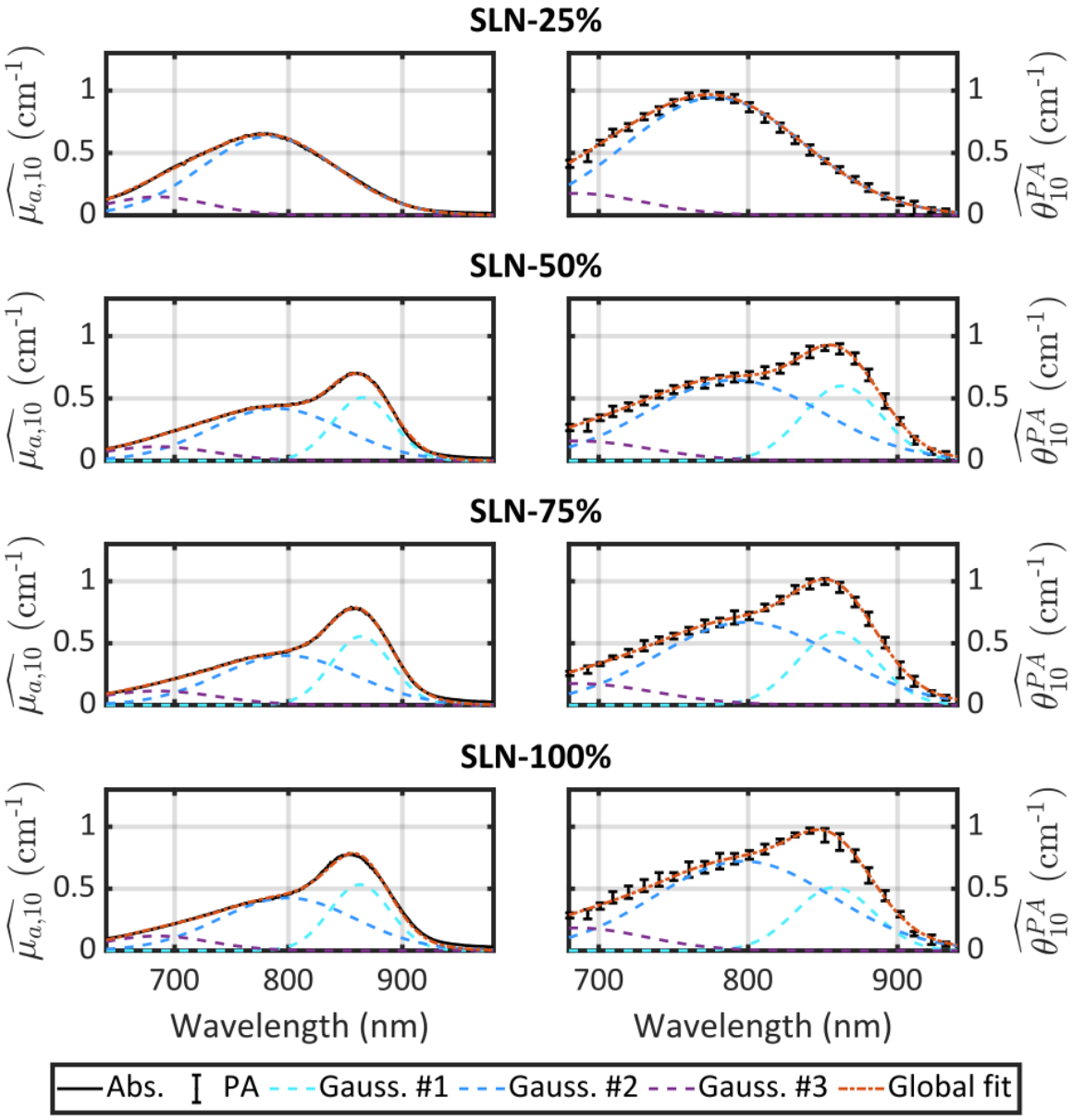

Fig. S15: Gaussian decomposition of absorption and PA spectra for SLN of Batch #1 with BY-aniline-Palm percentage above 25% (included)

Table S3 – Parameters determined for the Gaussian decomposition of the absorption spectra and the PA spectra of Batch #2 and BY-anilne-PLA-47%. a_i (cm^{-1}) is the amplitude of the gaussian curve, λ_i (nm) is the central wavelength and ω_i (nm) is proportional to the full width half maximum ($FWHM_i = 2\sqrt{2\ln 2}\cdot\omega_i$). BPAE was defined as in Table S2.

			SLN-2%	SLN-25%	SLN-50%	SLN-100%	BY-aniline-PLA-47%
Absorption	**Gaussian #1**	a_1			0.56	0.71	
		λ_1			875	859	
		ω_1			26.5	26.5	
	Gaussian #2	a_2	0.47	0.56	0.40	0.41	0.96
		λ_2	763	784	799	799	764
		ω_2	43.2	59.5	59.5	59.5	37.3
	Gaussian #3	a_3	0.50	0.14	0.14	0.13	0.43
		λ_3	685	685	685	685	685
		ω_3	54.0	45.3	46.5	46.2	40,3
PA	**Gaussian #1**	a_1'			0.61	0.66	
		λ_1'			870	854	
		ω_1			26.5	26.5	
	Gaussian #2	a_2'	0.56	0.81	0.59	0.55	1.00
		λ_2'	759	779	800	797	764
		ω_2	43.2	59.5	59.5	59.5	37.3
	Gaussian #3	a_3'	0.63	0.20	0.21	0.15	0.47
		λ_3'	685	685	685	685	685
		ω_3	54.0	45.3	46.5	46.2	40.3
BPAE	**Gaussian #1**	$\frac{a_1'}{a_1}$			1.09	0.93	
	Gaussian #2	$\frac{a_2'}{a_2}$	1.19	1.44	1.46	1.32	1.04
	Gaussian #3	$\frac{a_3'}{a_3}$	1.26	1.38	1.45	1.16	1.11

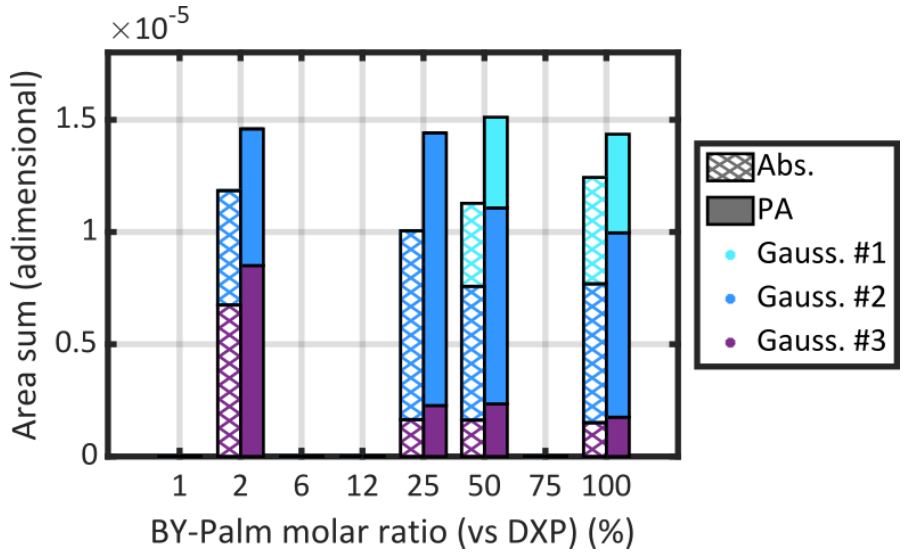

Fig. S16: Contribution of each Gaussian function (area) in the total spectrum area for each SLN of Batch #2. Absorption spectra are on the left-hand side in hatched bars while PA spectra are on the right-hand side in coloured bars.

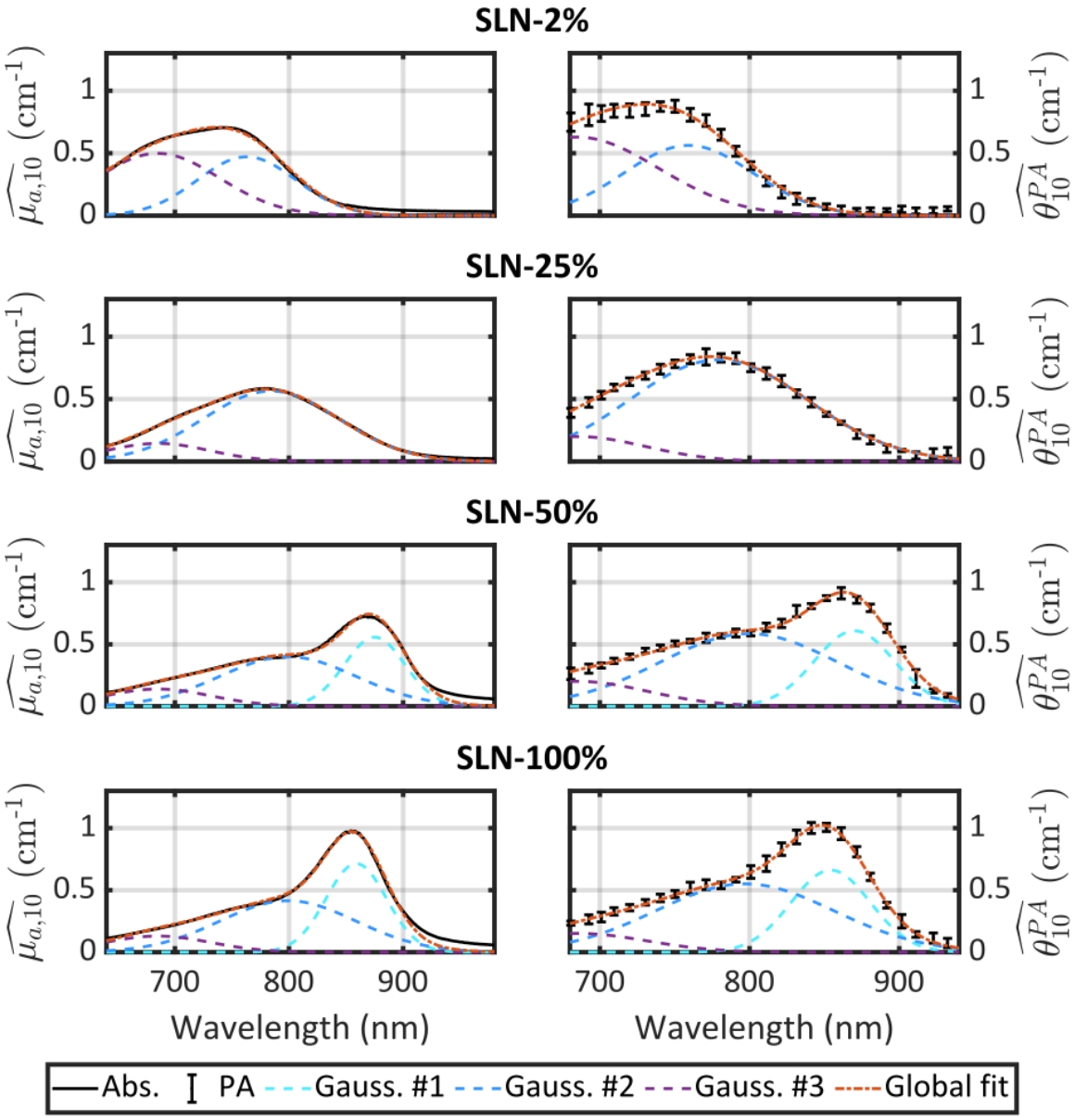

Fig. S17: Gaussian decomposition of absorption and PA spectra for SLNs of Batch #2.

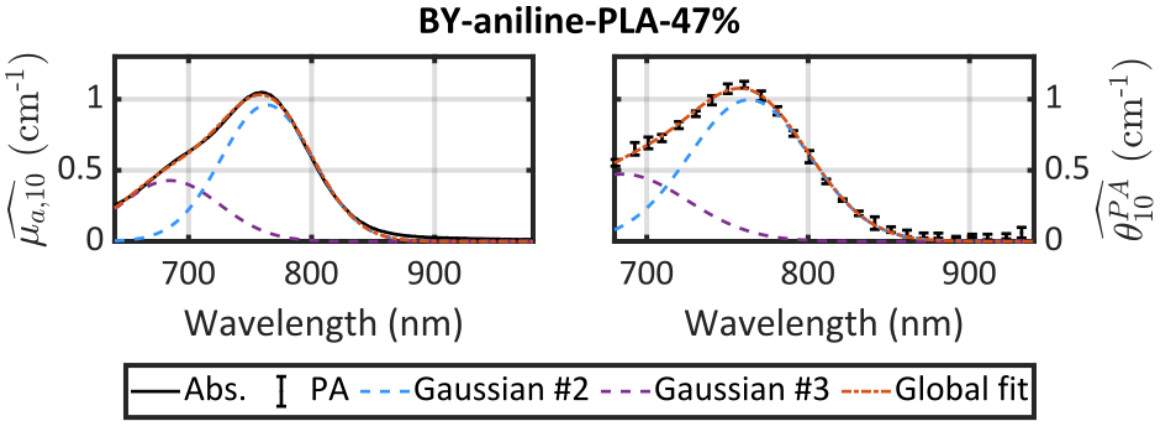

Fig. S18: Gaussian decomposition of absorption and PA spectra for BY-aniline-PLA-47%

2.6. Photoacoustic spectra at different temperatures

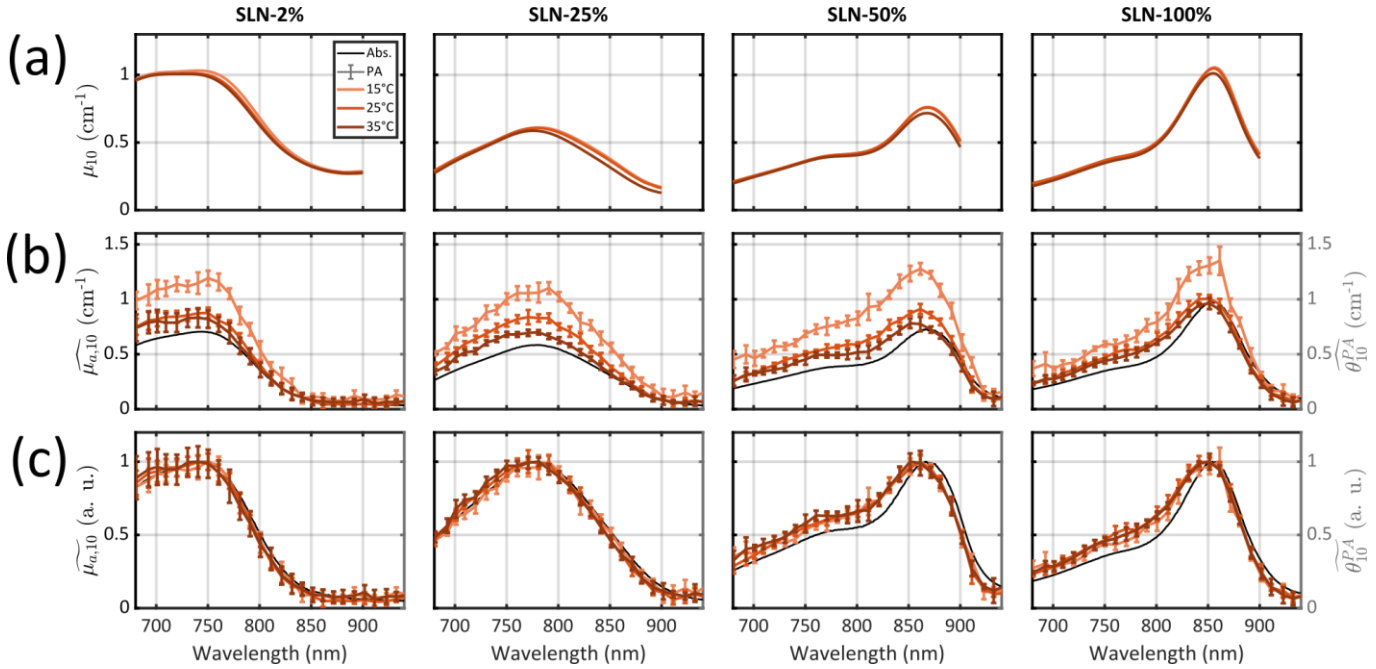

Fig. S19: (a) Absorbance spectra measured at three temperatures (15°C, 25°C and 35°C) of SLNs from batch #2. Measurements at different temperatures are color-coded with shades of orange. The scattering was not corrected. (b) PA spectra at three temperatures (15°C, 25°C and 35°C) of SLNs. The absorption spectra (corrected for scattering) are displayed with a black solid line. (c) Normalized absorption and PA spectra at three temperatures (15°C, 25°C and 35°C) of SLNs. Each spectrum is normalized by the spectrum maximum. The colour code is the same in (a) (b) and (c).

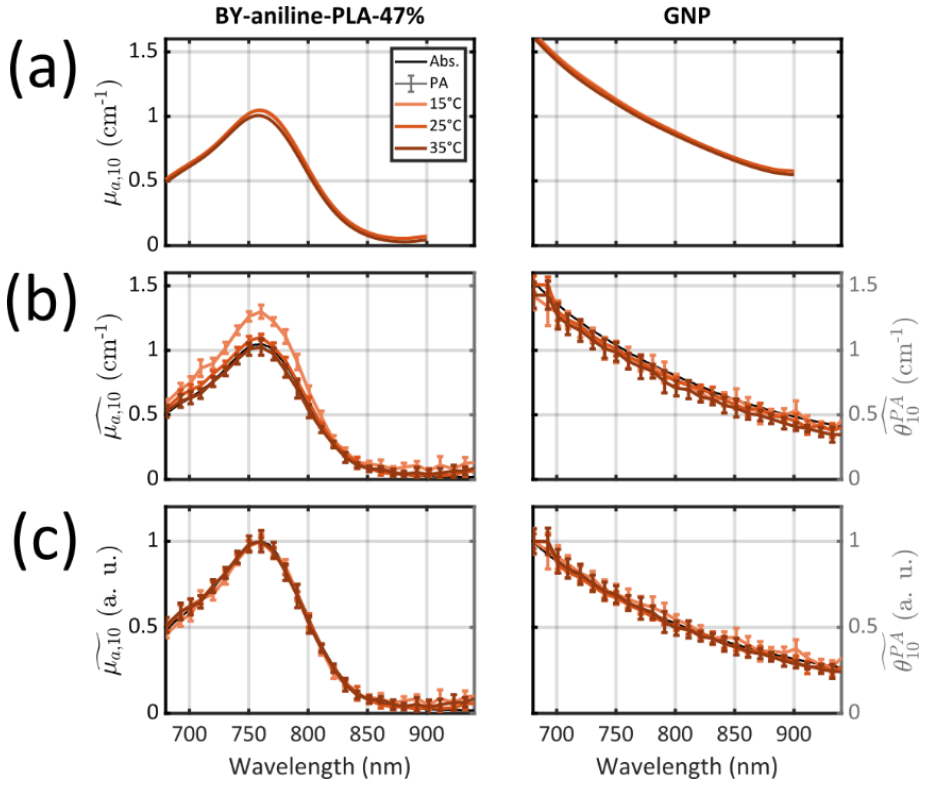

Fig. S20: (a) Absorbance spectra measured at three temperatures (15°C, 25°C and 35°C) of PLA NPs (left) and GNPs (right). Measurements at different temperatures are color-coded with shades of orange. The scattering was not corrected. (b) PA spectra at three temperatures (15°C, 25°C and 35°C) of PLA NPs (left) and GNPs (right). The absorption spectra (corrected for scattering) are displayed with a black solid line. (c) Normalized absorption and PA spectra at three temperatures (15°C, 25°C and 35°C) of PLA NPs (left) and GNPs (right). Each spectrum is normalized by the spectrum maximum. The colour code is the same in (a) (b) and (c).

Table S4 – Global photoacoustic efficiencies (GPAE) for Batch #1 were defined in the Material and Method section as the ratio between the integral of the PA spectrum and the integral of the equivalent absorption spectrum on the interval [680 nm, 920 nm]. It corresponds as the weighted mean PGE over the whole spectrum.

	SLN-1%	SLN-2%	SLN-6%	SLN-12%	SLN-25%	SLN-50%	SLN-75%	SLN-100%
GPAE 25°C	1.31	1.21	1.32	1.39	1.43	1.4	1.44	1.43

Table S5 – Global photoacoustic efficiencies (GPAE) for Batch #2, GNP and BY-aniline-PLA-47% were computed as defined in Table S4.

	SLN-2%	SLN-25%	SLN-50%	SLN-100%	BY-aniline-PLA-47%	GNP
GPAE 15°C	1.65	1.84	1.88	1.47	1.24	0.96
GPAE 25°C	1.2	1.4	1.34	1.15	1.05	0.97
GPAE 35°C	1.16	1.19	1.17	1.08	0.98	0.92

2.7. Photostability under laser excitation

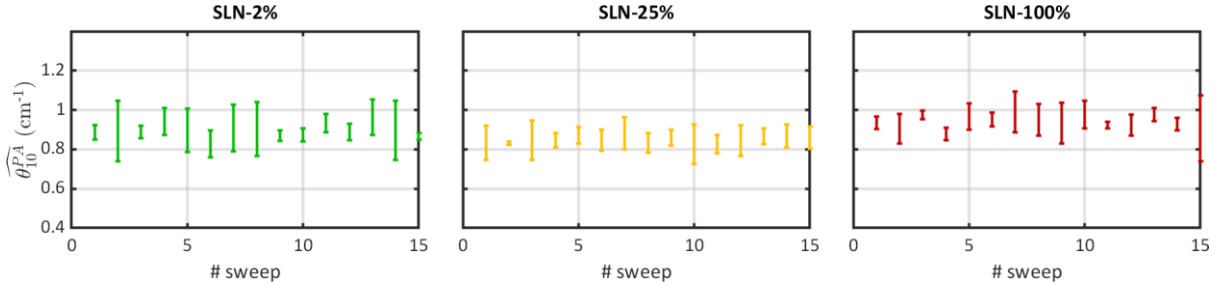

Fig. S21: Photoacoustic coefficients as a function of the number of the sweeps. The PA coefficients are measured at the maximum absorption wavelength, namely : 750 nm for SLN-2% (green), 780 nm for SLN-25% (yellow) and 960 nm for SLN-100% (red), respectively. For each sweep, the acquisition was performed simultaneously on four tubes and the displayed results corresponds to the median (with the MAD as error bar) over the 4 tubes.

2.8. Photoacoustic signal variability with the laser fluence

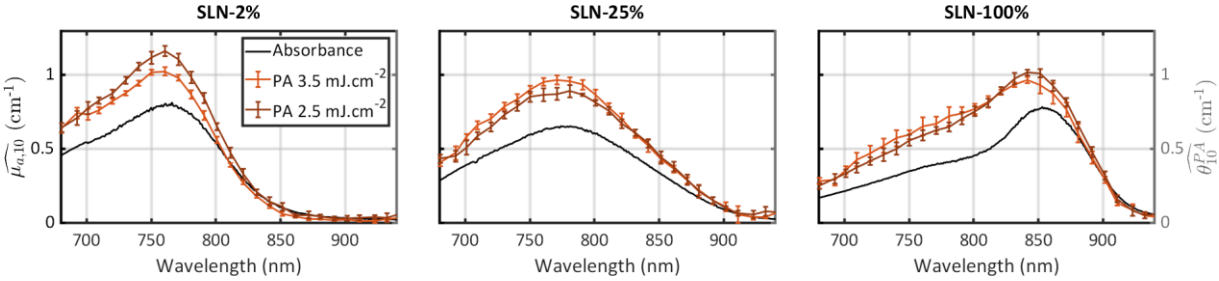

Fig. S22: Superimposition of the absorption and PA spectra for three formulations (SLN-2%, SLN-25% and SLN-100%). Two different laser fluences are used: 3.5 mJ.cm^{-2} (orange) and 2.5 mJ.cm^{-2} (brown). The mean variation between the spectra at the two fluences is below 10%.

2.9. Transient absorption signal

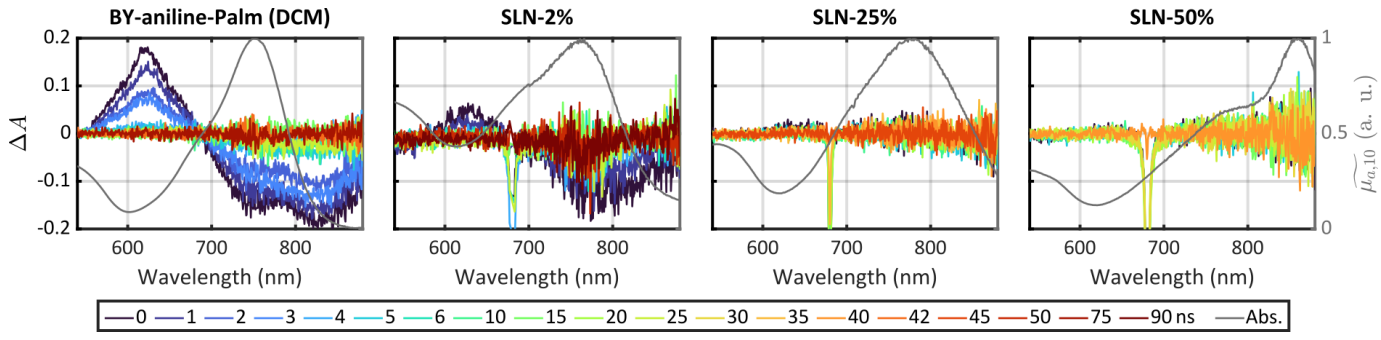

Fig. S23: Transient absorption spectra of BY-aniline-Palm in DCM, SLN-2%, SLN-25% and SLN-50% for different time delays (in ns and color-coded) between pump (at 680 nm) and probe. Normalized equivalent absorption spectra are displayed in grey.