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Microfluidic Assisted Flash Precipitation of Photocrosslinkable Fluorescent Organic Nanoparticles for Fine Size Tuning and Enhanced Photoinduced Processes

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ABSTRACT. Highly concentrated dispersions of fluorescent organic nanoparticles (FONs), broadly used for optical tracking, bioimaging and drug delivery monitoring, are obtained using a newly designed micromixer chamber involving high impacting flows. Fine size tuning and narrow size distributions are easily obtained by varying independently the flow rates of the injected fluids and the concentration of the dye stock solution. The flash nanoprecipitation process employed herein is successfully applied to the fabrication of bicomposite FONs designed to allow energy transfer. Considerable enhancement of the emission signal of the energy acceptors is promoted and its origin revisited as a result of polarity rather than steric effects. Finally, we exploit the high spatial confinement encountered in FONs and their ability to

encapsulate hydrophobic photosensitizers to induce photocrosslinking. Increase in the photocrosslinked FON stiffness is evidenced by measuring the elastic modulus at the nanoscale using atomic force microscopy. Such results pave the way toward the straightforward fabrication of multifunctional and mechanically photoswitchable FONs, opening novel potentialities in terms of sensing, multimodal imaging, and theranostics.

1. INTRODUCTION

For the past two decades, the technical development of scientific instruments has been accompanied by an increasing demand of miniaturization to gain spatial and time resolution and access to novel phenomena related to size reduction down to the nanometer scale. Assessing flow velocimetry in microfluidics,^[1] sensing local temperatures^[2], pH^[3] or chemicals^[4], and tracking cell^[5] or nanomedicine^[6] require nanometer-sized materials to follow local evolutions as a function of external parameters. In this context, emissive nanoparticles have been considered as attractive tools since they can easily be monitored by widespread wide-field or confocal fluorescence microscopes, offering high detection sensitivity and versatile implementation of the investigated devices or (bio)chemical systems.^[7] The search for bright but biodegradable and heavy-metal free nanoparticles is thus becoming a pressing demand, requiring progressive replacement of the broadly used luminescent silica nanoparticles and quantum dots, facing the abovementioned limitations. For these reasons, engineering bright fluorescent organic nanoparticles with large colloidal stability, low polydispersity index and additional functionality, stimulates a large area of investigations. Fluorescent nanolatexes, namely submicrometer-sized crosslinked polymer particles incorporating fluorophores, represent the most regarded family of

organic nanoparticles. Usually, they are synthesized by resorting to emulsion or dispersion-based fabrication processes.^[8] Their transformation into fluorescent nanolabels can be achieved by entrapping fluorophores before polymerization,^[9] or by inducing self-assembling of preformed amphiphilic block copolymers in the presence of hydrophilic or hydrophobic fluorophores.^[10] Another approach relies on polymer networks incorporating fluorophores as covalent side chains, which requires complementary reactive functions.^[11] However, the first approach faces extensive dye release in the aqueous media in the long term, especially for ionic and hydrosoluble fluorophores, thereby leading to false-positive. As for the second approach, the risk of self-quenching upon aggregation for hydrophobic dyes or the natural electrostatic repulsions between dyes of similar charges requires diluted dyes concentrations, leading to low-payload nanolabels. In this case, prolonged irradiation tends to conduct to the rapid loss of emission signal due to the weak density of dyes, amenable to photobleaching in the presence of singlet oxygen. To circumvent this issue of a low functionalization rate, an alternative approach has been proposed, based on the formation of non-doped fluorescent nanoparticles, resulting from the self-assembly of a high amount of small hydrophobic and fluorescent molecules, promoted by water addition. Two main structural strategies have been developed to still obtain emission after dye aggregation into nanoparticles. The first one led to the coined aggregation-induced-emissive (AIE) nanomaterials where emission arises from blocked intramolecular rotations of the dyes within the nanoparticles upon π - π stacking,^[12] or from the formation of H- and J-aggregates.^[13] The second one involves bulky or twisted fluorophores that emit in solution and keep emitting as nanoparticles thanks to the large intermolecular distance introduced by sterically hindered substituents, prohibiting the formation of dark π - π states.^[14] Whatever the adopted synthetic method, the resulting fluorescent organic nanoparticles (FONs) are formed upon solvent shifting,

namely essentially through manual injection into a large volume of water of a diluted solution of fluorophores dissolved in a water-miscible solvent. Such a fabrication process tightly depends on the operator and geometry of the precipitation container, which suffers from diluted solutions, small preparation volumes, and a lack of reproducibility and size control. To solve such issues, smart micromixers have been developed to ensure quick mixing and achieve high supersaturation and nucleation rate that are prerequisites for nanoparticle formation in the millisecond range following a nucleation-growth or nucleation-aggregation process.^[15] Two mixer geometries have been proposed by B. K Johnson and R. K. Prud'homme et al., named confined impinging jet (CIJ) mixer^[16] and multi-inlet vortex mixer (MIVM)^[17] to produce high turbulence, favoured by a high flow velocity or large Reynolds numbers above 1000. CIJ mixers consist in two sub-millimeter channels, placed face-to-face across a large mixing chamber and connected to gas-tight syringes that contain each water or organic solution and are attached with each other to exert equal injecting pressure (Figure S1A). Although CIJ mixers are simple and hand-operated, the momenta of the organic solution and anti-solvent (here water) are necessarily identical, limiting their contribution to the control of the resulting nanoparticle diameters and the use of concentrated solutions at the risk of causing dye precipitation. MVIM systems overcome these restrictions thanks to a conical mixing chamber connected to two or four flow inlets, placed in a symmetrical and tangent manner around the chamber, which affords separate control of the stream velocity, continuous fluorescent nanoparticle production and easy variation of the solution concentration (Figure S1B-C).^[18] Although both CIJ and MVIM technical solutions are elegant, the mixer dimensions are still too large to be implemented in the future on a microscope stage and allow for straightforward optical follow-up to gain understanding in the mixing dynamics. Moreover, they all imply the use of surfactants or amphiphilic polymers in large concentrations

to stabilize the generated nanoparticles,^[10b] which can impact the flow dynamics and provide dye-free micelles in the case of bad mixing. In this respect, a novel compact micromixer, designated as Microsphere500, has been developed in the past for the fabrication of surfactant-free oil-in-water emulsions at high flow rates and under highly impacting flows.^[19] Its original geometry, largely detailed in previous studies,^[19] is based on two opposing channels with distinct sections, enabling high-impact impinging flows. The mutual position of the channels, in a slight offset with regard to the longitudinal stream direction, creates a vortex stream that superimposes the flow instabilities due to the high energy impact, hence very efficient mixing of biphasic mixtures is obtained.^[20] We thus want to demonstrate that FONs with controllable diameter can easily be obtained out of small fluorophores by resorting to the simple Microsphere500 micromixer, combining the advantages of both CIJ and MVIM systems, namely high flow momenta, continuous nanoparticle production and technical simplicity. Issues regarding FON homogeneity and composition as a function of the experimental conditions of fabrication are addressed by steady-state and time-resolved photophysical studies. To validate the versatility of the current microfluidic device, we show that bicomposite FONs can be obtained by flash coprecipitation, allowing for tunable emission color thanks to the large structural confinement effects encountered in FONs and responsible for efficient photoinduced electronic energy or electron transfer.^[21] Finally, we harness such confinement effects to show the possibility of chemical reaction within pre-formed FONs. To this aim, we perform photocrosslinking assays using FONs comprising bis-acrylate fluorescent monomers and tetrathiol crosslinker to offer in the future easily accessible emissive nanotracers as an alternative to crosslinked polystyrene and silica nanoparticles.^[22]

2. EXPERIMENTAL SECTION

2.1. Chemicals and solution preparation

Both fluorophores employed in these studies, **ALD** (bis(2-(acryloyloxy)ethyl) O,O'-((((4-formylphenyl)azanediyl)bis([1,1'-biphenyl]-4',4-diyl))bis(ethane-1,1-diyl)) disuccinate) and **VIN** (bis(2-(acryloyloxy)ethyl) O,O'-((((4-(2,2-dicyanovinyl)phenyl)azanediyl)bis([1,1'-biphenyl]-4',4-diyl))bis(ethane-1,1-diyl)) disuccinate), were synthesized according to literature procedure.^[23] Spectroscopic grade and inhibitor-free THF, used for all preparations and photophysical studies, and photocrosslinking respectively were purchased from Aldrich. Three **ALD** solutions in THF with varying concentrations (0.5, 1 and 2 wt. %) were initially prepared to cover all flash nanoprecipitation conditions. The bicomposite solutions comprising **ALD** and **VIN** to generate FONs with tunable emission wavelength were prepared from two separate stock solutions in THF, containing each **ALD** and **VIN** at a 0.5 wt. % concentration. The final solutions (2 mL) to be injected in the micromixer were prepared by adjusting the required volumes of the **ALD** and **VIN** stock solutions and completing with 1 mL of THF, so that distinct **ALD:VIN** ratios of 100:0, 95:5, 80:20 and 0:100 could be obtained with a total dye concentration fixed at 0.25 wt. %.

2.2. Flash nanoprecipitation

Manual method involving magnetic stirring. A solution of organic compounds (100 μ L) was added in one shot with a micropipette (Acura® manual 825) into a 18 mL-large amber glass vial, containing water (2.5 mL) while stirring with a vortex mixer (IKA VORTEX Genius 3) to afford FONs.

Method involving the Microsphere500 micromixer. The nanoparticles were fabricated via a microfluidic circuit including the Microsphere500^[19] mixing chamber made from aluminum plates, pump syringes from Harvard Apparatus (Pump Elite 11) and single-use plastic syringes 2 or 5 mL, and 50 mL (HSW SOFT-Ject® Luer Lock) connected to the mixing chamber via Teflon tubing. The two smallest syringes were used for the fluorophore solution in THF and the 50 mL one for water. The organic solution was injected through the smaller square channel (300 μm wide) of the microcircuit while water was injected through the larger square channel (600 μm wide). Solution volumes of 2 mL were at least needed for each measurement due to the dead volume. FON dispersions were collected from the mixing chamber outlet via two Teflon tubes, placed in mutual opposite directions. The microfluidic circuit was cleaned multiple times with THF and water between each measurement, except for experiment **A** where the concentrations were the same for every sample. The Microsphere500 mixing chamber could be used as many times as needed, and tubes were renewed only if fluorophore traces remained after thorough washing.

2.3. Photocrosslinking reaction

Preparation of solutions for photopolymerization as FONs and thin films. Three distinct solutions of **ALD** (15.0 mg, 3.3 mmol), pentaerythritoltetrakis(3-mercaptopropionate) (**PT-SH**) (8.1 mg, 3.3 mmol), and 2,4,6-trimethylbenzoyl-diphenylphosphine (**TPO**) (1.4 mg, 0.8 mmol) in THF (5 mL) were separately prepared. Three daughter solutions (**A** and **B**), completed to a 2 mL total volume with THF were made out of them following the **TPO:PT-SH:ALD** volume composition (in mL) as follows: **P0**) 0:0:1 in THF (1 mL), **P1**) 0.1:0.1:1 in THF (0.8 mL) and **P5**) 0.1:0.5:1 in THF (0.4 mL). Such solutions were directly deposited on a glass slide and let dry to form homogeneous thin films. Alternatively, **FON₀**, **FON_{0.1}** and **FON_{0.5}** could be produced by manual

addition in water (2.5 mL) under vortex stirring of a small amount (100 μ L) of solutions P0, P1 or P5 respectively.

Photocrosslinking conditions. Photocrosslinking was performed in 1 cm-wide quartz cell under constant stirring, using a UV light source Xe-Hg lamp (Hamamatsu LC8) paired with a 365 nm bandpass filter. The lamp power used for all experiments was set at 30 mW.cm⁻² and measured with a photodetector (UV-vis Nova II Ophir). Irradiation was performed for 5 min. Photocrosslinking in deoxygenated conditions was carried out *via* argon bubbling for 1 min. Then, a constant flow of argon was settled in the quartz cell for 2 min before photoirradiating the solution.

Photoirradiation of FONs, prepared here upon manual precipitation of a TPO:PT-SH:ALD mixture into water, was performed at 365 nm in a quartz cell under constant stirring using an irradiation lamp equipped with an interferential filter. FONs devoid of TPO or PT-SH were also prepared to serve as control samples. Two aeration conditions were examined: without and with preliminary deoxygenation for 2 min by argon bubbling of the FON suspensions. We fixed the irradiation time and power at 5 minutes and 30 mW.cm⁻² respectively, which offered the best compromise in terms of photocrosslinking efficiency and persistence of fluorescence from preliminary solid-state experiments. After irradiation, all samples were diluted in the same way to yield absorbance such that the risks of inner filter effects in emission (less than 0.1 where the emission and absorption spectra overlap) and photoinduced photopolymerization during excitation (excitation slits with limited width were set) were minimized.

2.4. FON structural investigations

Dynamic light scattering (DLS). FON size measurements in aqueous dispersions were performed via DLS using the VASCO KinTM nanoparticle sizer provided by Cordouan Technologies, and equipped with a 45 mW diode laser operating at 638 nm. The laser power was manually adjusted for each sample before any measurement to get satisfying results. All measurements were collected on aqueous FON dispersions at 25 °C in a backscattering mode at a 170° angle, over 2 min with a 5 Hz acquisition frequency, namely every 200 ms. Acquisitions and analyses were done with the Nano KinTM software, provided by Cordouan Technologies, Cumulant-based algorithm in intensity was privileged to infer size distributions. A logarithmic scale was adopted for clarity reason, improving visualization of all samples on the same scale, with no impact on the fitted parameters.

Zetametry. Measurements of surface potential ζ , were carried out by means of a Zetasizer Nano ZS ZEN 3600 (Malvern). The samples were placed in disposable folded capillary cells (DTS1070). Two sets of three measurements were realized for each sample and the zeta potential was calculated from electrophoretic mobility dispersion fitted by the Smoluchowski model.

Transmission electron microscopy. FON size measurements as dry samples were performed using a MO-Jeol 1230 microscope (80 kV) and diluted samples deposited on holey carbon-coated copper grids (300 mesh), purchased from Agar Scientific.

2.5. Photophysical studies

Steady-state measurements. UV–visible absorption spectra were recorded using a Varian Cary 5E spectrophotometer (Agilent). Emission spectra were recorded using a Fluorolog 3 spectrofluorometer (Horiba). Correction for the emission spectra with regard to the spectral

response of the detector was automatically applied. Fluorescence quantum yields were determined in solution, referred to coumarine 540A in ethanol ($\Phi_f = 0.38$).

Time-resolved fluorescence measurements. Fluorescence time decays were measured in water using the fully automated spectrofluorometer (model Fluotime 300, PicoQuant) following the time-correlated single photon counting method. Excitation was performed using a pulsed laser diode (LDH-D-C-450B) working at 450 ± 10 nm (70 ps fwhm time resolution), or a pulsed LED (PLS 340) working at $340 \text{ nm} \pm 10$ nm (500 ps fwhm time resolution). Fluorescence decays were recorded using a Hybrid-PMT detector combined with an acquisition temporal resolution up to 25 ps. Long-pass edged filter purchased from Semrock centered at 473 nm (BPL01-473R-25) or 355 nm (BPL01-355R-25) was used to discard any possible contribution of excitation light scattering by the organic nanoparticle dispersions. Time fluorescence decay modeling was performed with EasyTau 1 software developed by PicoQuant.

Resonance energy transfer (RET). Förster resonance energy transfer could reasonably be evoked to explain the spectral evolution of the emission signals. The Förster radius can be estimated from the spectral overlap factor J between the energy donor (**ALD**) and acceptor (**VIN**) (Eq. 2).

$$R_0^6 = \frac{0.2108\kappa^2\Phi_D^\circ}{n^4} \int_0^\infty I_D(\lambda)\varepsilon_A(\lambda)\lambda^4 d\lambda \quad \text{with } J = \int_0^\infty I_D(\lambda)\varepsilon_A(\lambda)\lambda^4 d\lambda \quad (2)$$

Φ_D° : fluorescence quantum yield of the donor in the absence of acceptor, n : refractive index of the solution, κ^2 : orientation factor with $\kappa^2 = 0.476$ for dipoles in a rigid medium like FONs, I_D : emission intensity of the donor, ε_A : molar absorption coefficient of the acceptor.

The energy transfer quantum efficiency Φ_{FRET} was calculated from the donor emission signal, through the expression $\Phi_{\text{FRET}} = 1 - I_{\text{D}}/I_{\text{D}}^{\circ}$, where I_{D}° and I_{D} feature the emission intensity in the absence and presence of acceptor, respectively.

2.6. AFM quantitative mechanical measurements

Sample preparation. Freshly cleaved mica squared plates (1 inch, Bruker) were treated with a 0.01 % (w/v) poly-L-lysine solution (Aldrich) for 15 min, thoroughly rinsed with Millipore water and finally dried under a nitrogen flow. They were subsequently incubated for 30 min with FON dispersions that were previously diluted 100 times, and washed again with Millipore water. The dispersions were let dry in air before AFM measurements.

Atomic force microscopy (AFM). All AFM experiments were performed at room temperature (20 °C) using a NanoWizard® atomic force microscope (JPK, Germany) equipped with inverted optical microscope and x-y closed-loop control system. Cantilevers with calibrated spherical carbon-glassy tips (Biosphere™ series purchased from Nanotools) characterized by a $100 \text{ nm} \pm 10 \text{ nm}$ tip radius and 2.8 N.m^{-1} spring constant were first used for imaging in intermittent contact mode to select nanoparticles to be probed. Force mapping using a scanning mode was privileged over repeated measurements on single nanoparticles to avoid tip positioning uncertainty relative to the nanoparticles. It offers the additional advantage to specifically select the force curves recorded at the nanoparticle center in a post-treatment procedure. Typically, 32×32 force mapping curves were recorded around the selected nanoparticle ($500 \text{ nm} \times 500 \text{ nm}$) and force *versus* piezo movement (F-Z) curves, located at the nanoparticle center, were extracted in an offline procedure. The raw F-Z approach curves were converted into force *versus* tip-sample distance curves to apply a contact mechanics model for a sphere-sphere contact geometry. The

resulting apparent elastic modulus E was then obtained by fitting these curves with the Hertz model.

3. RESULTS AND DISCUSSION

3.1. FON fabrication: manual addition vs microfluidic mixing chamber

In order to generate photocrosslinkable FONs out of small molecules following high impact-flow microfluidics, we resorted to push-pull structures **ALD** and **VIN** that had been designed to display both fluorescence and photocrosslinking properties in the solid state for solution-processed OLEDs (Figure 1).^[23-24] Such compounds display large structural similarity and distinct emission ranges, namely greenish-blue for **ALD** and orange-red for **VIN** as a result of their aldehyde and dicyanovinylidenyl groups respectively, that will be exploited latter in the manuscript to fabricate color-tunable FONs.

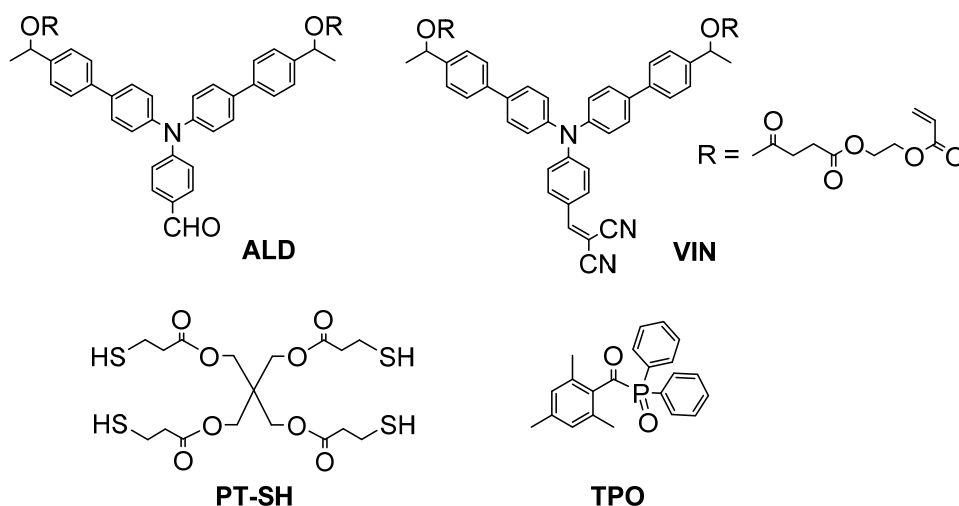


Figure 1. Structures of fluorophores processed as color-tunable and photocrosslinkable FONs.

The propeller shape of the triphenylamino core and the introduction of two unresolved chiral centers at the benzylic carbon atoms, yield racemic mixtures, and advantageously limit the formation of dark π – π aggregates. In this way, fully amorphous material, characterized by a glass transition temperature T_g measured at 6.4 °C by differential scanning calorimetry, is obtained and showed a thick oily-like state. This low glass transition helps in the formation of spherical organic nanoparticles upon nanoprecipitation in water to minimize surface tension effects and prevent recrystallization in the course of time that could impart FONs with undesirable aggregation effects or various crystalline phases.^[25] FON nanoprecipitation typically proceeds at room temperature from the quick addition and mixing of a concentrated dye solution in tetrahydrofuran (THF) within a larger volume of water using magnetic stirring, to cause efficient dispersion (Figure 2A).

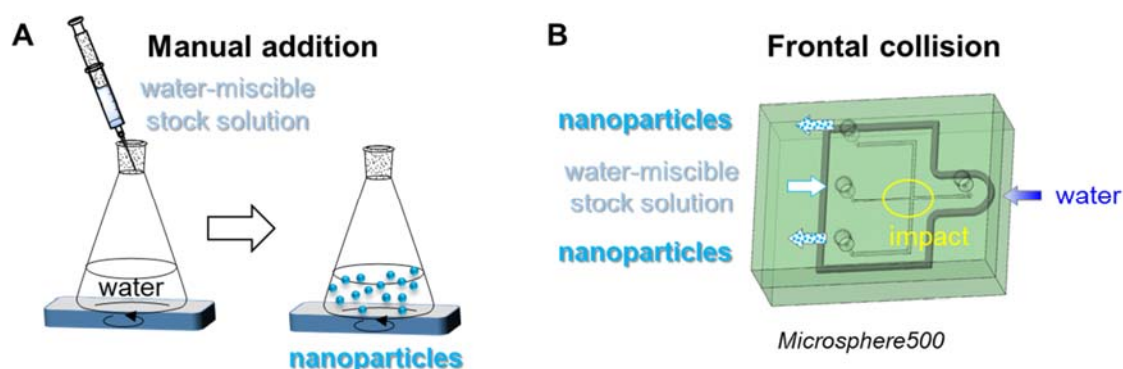
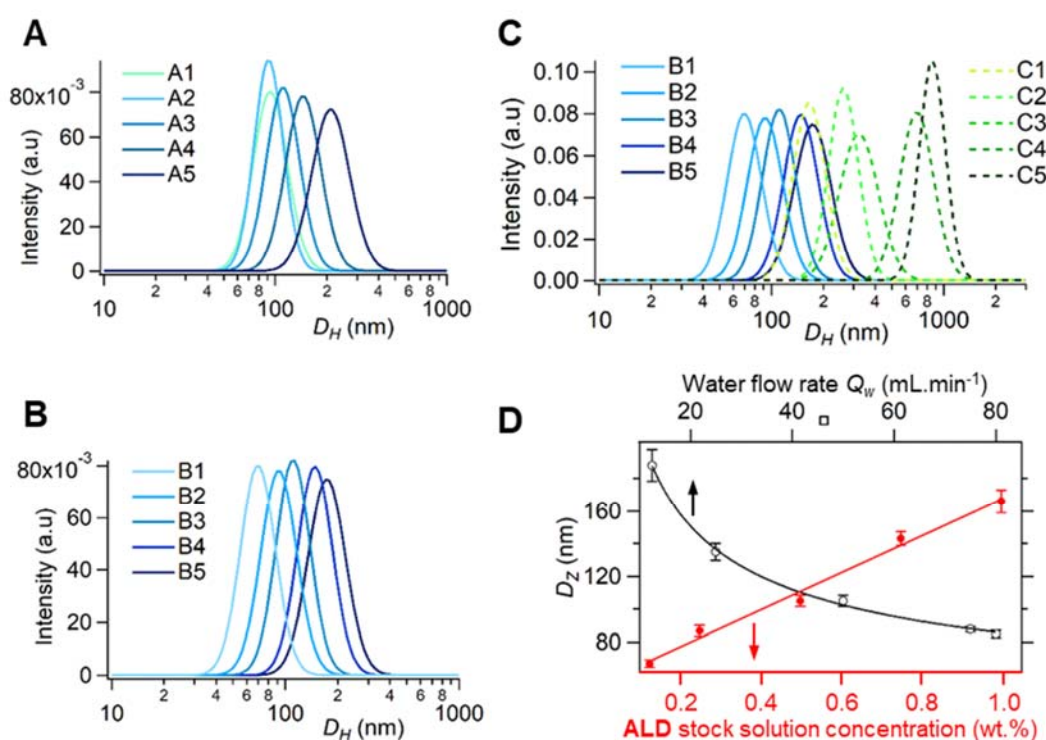


Figure 2. FON fabrication following flash precipitation. A) Quick manual addition of a stock fluorophore solution into a large volume of water under vortex stirring. B) Transparent 3D-view of the Microsphere500 micromixer allowing for high-impact frontal collision of two opposite flows, containing respectively the stock solution (channel with a 300 $\mu\text{m} \times 300 \mu\text{m}$ square section) and water (channel with a 600 $\mu\text{m} \times 600 \mu\text{m}$ square section). The thicker gray line features the rubber seal, sandwiched between the two aluminum plates (green), to avoid leakage

due to high-pressure injection, and the thinner line corresponds to the microfluidic circuit itself, etched in the bottom plate. All tubing inlets and outlets are fixed on the top plate and indicated by a circular aperture.

Controlling two main criteria like the concentration of the organic stock solution and the mixing rate thus appears crucial. In order to independently investigate their influence in a defined manner, we resorted to the Microsphere500 mixing chamber comprising one channel (600 μm -large section), supplied with water and slightly up-shifted with regard to an opposite smaller channel (300 μm -large section), funneling the organic stock solution (Figure 2B). On the basis of previous studies involving manual injection in water of a 0.1 wt. % dye solution in THF, we considered an initial 0.5 wt. % dye concentration as a median value and varied this mass fraction from 0.025 up to 1 wt. %. The mixing rate was indirectly tuned by changing independently the flow rates of each channel and the respective flow rate ratio, which controls the mass ratio. We thus carried out two distinct experiments **A** and **B**, corresponding to the conditions detailed in Table S1, namely variable organic solution (o) and water (w) flow rates (constant flow rate ratio (o:w) and stock solution concentration), and variable stock solution concentration (constant (o) and (w) flow rates, and flow rate ratio (o:w)) respectively. For each experiment **A** and **B**, the samples were collected 4 s after starting the mixing process to avoid composition inhomogeneity. FON size distribution was measured by dynamic light scattering (DLS) right after the sample collection. For experiment **A**, the FON Z-average size (diameter) D_z decreased from 193 to 86 nm (with a polydispersity index (PDI) averaged over the five measurements found around 0.215) when the flow rate increased by a factor of 5 (Figure 3A). In the same way, the D_z values for experiment **B** decreased from 166 to 66 nm (with a similar averaged PDI around 0.219) along with the reduction of the injected stock organic solution (Figure 3B). Such evolution recalls

previous results obtained with a 3D flow focusing chamber although in the latter case, control of the size polydispersity was delicate to be achieved with high flow focusing ratios.^[26] By contrast to D_Z hydrodynamic diameters, the dry diameter D_{TEM} values, determined from statistical numbering of TEM-imaged FONs for samples issued from experiment **B** (Figure 5E) were found quite constant at around 61-66 nm with a relatively narrow size dispersion (Table S2 and Figure S2). Such large distinct evolutions may appear quite puzzling at first sight and could suggest aggregation at higher concentration to explain the size increase, which appears quite incompatible with the well-defined nanoparticles observed by TEM or later on by AFM. As mentioned above, compound **ALD** displays quite a low T_g below room temperature. We suspect fragmentation in the TEM chamber of the honey-like FONs into smaller nanoparticles with more stable geometry, hence very similar size distributions are obtained.



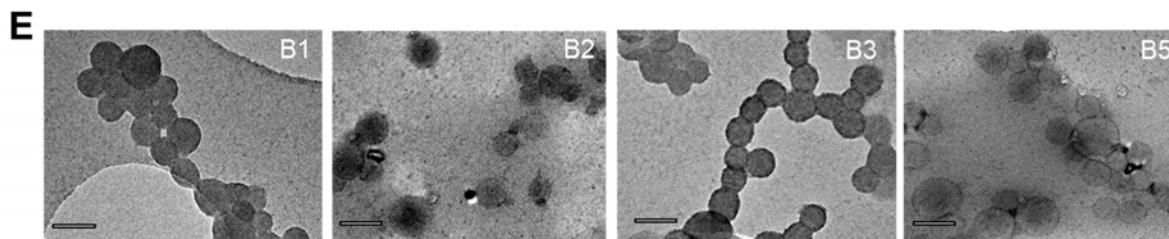


Figure 3. DLS measurements of FON dispersions made of **ALD** fluorophores and obtained by means of the Microsphere500 mixing chamber: A) experiment **A**, B) experiment **B**, and by means of vortex stirring: C) experiment **C**. D) Variation of the Z-average size (diameter) D_Z of FONs as a function of the water flow rate Q_w (experiment **A**) and stock solution concentration (experiment **B**); error bars were calculated using a 95% confidence interval. E) TEM imaging of FONs issued from experiment **B** (sample B4, not represented here, gave intractable images). Scale bar: 100 nm.

As for the D_Z values, , it was interesting to find that they decreased as a function of the water flow rate Q_w , following a power law $D_Z = 544Q_w^{-0.424}$, as commonly reported for micromixers where one flow rate predominates, even in the case of systems with distinct geometry or smaller flow velocity.^[27] By contrast, the D_Z values evolve quite linearly as a function of the stock solution concentration (Figure 3D). Hence, we can suggest that high flow rates favor efficient dispersion of the organic constituents and nucleation upon supersaturation, conducting to smaller nanoparticles with a narrow size distribution. Transmission electron microscopy imaging showed indeed smooth and well spherical nanoparticles whose size appears very homogeneous. Interestingly, compared to identical final **ALD** concentrations (experiment **B**), nanoparticles obtained upon manual addition in water of **ALD** solutions with a distinct stock solution concentration (experiment **C**) systematically showed larger mean size and size polydispersity (averaged PDI equal to 0.230) (Figure 3C, Table S1). Finally, we tested the

reproducibility of experiment **A** where flow impact rather than stock solution concentration exerts higher influence on FON size. We found that the diameter evolution of the second series differs by only 5 % compared to the first one. All these elements let us propose that FON fabrication using the Microsphere500 mixing chamber yields high-quality and reproducible samples.

Size measurements were completed with surface potential ζ measurements by means of zetametry to investigate FON colloidal stability in water. Surprisingly, largely ζ negative values of -39 ± 8 mV were obtained. Given the FON size, ζ values larger than -30 mV, indicate great colloidal stability in water which was indeed found over one year at least (Figure S3). This apparently surprising result, despite the large **ALD** hydrophobic character and absence of ionizable units, has already been observed and ascribed to the adsorption at the FON surface of traces of fatty acids, present as contaminants.^[28]

To conclude on this part, it is worth noting that the rate of the impacting organic flow used in our case is significantly reduced by a factor of 4 to 5 comparatively to the CIJ and MVIM mixer geometries used in literature, while nanoparticles with size of similar Z-average ($D_Z \approx 60$ -200 nm) are obtained.^{10b} This comparison is based on literature data since we could not benefit from direct assay on the last two mixers. This lets us propose that the Microsphere500 mixer, allying ultrafast opposite impacting flows, vortex mixing and easy modulation of the kinetic energy, would represent an attractive setup to significantly limit the consumption of precious organic materials in the formulation of active nanoparticles.

3.2. Evolution of the FON photophysical properties as a function of the mixing rate

Since fabricated FONs in these studies are devoid of any additional surfactant or polymer, direct assessment of their photophysical properties as a function of their intrinsic size and method of

preparation can be performed. All nanoparticle dispersions displayed bright bluish-green emission, centered at 500 nm upon excitation in the UV absorption band composed of a shoulder at 375 nm and a maximum at 345 nm that are related to charge transfer from the triphenylamino center to the formyl and biphenyl moieties, respectively.^[23] Interestingly, this emission maximum is quite similar to that reported for non-doped thin films fabricated by spin-coating a 1 wt. % dye solution in chloroform and subsequently vacuum-dried,^[23] thereby indicating that local polarity effects originate from the dyes themselves, independently of the surrounding hydration shell. The constant energy value for the emission signal of all nanoparticles lets us suggest quite similar dye spatial arrangements from one FON batch to another since any significant difference in local surroundings would cause notable energy shift of the transition. At this step, performing fluorescence microscopy imaging on single nanoparticles could be very stimulating to infer their proper responses, but such experiments represent an entire field of investigations *per se*, beyond the scope of the current studies. However, particular attention paid to the emission intensity and dynamic relaxation of the excited state revealed significant difference between the fabrication methods **A** and **B**. Each sample was diluted with Millipore water in the same amount before measurements to limit inner filter effects, especially in excitation. In agreement with the constant concentration of fluorescent material in all samples of experiment **A**, the absorption and emission spectra display almost the same intensity, except for sample A5. The latter contains larger FONs formed under larger flow rates, and show significant contribution of light scattering (Figure 4A). At this step, we must admit that we have no reasonable explanation to support the observed bathochromic and hypochromic shifts. Formation of π - π aggregates that is commonly evoked is indeed contradictory with the almost constant value of the fluorescence quantum yields Φ_f measured to be 0.16 on average. As for the samples of experiment **B** where the concentration of

injected fluorophores is twice as high from one sample to another, the intensity of the absorption and emission spectra logically increased proportionally (Figure 4B). No change in the general shape occurred, hence intermolecular interactions within the nanoparticles are similar between the different batches. The fluorescence quantum yield Φ_f was found very close to that for samples **A**, namely at 0.17 on average. Since **ALD** fluorophores, characterized by strong charge transfer in their radiative transitions,^[23] are very sensitive to local polarity effects in their excited states, any strong structural changes in the fluorophore surroundings would be accompanied by notable modification of their emission energy and intensity as well. The constant value found for Φ_f tends to indicate similar dye self-assembling within FONs whatever the flow energy impact.

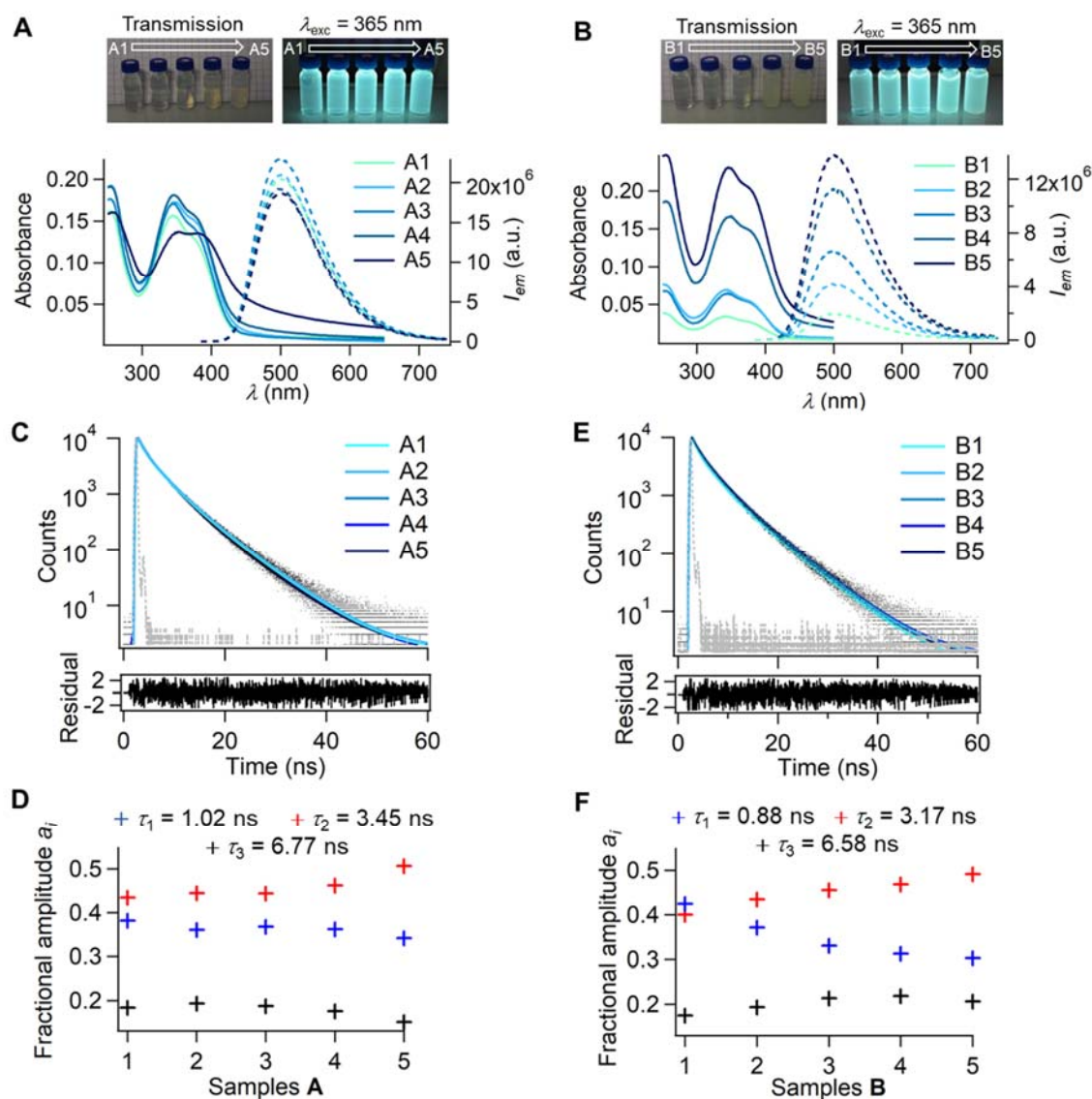


Figure 4. ALD FON dispersions under ambient light and 365 nm excitation (top) and UV-vis absorption and emission spectra ($\lambda_{exc} = 375$ nm) (bottom) of aqueous ALD FON dispersions: A) issued from experiment A, B) issued from experiment B. Fluorescence time decays (middle) ($\lambda_{exc} = 340$ nm, $\lambda_{em} = 501$ nm) and evolution of the fractional amplitudes a_i after reconvolution from the excitation pulse response (gray trace) and global fitting following a three-exponential mathematical law (bottom) of aqueous ALD FON dispersions issued from: C-D) experiment A, E-F) experiment B.

and E-F) experiment **B** (residual traces of the global fitting are indicated on the bottom of the lifetime decays in Figures C and E).

Yet, accurate examination of the FON fluorescence decays revealed subtle evolutions with the FON diameter. The decays, obtained after excitation at 340 nm and recording the emission signal at 501 nm, required a multi-exponential fit and provided three distinct lifetimes using global fitting analyses ($\tau_1 = 0.88$ (**A**), 1.02 (**B**) ns; $\tau_2 = 3.17$ (**A**), 3.45 (**B**) ns; $\tau_3 = 6.58$ (**A**), 6.77 (**B**) ns) for the samples of both experiments, recalling previous behaviors investigated in solid thin film (Figure 4C-D). The smaller lifetimes found overall for the samples **A** compared to those of samples **B** may be related to the slightly distinct surroundings in terms of water:THF composition in the final mixture since the injected THF volume increased from sample B1 through sample B5. As for the evolution of the lifetime fractional amplitudes a_i along with the FON diameter, two opposite evolutions could clearly be observed (Figure 4E). The a_2 contribution of the intermediate lifetime τ_2 globally increased with the FON size. Conversely, the a_1 component of the shorter lifetime τ_1 decreased, even more neatly for samples **B**. Based on previous description of FONs in water, molecules at the interface undergo significant quenching in contact with water due to vibrational relaxations and would be representative of the shorter lifetime τ_1 .^[29] On the contrary, the intermediate lifetime τ_2 , whose contribution dominates for fluorophores processed as neat thin films, would suggest a bulk origin. So, observing a raise of the shorter lifetime component τ_1 and a drop of the intermediate lifetime component τ_2 with the FON size reduction agrees with an increase in the surface-to-volume ratio, showing the sensitivity of **ALD** fluorophores toward their close surrounding.^[30] We thus harnessed such sensitivity to show that bicomposite FONs can also be obtained using the high-impact Microsphere500 microfluidic

chamber with little risks of demixing into separated components caused by high dye concentrations in stock solutions.

3.3. Bicomposite FONs with tunable color emission and amplified emission intensity as a result of dye coprecipitation

To this aim, we targeted the generation of bicomposite FONs out of energy donors and acceptors, which can promote efficient energy transfer for spatially confined fluorophores. We thus employed the red-emissive fluorophore **VIN**, displaying large structural similarity with the bluish-green fluorophore **ALD** and yielding amorphous material ($T_g = 11.9\text{ }^{\circ}\text{C}$), so that high mixing entropy is favored in the final bicomposite FONs (Figure 1). Its absorption spectrum broadly overlaps the emission spectrum of the bluish-green emitter, yielding efficient Förster resonance energy transfer (FRET), with a Förster radius R_0 valued to be 39 Å in tetrahydrofuran from calculations. To validate our approach, we prepared four stock solutions in THF with a varying **ALD:VIN** composition: 100:0, 95:5, 80:20 and 0:100 %. We fixed the flow rates of the microchannels funneling water and THF solution respectively at 50 and 2 mL.min⁻¹, and **ALD:VIN** solutions at a 0.25 wt. % total dye concentration, which represents a median value among the previous assays. All samples investigated by TEM formed well-defined nanoparticles, the **VIN** FONs being a little less defined (Figure 5C). Their zeta potential values ζ were found to be around $-42 \pm 4\text{ mV}$, supporting again large colloidal stability in water. Lower diameters could be noticed for all batches compared to the size distribution measured for experiment B2 involving a similar dye concentration. This is likely to be due to a dilution effect, slightly higher than expected during solution preparation, thereby generating smaller FONs. Nevertheless, this does not question the logic size evolution obtained through a series of samples, as demonstrated by the above reported reproducibility assay, provided that FON are generated in the row, using the same

tubing, solvents and plastic syringes as experimented here. It is worth noting that the FON diameter varied despite the constant amount of injected organic material, which can be ascribed to distinct dipolar interactions established between the fluorophores and water molecules (Figure 5B).

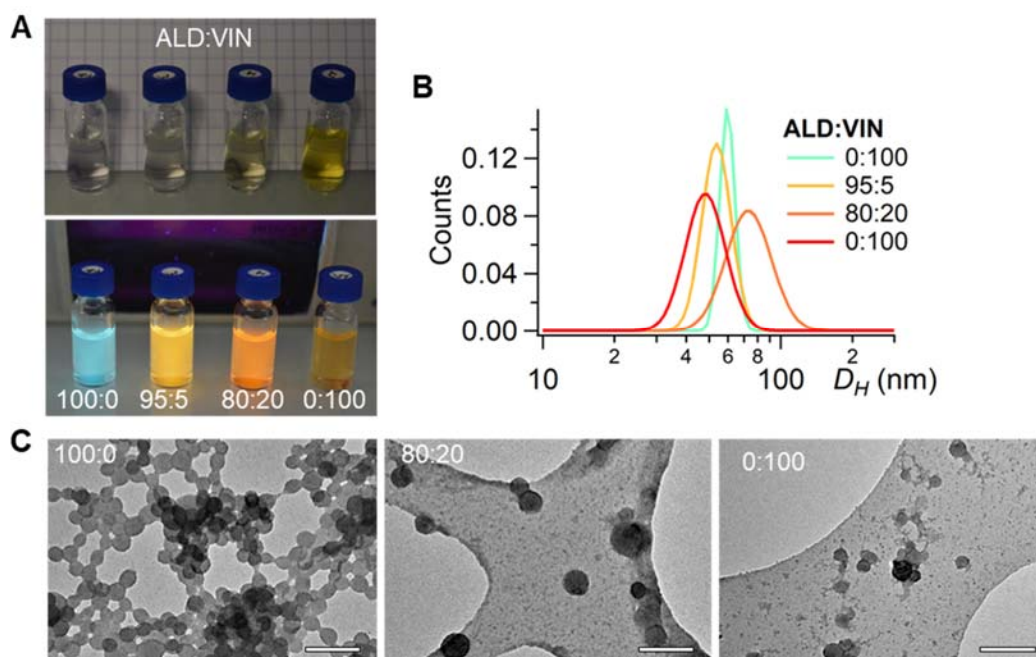


Figure 5. ALD:VIN FON dispersions obtained from the Microsphere 500 mixing chamber for distinct compositions 100:0, 95:5, 80:20 and 0:100. A) Vials in transmission (top) and under 365 nm excitation (bottom). B) DLS distributions. C) TEM imaging using holey carbon-coated copper grids (sample 95:5 was not imaged for priority reasons). Scale bar: 200 nm.

In agreement with the increasing amount of compound **VIN**, an absorption band centered at 452 nm rose (Figure 6A), typical of the lower charge transfer excited state calculated from TDDFT computations.^[23] Given the absence of spectral overlap in the visible between the absorption spectra of the **ALD** blue and **VIN** red emitters and the match at 375 nm between a maximal absorption for **ALD** and a minimal absorption for **VIN**, the emission spectra were

recorded upon excitation at 375 nm and 452 nm to distinguish the contribution of both dyes and assess the extent of energy transfer. For $\lambda_{\text{exc}} = 375$ nm, the emission band centered at 500 nm was typical of pure FON_{ALD} and dramatically shifted to 589 nm for FONs comprising only a 5 mol. % amount of **VIN** (Figure 6B), yielding bright sun yellow emission (Figure 6A).

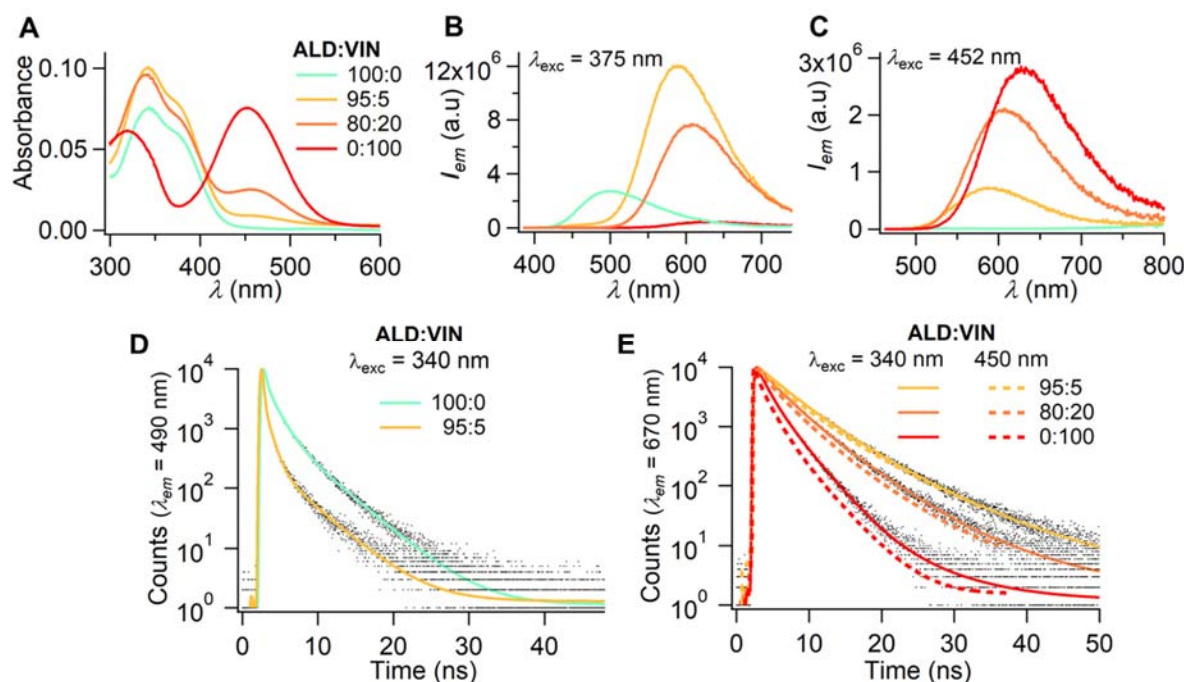


Figure 6. Aqueous **ALD:VIN** FON dispersions obtained using the Microsphere500 mixing chamber with carrying composition 100:0, 95:5, 80:20 and 0:100: A) UV-vis absorption spectra, B) emission spectra upon excitation at $\lambda_{\text{exc}} = 375$ nm where mainly **ALD** absorbs, C) emission spectra upon excitation where mainly **VIN** absorbs, D) Fluorescence time decays upon excitation at $\lambda_{\text{exc}} = 340$ nm and recording at $\lambda_{\text{em}} = 490$ nm where **ALD** exclusively emits light, E) fluorescence time decays upon excitation at $\lambda_{\text{exc}} = 340$ and 450 nm and recording at $\lambda_{\text{em}} = 670$ nm where **VIN** exclusively emits light.

Its intensity appeared to be 17 times higher compared to that obtained upon direct excitation at 452 nm and 700 times higher if **VIN** alone were excited at the same concentration (Figure 6C).

From the donor emission spectra, the energy transfer quantum efficiency Φ_{FRET} from **ALD** to **VIN** could be assessed to be 74 and 96 % for FON compositions of 95:5 and 80:20 respectively. This large value could be confirmed through time-resolved fluorescence measurements, by exciting at two distinct wavelengths, namely 340 and 450 nm, and recording the fluorescence decays at 490 (Figure 6D) nm or 670 nm (Figure 6E). A strong decay of the blue-greenish signal was again noted for the 95:5 FON composition where the amplitude-averaged lifetime $\langle\tau\rangle_a$ indeed decreased by 75 % from 0.81 to 0.23 ns. Very interestingly, the fluorescence decay at 670 nm shortened ($\langle\tau\rangle_a$ dropped from 4.46 to 1.45 ns) for increasing amounts of red emitter **VIN**, whatever the excitation at 340 or 450 nm, whereas similar time constants would have been expected (Table 1). Unfortunately, we were not allowed to distinguish any negative time contribution in the fluorescence decay of **VIN** upon exciting at 340 nm, which features very efficient energy transfer from **ALD** to **VIN** molecules, namely less than a dozen of ps, since the instrumental response function of the acquisition card is not higher than 120 ps. This trend is actually to be related to distinct polar surroundings undergone by the red dyes as a function of the FON composition. This is illustrated by the progressive bathochromic shift (589, 607, and 630 nm) of the emission band undergone for larger amounts (5, 20 and 100 %) of **VIN** whose dipole moment was calculated to be 9.68 D against 7.93 D for **ALD**. Since **VIN** is strongly solvatochromic and known to be significantly quenched in polar surroundings (water included) upon strong twist in the excited state,^[31] color tuning is here caused by the change in polarity^[32] around the red emitters with the composition, rather than by overlapping emission signals.^[21c] Nanoprecipitation of pure **ALD** or **FVIN** FONs out of fluorophore mixes could be ruled out on the basis of the above calculated Förster radius value, which is not compatible with the FON

diameter, larger than 40 nm, and the almost quantitative energy transfer observed for only a 5 % amount of **FVIN** energy acceptors with regard to the **ALD** energy donors.

Table 1. Size distributions and photophysical properties of color-tunable FON dispersions made of **ADL:VIN** of varying composition.

FON ALD:VIN	D_z (nm) (PDI)	$\lambda_{\max}(\text{abs})$ (nm)	$\lambda_{\max}(\text{em})$ (nm) ^[a]	Φ_f ^[b]	$\langle \tau \rangle_a$ ^[c] (ns)		
					$\lambda_{\text{exc}}=340 \text{ nm}$ $\lambda_{\text{em}}=490 \text{ nm}$	$\lambda_{\text{exc}}=340 \text{ nm}$ $\lambda_{\text{em}}=670 \text{ nm}$	$\lambda_{\text{exc}}=340 \text{ nm}$ $\lambda_{\text{em}}=670 \text{ nm}$
100:0	61 (0.019)	375, 342	500	0.13	0.93	-	-
95:5	52 (0.069)	452, 375, 342	589	0.34	0.23	4.54	4.46
80:20	70 (0.250)	456, 375, 340	607	0.22	-	3.48	3.04
100:0	44 (0.142)	452, 320	630	0.10	-	1.93	1.45

[a] Excitation indifferently at 375 or 452 nm (except for neat **ALD** FONs). [b] Valued using coumarin 540A as a fluorescence standard ($\Phi_f = 0.38$). [c] Fluorescence decays were modeled through a triexponential law:

$$I(t) = \sum_{i=1}^3 \alpha_i \exp(-t / \tau_i) \quad \text{with the fractional amplitudes} \quad a_i = \alpha_i / \sum_j \alpha_j \quad \langle \tau \rangle_a = \sum_{i=1}^3 a_i \tau_i$$

Comparative photophysical experiments performed on mixtures of pristine **ALD** FONs and **VIN** FONs following the same **ALD:VIN** ratios than those reported for FON blends indeed evidenced the absence of significant energy red shift upon exciting **ALD** FONs for a 95:5 **ALD:VIN** ratio (Table S3 – Figure S4). Only the 80:20 **ALD:VIN** composition may suggest slight interactions between the separated FONs as a consequence of favorable hydrophobic

interactions rather than any interactions between solvated individual dyes whose photophysical properties remain unchanged (Figure S5). Indeed, partial energy shift of the **ALD** emission band that broadens due to the **VIN** contribution and possible inner filter effects was observed (Table S3 – Figure S4B-C and associated comments), while the amplitude-averaged lifetime $\langle \tau \rangle_a$ of the **ALD** units was reduced by half (Figure S6). However, in that case, energy transfer that would then operate at the interface between **ALD** and **VIN** FONs is by no way comparable to that observed for bicomposite FONs. All these observations thus support the fact that FRET operates within the bicomposite FONs as a result of **ALD** and **VIN** coprecipitation within the same nanoparticles that do not demix into separate FONs. Modeling the kinetics of such an energy process in FONs as a function of the amount of energy acceptors with respect to that of the energy donors would require more complex approaches, taking into account energy migration through space and through bond, namely through dipole-dipole interactions or hopping, as described in literature.^[33]

To conclude on this part, diluting red-emissive dyes in hydrophobic matrices, serving as energy antenna, appears as a very attractive and ubiquitous approach to generate bright far-red FONs, thereby avoiding competitive radiationless deactivation of low-energy emitters in polar surroundings. Such observation echoes the recent theoretical developments on fluorescence quenching in solution and fluorescence recovery in AIEgene nanoparticles. Such studies point out the dramatic energy shift of the conical intersection (CI) between the S_1 excited state and S_0 ground state undergone along the potential energy surface for self-assembled fluorophores, thereby reducing the radiationless deactivation pathway.^[12b] Based on previous transient absorption spectroscopy experiments carried out on a similar compound, we herein suspect that such CI shift may operate to explain the considerable dye emission amplification observed for

VIN fluorophores embedded in **ALD** FONs compared to dyes dissolved in THF (Figure S5) or self-assembled in “monocompound” FONs, which would result not from steric effects but from strongly exacerbated polarity effects. To go a step further and harness the high spatial confinement of dyes in organic nanoparticles, we finally tested the ability of photocrosslinkable fluorophores like **ALD** to be reactive after being processed as FONs, which to our knowledge as never been explored so far, despite the great interest in the generation of photopolymerized functional nanoarchitectures.^{[34],[35]}

3.4. Photoreaction performed in pre-formed FONs

We here based our approach on the fact that **ALD**, processed as thin films, could undergo solid-state through a photoinitiated radical polymerization of its acrylate units.^[23] In order to limit the action of oxygen acting as a radical scavenger, we privileged the well-documented thiol-ene reaction, highly compatible with water and ambient air and temperature conditions.^[36] Pentaerythritoltetrakis(3-mercaptoprioionate) (PT-SH), containing four thiol units, was chosen as a crosslinker while 2,4,6-trimethylbenzoyl-diphenylphosphine (TPO) was retained as a hydrophobic photoinitiator (Figure 1). The latter choice was motivated by previous results showing high deactivation of TPO photodissociation upon competitive energy transfer toward low-energy lying red emitters like **VIN**.^[24] Thanks to the aforementioned results emphasizing amplified energy transfer in FONs, we thus privileged the use of **ALD** instead of **VIN**, despite a TPO excitation in the UV range, namely at 365 nm, overlapping the **ALD** absorption spectrum. Based on preliminary assays performed on thin films to obtain efficient crosslinking within a reasonable irradiation time, two distinct PT-SH:**ALD** composition with a molar ratio of 0.1:1 and 0.5:1 (FON_{0.1} and FON_{0.5}), corresponding respectively to a 20 % and equimolar content of thiol units compared to the acrylate ones, were tested while keeping constant at 2.5 mol. % the amount

of TPO photoinitiator with regard to **ALD**. FONs devoid of TPO and PT-SH were also prepared to serve as control samples (FON₀).

From the absorption and emission spectra before photoirradiation, the shape and intensity maxima of the bands were found to be almost the same, given the experimental uncertainty upon dilution or precipitation in water, showing weak impact of the additional components like PT-SH known to promote fluorescence deactivation upon reductive electron transfer (Figure 7B and 7D).^[37] After photoirradiation in deoxygenated conditions, noticeable changes in absorption could be observed, especially for FON_{0.5} with a higher PT-SH content, where the low-energy shoulder at 375 nm, ascribed to the charge transfer excited state responsible for emission, is reduced in intensity (Figure 7C).

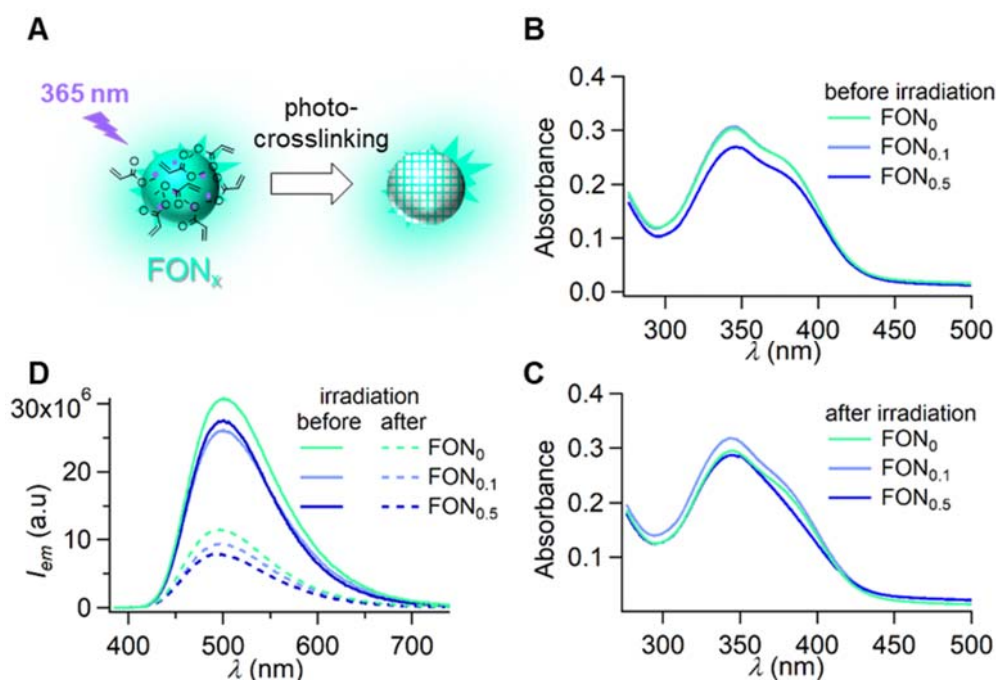


Figure 7. A) Schematic description of photocrosslinking of FONs made of TPO:PT-SH_x:ALD upon irradiation at 365 nm for 5 min. (30 mW.cm⁻²). UV-vis absorption spectra: B) before irradiation, C) after irradiation, and D) emission spectra ($\lambda_{exc} = 375$ nm) of FONs with varying

molar compositions of PT-SH (**ALD** alone: $x = 0$; TPO:0.1 PT-SH:**ALD** $x = 0.1$; TPO:0.5 PT-SH:**ALD** $x = 0.5$) where TPO is employed at a 2.5 mol. % ratio with respect to **ALD**. Irradiation was performed on deoxygenated FON dispersions by prior argon bubbling.

Particular focus on control FON₀ made exclusively of **ALD** showed a similar reduction, albeit much less pronounced than for any other composition (Table 1). When looking at the emission spectra, all samples remained very emissive, even though the signal intensity appeared to be about 30-36 % of the initial value. Again, the most significant reduction is noticed for FON_{0.5} containing TPO and the higher amount of PT-SH crosslinker. We thus here suspect that photoredox processes assisted by UV irradiation or electron transfer from the thiol units, rather than the photocrosslinking reaction itself, are responsible for the photodegradation of the fluorophores promoted in their excited state. Indeed, photoirradiation of the FON dispersions in the absence of prior argon bubbling caused dramatic loss of the emission intensities to 6-16 % of their initial values.

In order to prove the reality of photocrosslinking, structural nanometer scale investigations were carried out first by resorting to TEM imaging. While the aspect of FON₀ and FON_{0.5} appeared smooth and round before irradiation, it systematically evolved to a more grainy structure while keeping its overall spherical shape, which lets us again suggest homogeneous composition of the precipitated composite FONs during fabrication (Figure 8).

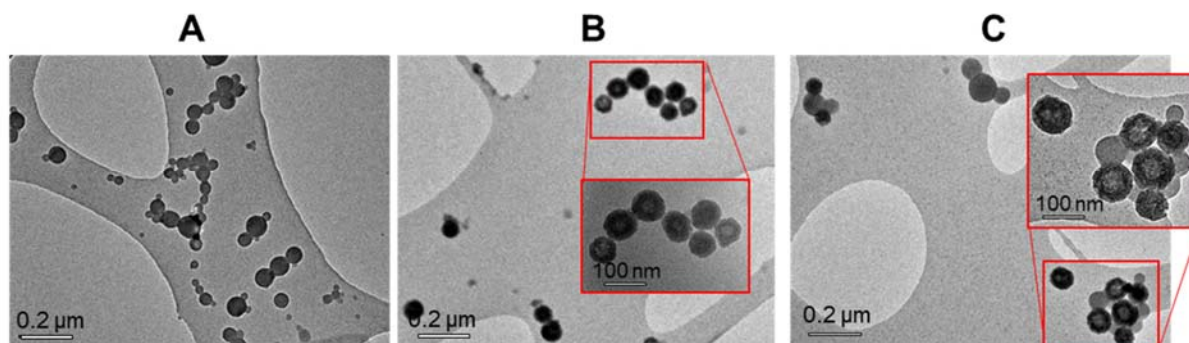


Figure 8. TEM images of FON dispersions deposited on holey carbon-coated copper grids. A) $\text{FON}_{0.5}$ before irradiation, B) $\text{FON}_{0.5}$ after irradiation, C) FON_0 before irradiation at 365 nm.

Interestingly, for sample $\text{FON}_{0.5}$, all nanoparticles systematically appeared darker after photoirradiation, featuring a higher electronic contrast. This evolution could evoke denser systems, as a result of significant shrinkage due to photocrosslinking. When compared to model FON_0 , such geometry evolution is much less marked in agreement with little reactive species in the absence of TPO, indicating that dye degradation cannot be the sole pathway toward the noted structural change.

We thus performed atomic force microscopy measurements (AFM) to address at the nanometer scale some possible mechanical differences between non-photoirradiated and photoirradiated photocrosslinkable FONs. As FONs largely differ from rigid crosslinked polystyrene or silica nanoparticles often used as bioimaging probes, we decided to adopt an approach based on nanoparticle deformation upon compression rather than the common indentation method, especially valid on hard and non-deformable nanosystems.^[38] We thus used calibrated spherical glassy carbon AFM probes with tip radius (100 ± 10 nm) comparable or higher than the radius of probed FONs, which favors compression instead of indentation during the conventional force curve measurements. In order to obtain reliable measurements, we carried

out statistical analyses on a dozen of air-dried nanoparticles after deposition from highly diluted dispersions on cleaned mica surfaces, pretreated with lysine to favor FON attachment and avoid nanoparticle rolling effects. Under these controlled conditions, AFM force investigations of FON_{0.5} containing the higher PT-SH molar fraction before and after photoirradiation displayed significant mechanical differences in terms of E elastic modulus. E values increased by 62 % from non-photoirradiated FONs to photoirradiated ones, namely from 105 ± 11 to 170 ± 10 MPa (Figure 9).

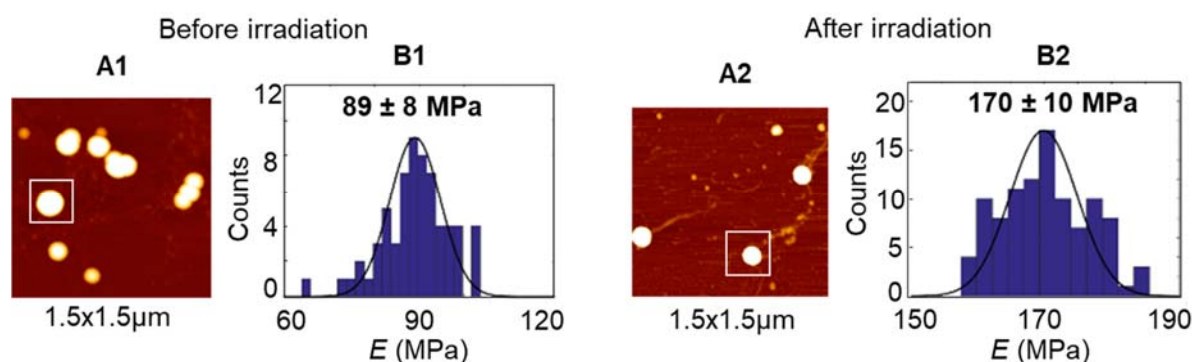


Figure 9. FON_{0.5} (composition TPO:0.5 PT-SH:ALD) deposited on lysine-treated mica surface before (1) and after irradiation (2) at 365 nm. A) AFM imaging of several nanoparticles. B) Elastic modulus E distribution upon force mapping investigations of one single nanoparticle (selected by a white square on Figure 9A).

Such significant changes well match the expected mechanical evolution when going from softer nanostructures to stiffer ones, as expected upon photocrosslinking. The revisited exploration method with compression rather than indentation presented here for quantitative mechanical measurements of quite soft nanoparticles by means of AFM, appeared as a nice approach to inform on FON rigidification, as a consequence of photocrosslinking.

4. CONCLUSION

These studies have evidenced the versatile approach of generating FONs devoid of surfactant and displaying colloidal stability by means of a novel model of micromixer, Microsphere500, allowing for straightforward variation of the mean diameter as a function of the flow velocities or stock solution concentration. Compared to classical manual addition, this flash nanoprecipitation technique yields highly concentrated FON dispersions, characterized with much smaller size, narrower polydispersity, and high fabrication reproducibility without causing subsequent precipitation. The fabrication of monocomposite FONs has successfully been extended to the microfluidic production of bicomposite FONs without causing demixing or precipitation into separated nanoparticle despite the high concentration used. Such coprecipitation process was proved by implying energy donors and acceptors amenable to efficient electronic energy transfer that led to considerably enhanced emission of red emitters as a result of the tight confinement and mutual polarity effects. Finally, we harnessed such confinement effects for the first time to our knowledge to promote reaction within FONs containing fluorophores with photopolymerizable units, and therefore obtain photocrosslinked FONs based on photoinitiated thiol-ene reaction. The results point out the difficult balance between photoinitiation, and fluorophore photodegradation upon irradiation due to competitive excitation absorption. Quantitative mechanical studies performed at the nanometer scale by resorting to AFM revealed FON stiffening after photoirradiation, confirming the possibility to perform reaction with self-assembled molecules. Future studies will explore the possibility of trapping or post-grafting various molecules of interest using coupled microfluidic devices to impart FONs with theranostic properties and high colloidal stability in biological media if required. Further investigations of the mixing dynamics within the Microsphere500 chamber,

beyond the scope of the current studies, will also be performed by replacing the aluminum plates with optically transparent windows and using a camera with ultra-high acquisition rate.

SUPPORTING INFORMATION

Supporting Information is available from the Wiley Online Library or from the author.

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KEYWORDS. Fluorescent organic nanoparticles • microfluidics • size and control modulation • photophysics • photochemistry

CONFLICTS OF INTEREST

There are no conflicts to declare.

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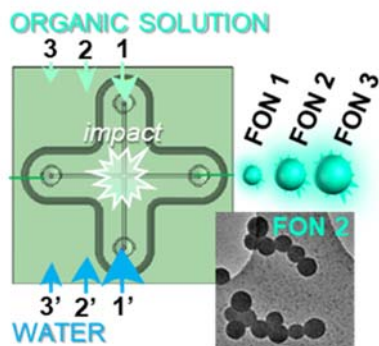
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TABLE OF CONTENTS GRAPHIC



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Authors: Stéphane Hoang, Simon Olivier, Stéphane Cuenot, Agnès Montillet, Jérôme Bellettre, and Eléna Ishow*

Title: Microfluidic Assisted Flash Precipitation of Photocrosslinkable Fluorescent Organic Nanoparticles for Fine Size Tuning and Enhanced Photoinduced Processes

Dispersions of bright fluorescent organic nanoparticles are fabricated at high concentration using high-impact mixing chamber allowing for fine size tuning and narrow size distribution. Dye spatial confinement within bicomposite nanoparticles enables amplified emission upon energy transfer due to polarity effects, and photopolymerization addressed at the nanoscale by atomic force microscopy.