# Supporting Information

**A Bottom-Up Approach to Red-emitting Molecular-Based Nanoparticles with Naturally Stealth Properties and their Utility for Single Particle Tracking Deep in Brain Tissue**

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1. Experimental procedures

*a. Synthesis:* **$Q_{BDF}$** dye was synthesized according to the method reported by Li *et al*.[1]

*b. FONs preparation:* 200 µL of dye solution at concentrations of 0.1, 0.4, 1, 2, 4 or 5 mM in THF (spectroscopic grade, Thermo Fisher) were rapidly added to 19.8 ml of freshly distilled water at room temperature under pulsed (1 Hz) or continuous sonication (10 W) for 3 min. Of note, a 0.1 mM stock concentration does not yield stable nanoparticles and a 5 mM stock concentration yields turbid solutions. For imaging experiments (TEM or fluorescence microscopy), FONs were freshly prepared on the day of the experiment. To assess the stability of FONs over time (Figure S3), FONs were stored at room temperature (RT).

*c. Photophysical measurements:* Absorption spectra of FONs were recorded at RT on a Jasco V-670 UV/Vis spectrophotometer. Spectra are displayed without correcting the baseline for diffusion. Emission spectra were obtained from diluted solutions (optical density at absorbance maximum set to $\leq$ 0.1) excited around the absorbance maximum using a Fluorolog spectrofluorometer. 1 cm quartz cuvettes were used. Fluorescence quantum yields were calculated as follows: $\Phi_f = \Phi_{ref} * \frac{E}{E_{ref}} * \left(\frac{1-10^{-Aref}}{1-10^{-A}}\right) * \left(\frac{n}{n_{ref}}\right)^2$ where $\Phi_f$ is the fluorescence quantum yield of the product, $\Phi_{ref}$ is the fluorescence quantum yield of the reference (4-(Dicyanomethylene)-2-methyl-6-(4-dimethylaminostyryl)-4H-pyran (DCM) in ethanol, $\Phi_{ref}$ = 0.437), E is the area under the curve of the emission spectrum of the sample excited at $\lambda_{ex}$, $E_{ref}$ is the area under the curve of the emission spectrum of the reference excited at $\lambda_{exRef}$, A is the absorbance of the sample at $\lambda_{ex}$, $A_{ref}$ is the absorbance of the reference at $\lambda_{exRef}$, n is the refractive index of the sample solvent ($n_{H2O}$ = 1.333) and $n_{ref}$ is the refractive index of the reference solvent ($n_{EtOH}$ = 1.36).

*d. Molar attenuation coefficient determination*: Molar attenuation coefficients (ε) were determined by absorbance (A) using the Beer-Lambert relationship $A = \varepsilon.l.C$. The molar concentration of **$Q_{BDF}$** dye within FONs ($C_{dye}$) was determined experimentally and averaged

from 2 different FONs preparations per condition. To do so, 3 mL of FONs were lyophilized, the obtained dye was re-suspended in 3 mL $CHCl_3$ and $C_{dye}$ was determined by absorbance using $\varepsilon_{dye}^{CHCl_3}$ = 42000 $M^{-1}.cm^{-1}$ at $\lambda_{abs}^{max}$ = 420 nm. The dye molar attenuation coefficient in FONs ($\varepsilon_{dye}$, provided in Table S1) was then determined by absorbance using $C_{dye}$ as concentration value. The molar concentration of nanoparticles ($C_{NP}$) was determined as $C_{NP} = \frac{C_{dye}}{N}$ where N is the number of dye molecules per NP. N was calculated using the following formula: $\frac{4}{3} * \pi * \left(\frac{\emptyset_{TEM}}{2}\right)^3 * \frac{\rho}{M} * N_A$ where $\emptyset_{TEM}$ is the mean NP diameter determined by transmission electron microscopy (see below), ρ is the density of the NP assumed to be equal to 1 $g.cm^{-3}$, M = 1023.44 $g.mol^{-1}$ is the molecular weight of the dye and $N_A = 6.022*10^{23}$ $mol^{-1}$ is the Avogadro constant. N is provided in Table 1. The FONs molar attenuation coefficient in water (ε, provided in Table 2) was determined by absorbance using $C_{NP}$ as concentration value.

*e. Zeta potential measurements:* Zeta potentials were determined using a Nano Particle Analyser SZ-100 (Horiba Scientific) on 3 mL undiluted FONs samples. Values provided are the average zeta-potential readings of 3 to 5 different FONs preparations per condition, each reading being itself the average of 5 to 10 reproducible measurements from a given sample.

*f. Transmission Electron Microscopy:* Transmission Electron Microscopy was performed on a Hitachi H7650 electron microscope at the Bordeaux Imaging Centre core facility on 2 to 3 different FONs preparations. Carbon-membrane coated copper grids were ionized to make them positively charged to favor electrostatic interactions with the negatively charged FONs. A drop of undiluted FONs was deposited on such grids for 1-3 min, dried, contrasted with a drop of Nano-W® (methylamine tungstate) or a drop of uranyl-acetate for 1-3 min and left to dry before imaging. For analysis, nanoparticles were detected on the obtained images either by hand or using a custom-written Matlab script calling the "imfindcircles" function. Diameters were

pooled in histograms using the Matlab script and are shown in Figure 1 and Figure S1. The mean values are reported in Table 1. Other statistics are provided in Table S1.

*g. Hydrodynamic diameter measurements by Nanoparticle Tracking Analysis (NTA):* FONs were diluted 20 times in water or buffered saline solutions supplemented with 10% Fetal Bovine Serum (FBS). The solutions were either Phosphate Buffered Saline (PBS, Invitrogen), MES Buffered Saline (containing (in mM): 135 NaCl, 5 KCl, 1.8 CaCl2, 0.4 MgCl2, 20 MES and 1 D-glucose) or Artificial Cerebro-Spinal fluid (ACSF) (containing (in mM): 130 NaCl, 2.5 KCl, 2.2 CaCl2, 1.5 MgCl2, 10 HEPES and 10 D-glucose). Hydrodynamic diameters were measured using a NanoSight device (NS3000, Malvern). 4 videos of 60s were recorded using the following parameters: excitation source 405 nm, temperature setting 25°C, syringe pump flow 25, fluorescence filter 565 nm, camera level 15 or 16. Particles were tracked using the provided software (verison 3.4.003) using a detection threshold of 3. Blur size and max jump distance were set automatically.

*h. Cell culture:* HeLa cells were subcultured in DMEM (Pan Biotech) supplemented with 10 % Fetal Bovine Serum (FBS) 3 times a week and maintained in an incubator at 37 °C, 5% $CO_2$. 24 h prior to an imaging experiment, glass coverslips were sterilized in ethanol and cleaned by plasma (Harrick Plasma, 3 min). 40.000 HeLa cells per well were then seeded on coverslips placed in a 12-well plate. On the day of the experiments, FONs were diluted 100 times in pre-warmed DMEM + 10% FBS and incubated on the cells at 37°C for 24 h. The FONs containing medium was then removed and the coverslips washed with PBS + 10% FBS before being transferred to a Ludin chamber (Life Imaging Services) in which the imaging was performed.

*i. Organotypic slice preparation:* Sprague-Dawley rats were sacrificed postnatal day 5. Hippocampal organotypic slices were prepared from their brains as previously described.[2] In short, 350 µm thick slices (cut using a Mc Ilwain tissue chopper) were collected in pH 7.3 dissection medium containing (in mM, from Sigma): 175 sucrose, 25 D-glucose, 50 NaCl, 0.5 $CaCl_2$, 2.5 KCl, 0.66 $KH_2PO_4$, 2 $MgCl_2$, 0.28 $MgSO_4$-$7H_2O$, 0.85 $Na_2HPO_4$-$12H_2O$, 2.7

$NaHCO_3$ and 0.4 HEPES. Slices were then transferred in 6-well plates containing culture medium (50% BasalMedium Eagle, 25% Hank's balanced salt solution 1× (with $MgCl_2$ and $CaCl_2$), 25% heat-inactivated horse serum, 0.45% D-glucose and 1mM L-glutamine) on hydrophilic polytetrafluoroethylene (FHLC) membranes (0.45 μm, Millipore) set on Millicell Cell Culture Inserts (0.4 mm, Ø30 mm, Millipore). Slices were cultured for 10 to 14 days at 35°C, 5% $CO_2$ with medium being changed 3 times a week. On the day of the experiments, FONs were diluted 100 times in culture medium and incubated on a slice for 2 h at 35 °C. The slices were then transferred to a Ludin chamber containing pre-warmed HEPES-based artificial cerebro-spinal fluid (ACSF) containing (in mM): 130 NaCl, 2.5 KCl, 2.2 $CaCl_2$, 1.5 $MgCl_2$, 10 HEPES and 10 D-glucose. Slices in ACSF were imaged for no longer than 1 hour.

*j. Fluorescence microscopy:* Imaging (incubation on HeLa cells, photostability in PBS, SPT in water or PBS + 10% FBS, and SPT in the ECS of organotypic brain slices) was performed on an upright epifluorescent microscope (Nikon) equipped with an Intensilight illuminator (Nikon Instruments Inc.) and an EMCCD camera (ProEM-HS, Princeton Instrument). When applicable, a 4× air objective (NA 0.1, Nikon) was used to check the overall position of the field of view within the slice (typically centered above the hippocampal CA1 region) under bright field illumination. Fluorescence images were collected using a water immersion 60× objective (NA 1.0, Nikon). Incident excitation and emitted wavelengths were filtered using the following filters and dichroic mirror (Semrock): excitation FF01-451/106, mirror FF520-Di02 and emission FF02-650/200 for both $\mathbf{Q_{BDF}}$ FONs and **QD-655** (Thermo-fisher, lot #1994870). Intensity of the excitation light was usually set to the lowest power that would yield good enough signal to noise, except for photobleaching experiments (Figure 2A) for which the intensity was purposefully set to the maximum (~67 $mW/cm^2$). Exposure times were fixed to 40 ms for snapshot images (Figure 2C) and to 10 ms for photobleaching and SPT (in solution and in slices) experiments.

*k. Photostability*: The photostability of $\mathbf{Q_{BDF}}(Ø_{12})$ and $\mathbf{Q_{BDF}}(Ø_{18})$ FONs was assessed in PBS. FONs in PBS alone, i.e. not supplemented with serum, stick to glass coverslips, assumedly by electrostatic interactions mediated by the salinity of the environment. Thus immobilized FONs were imaged under the maximum power of our Intensilight source (~67 mW/cm$^2$). Fluorescence quantification was performed on images previously background-subtracted using the rolling ball method in the Fiji-ImageJ image analysis software (Fiji > Process > Substract Background > Rolling ball radius: 10 pixels). The first image was thresholded so as to locate the FONs and the intensity in these puncta was quantified against time. The results are displayed as the normalized thus quantified average fluorescence intensity of 5 fields of view.

*l. Single Particle Tracking in solution*: FONs were diluted 100 times in water or in PBS supplemented with 10% FBS. To avoid local movements of liquid due to immersion of the objective in the sample, a coverslip was placed above the sample in the Ludin chamber and the objective was immersed in a drop of water placed on that coverslip. FONs were recorded for 50 s in regions of interest sized such that the acquisition of a single frame took less than 9 ms to ensure a 10 ms time interval between frames.

*m. SPT analysis*: FONs were tracked post-acquisition using the Mosaic/Particle Tracker 2D/3D Plugin in Fiji-ImageJ[3] on background-subtracted movies. Parameters for object detection were set to a radius of 4 pixels and a discrimination cutoff of 0.001 in solution and 0.015 in slices. The percentile of accepted bright pixels was adjusted for each recording depending on the noise level. Parameters for object tracking were set to a link range of 3 in solution and 2 in slices and a displacement of 3 in solution and of 4 in slices.

*n. Determination of hydrodynamic diameters*: Hydrodynamic diameters ($Ø_{SPT}$) were determined from trajectories of more than 10 data-points using an in-house Matlab script as described previously[4]. The calculation of $Ø_h$ derives from the Stokes-Einstein equation: $Ø_h = 2 * \frac{K_B.T}{6.\pi.\eta.D}$ , where $K_B$ = 1.38*10$^{-23}$ is the Bolzmann constant, T = 295 °K is the

temperature, $\eta$ = 1 in water or 1.01 in PBS + FBS is the medium viscosity and *D* is the nanoparticle's 2D diffusion coefficient. *D* was determined as the regression coefficient of the linear fit of the first 3 points of each trajectory's MSD curve.[5] Diameters were pooled in histograms using the Matlab script and are shown in Figure S5. The median values are reported in Table 1. Other statistics are provided in Table S2 and Table S3.

*o. Data representation and statistics:* **Figure 1C**: Analysis was performed on 20 fields of view from 3 FONs preparations on a total population of 4801 objects. **Figure 2A**: Region size: 6.9 µm. **Figure 2B** (Top): $\mathbf{Q_{BDF}}(Ø_{12})$ FON tracked for 4.5 s in water, i.e. 450 data points. Red sections indicate reconnections after gaps in detection. **Figure2B** (Bottom): $\mathbf{Q_{BDF}}(Ø_{39})$ FON tracked for 16 s in water, i.e. 16000 data points; Bigger FONs can typically be tracked for longer and with fewer gaps than smaller FONs. **Figure 3C**: Region size: 6.5 µm. All images displayed with the same contrast.

2. Animal handling

Sprague-Dawley rats (Janvier, France) were used to prepare organotypic brain slices, both male and female. All procedures were carried out in accordance with the guidelines of the University of Bordeaux/Centre National de la Recherche Scientifique Animal Care and Use Committee.

3. Supplementary Tables

**Table S1.** Statistics for TEM data of **$Q_{BDF}$** FONs

| NPs | n [a] | Mean [b] [nm] | Median [c] [nm] | σ [d] | SEM [e] | Skew [f] | COV [g] | Span [h] |
|---|---|---|---|---|---|---|---|---|
| $Q_{BDF}(Ø_{12})$ | 4801 | 12 | 11 | 4 | 0.1 | 0.3 | 35 | 0.9 |
| $Q_{BDF}(Ø_{18})$ | 3004 | 18 | 17 | 7 | 0.1 | 0.3 | 39 | 1.0 |
| $Q_{BDF}(Ø_{29})$ | 2624 | 29 | 15 | 26 | 0.5 | 0.5 | 88 | 3.6 |
| $Q_{BDF}(Ø_{29})$ Peak 1 * | 1053 | 13 | 14 | 3 | 0.1 | -0.1 | 22 | 0.5 |
| $Q_{BDF}(Ø_{29})$ Peak 2 * | 1571 | 59 | 54 | 23 | 0.6 | 0.2 | 39 | 1.0 |
| $Q_{BDF}(Ø_{39})$ | 1236 | 39 | 38 | 30 | 0.9 | 0.0 | 77 | 1.6 |
| $Q_{BDF}(Ø_{39})$ Peak 1 * | 594 | 14 | 14 | 4 | 0.2 | 0.1 | 25 | 0.6 |
| $Q_{BDF}(Ø_{39})$ Peak 2 * | 642 | 61 | 56 | 26 | 1.0 | 0.2 | 42 | 0.8 |

* **$Q_{BDF}(Ø_{29})$** and **$Q_{BDF}(Ø_{39})$** have a bimodal distribution. The statistics relevant to each peak is provided in the shaded lines. For **$Q_{BDF}(Ø_{29})$**, Peaks 1 and 2 are defined as smaller or bigger than 25 nm diameter respectively. For **$Q_{BDF}(Ø_{39})$**, Peaks 1 and 2 are defined as smaller or bigger than 30 nm diameter respectively. [a] Number of measured nanoparticles; [b] Mean diameter; [c] Median diameter; [d] Standard deviation; [e] Standard Error to the Mean ($\sigma/\sqrt{n}$); [f] Skew ((mean-median)/σ); [g] Coefficient of variation ((σ/mean)*100); [h] Span ($(D_{90}-D_{10})/D_{50}$, with $D_x$ the size value below which x% of the distribution lies. (e.g. $D_{50}$ = median)).

**Table S2.** Photophysical properties of **$Q_{BDF}$** dye dissoleved in THF and as subunits of FONs of increasing sizes.

| Dye | $\lambda_{abs}^{max}$ [a] [nm] | $\varepsilon_{dye}^{max}$ [b] [$10^4$ $M^{-1}$ $cm^{-1}$] | $\lambda_{em}^{max}$ [c] [nm] | $\Phi_f$ [d] [%] | $\tau_f$ [e] [%] | $\varepsilon_{dye}^{max}.\Phi_f$ [f] [$10^3$ $M^{-1}$ $cm^{-1}$] |
|---|---|---|---|---|---|---|
| $Q_{BDF}$ in THF | 450 | 3.0 | 621 | 64 | 5.5 | 19.2 |
| $Q_{BDF}$ in $Ø_{12}$-FONs | 458 | 2.6 | 598 | 29 | 4.6 (0.60)<br>1.9 (0.40) | 7.5 |
| $Q_{BDF}$ in $Ø_{18}$-FONs | 458 | 2.8 | 594 | 30 | 5.0 (0.62)<br>2.1 (0.38) | 8.3 |
| $Q_{BDF}$ in $Ø_{29}$-FONs | 462 | 2.9 | 595 | 33 | 5.5 (0.68)<br>2.6 (0.32) | 9.5 |
| $Q_{BDF}$ in $Ø_{39}$-FONs | 463 | 3.1 | 593 | 34 | 5.5 (0.69)<br>2.5 (0.61) | 10.4 |

[a] Absorbance maximum wavelength; [b] Molar attenuation coefficient of the dye at the absorbance maximum $\lambda_{abs}^{max}$; [c] Emission maximum wavelength; [d] Fluorescence quantum yield; [e] Fluorescence lifetime. When the lifetime is multi-exponential, the contribution of each lifetime value to the fit is indicated between brackets; [f] Brightness

**Table S3.** Statistics for SPT in water data of **$Q_{BDF}$** FONs

| NPs | n [a] | Mean [b] [nm] | Median [c] [nm] | σ [d] | SEM [e] | Skew [f] | COV [g] | Span [h] |
|---|---|---|---|---|---|---|---|---|
| $Q_{BDF}(Ø_{12})$ | 1792 | 73 | 57 | 188 | 5 | 0.1 | 257 | 1.1 |
| $Q_{BDF}(Ø_{18})$ | 1692 | 88 | 72 | 124 | 3 | 0.1 | 141 | 1.2 |
| $Q_{BDF}(Ø_{29})$ | 1923 | 105 | 84 | 139 | 3 | 0.2 | 133 | 1.4 |
| $Q_{BDF}(Ø_{39})$ | 767 | 130 | 100 | 212 | 8 | 0.1 | 163 | 1.5 |

[a] Number of measured nanoparticles; [b] Mean diameter; [c] Median diameter; [d] Standard deviation; [e] Standard Error to the Mean ($\sigma/\sqrt{n}$); [f] Skew ((mean-median)/σ); [g] Coefficient of variation ((σ/mean)*100); [h] Span (($D_{90}$-$D_{10}$)/$D_{50}$, with $D_x$ the size value below which x% of the distribution lies. (e.g. $D_{50}$ = median)).

**Table S4.** Statistics for SPT in PBS + 10%FBS data of **$Q_{BDF}$** FONs

| NPs | n [a] | Mean [b] [nm] | Median [c] [nm] | σ [d] | SEM [e] | Skew [f] | COV [g] | Span [h] |
|---|---|---|---|---|---|---|---|---|
| $Q_{BDF}(Ø_{12})$ | 1010 | 83 | 72 | 123 | 4 | 0.1 | 148 | 1.0 |
| $Q_{BDF}(Ø_{18})$ | 1525 | 113 | 89 | 154 | 4 | 0.2 | 137 | 1.4 |
| $Q_{BDF}(Ø_{29})$ | 1351 | 119 | 97 | 109 | 3 | 0.2 | 92 | 1.4 |
| $Q_{BDF}(Ø_{39})$ | 876 | 134 | 112 | 122 | 4 | 0.2 | 91 | 1.3 |

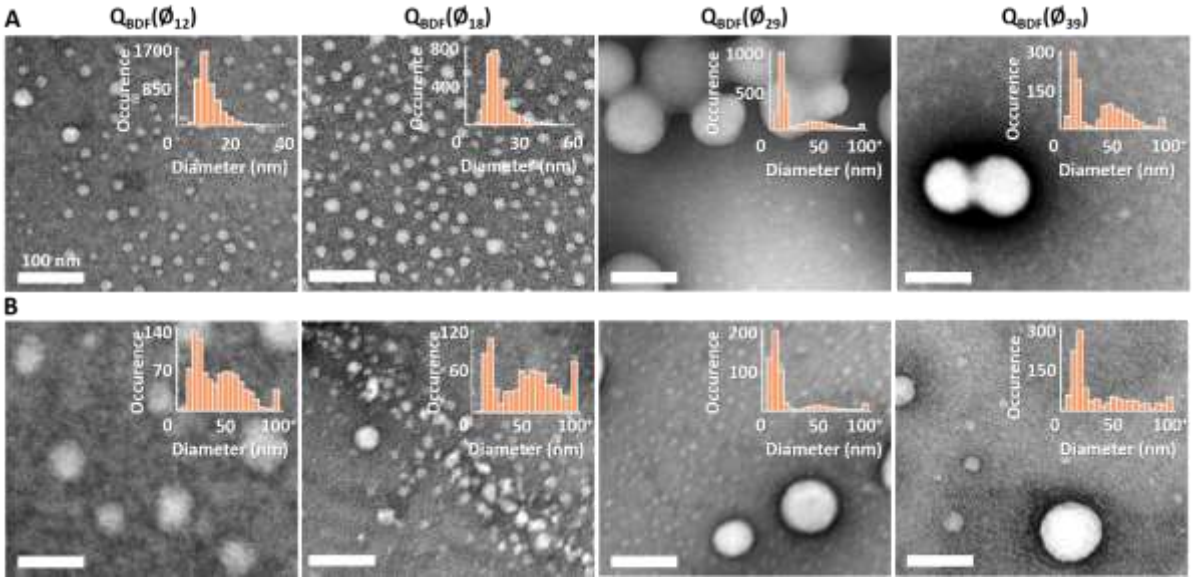
[a] Number of measured nanoparticles; [b] Mean diameter; [c] Median diameter; [d] Standard deviation; [e] Standard Error to the Mean ($\sigma/\sqrt{n}$); [f] Skew ((mean-median)/σ); [g] Coefficient of variation ((σ/mean)*100); [h] Span (($D_{90}$-$D_{10}$)/$D_{50}$, with $D_x$ the size value below which x% of the distribution lies. (e.g. $D_{50}$ = median)).

**Table S5.** Hydrodynamic diameters of **$Q_{BDF}$** FONs in various media measured by Nanoparticle Tracking Analysis

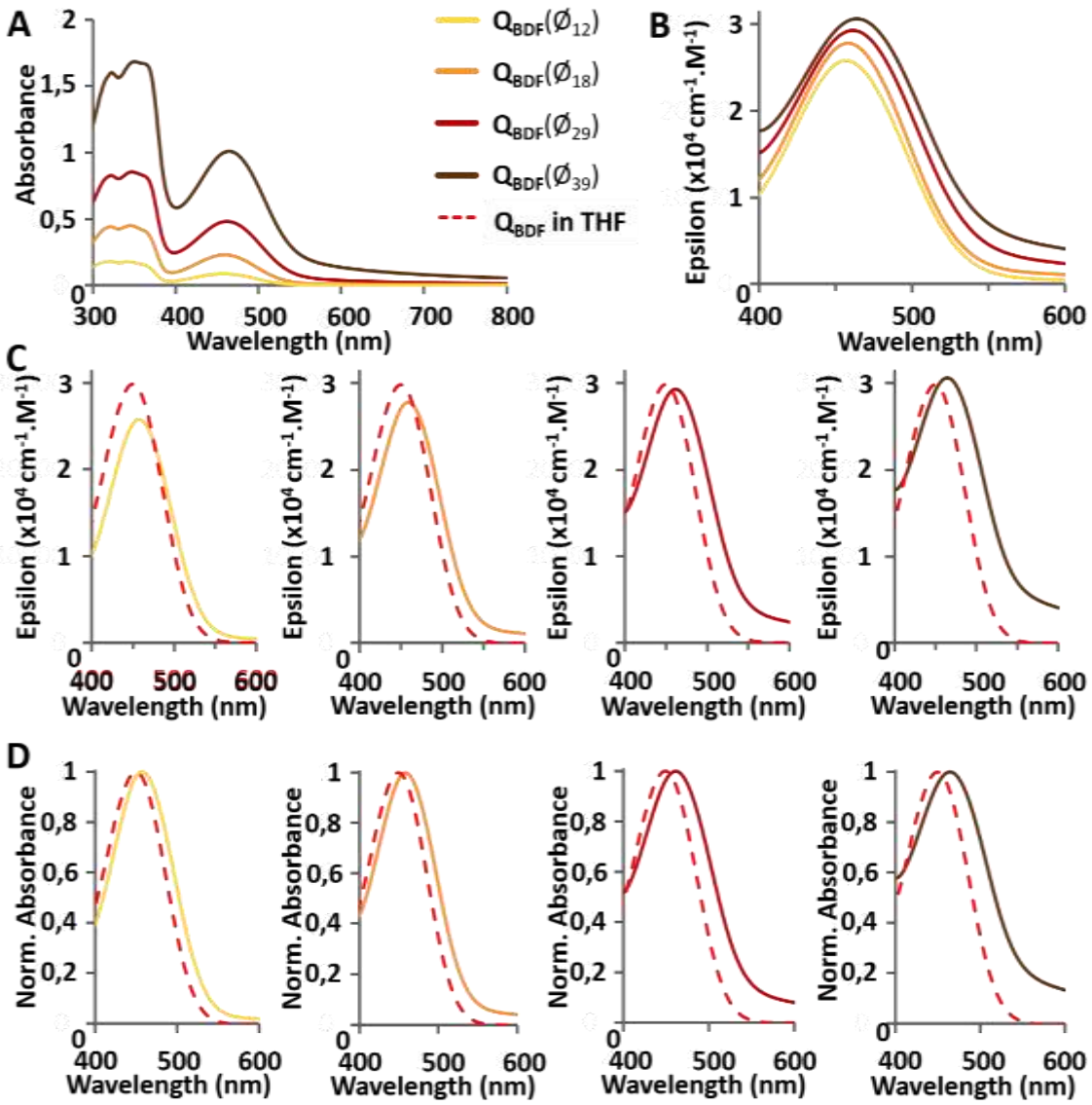
| NPs | [a] in $H_2O$ [nm] | [b] in PBS + FBS [nm] | [c] in MBS + FBS [nm] | [a] in ACSF + FBS [nm] |
|---|---|---|---|---|
| $Q_{BDF}(Ø_{12})$ | 36 | 53 | 51 | 50 |
| $Q_{BDF}(Ø_{18})$ | 66 | 80 | 79 | 79 |

[a-d] Median hydrodynamic diameters of **$Q_{BDF}(Ø_{12})$** and **$Q_{BDF}(Ø_{18})$** determined in water (a), in PBS supplemented with 10% FBS (b), in MBS supplemented with 10% FBS (c) or in ACSF supplemented with 10% FBS (d).

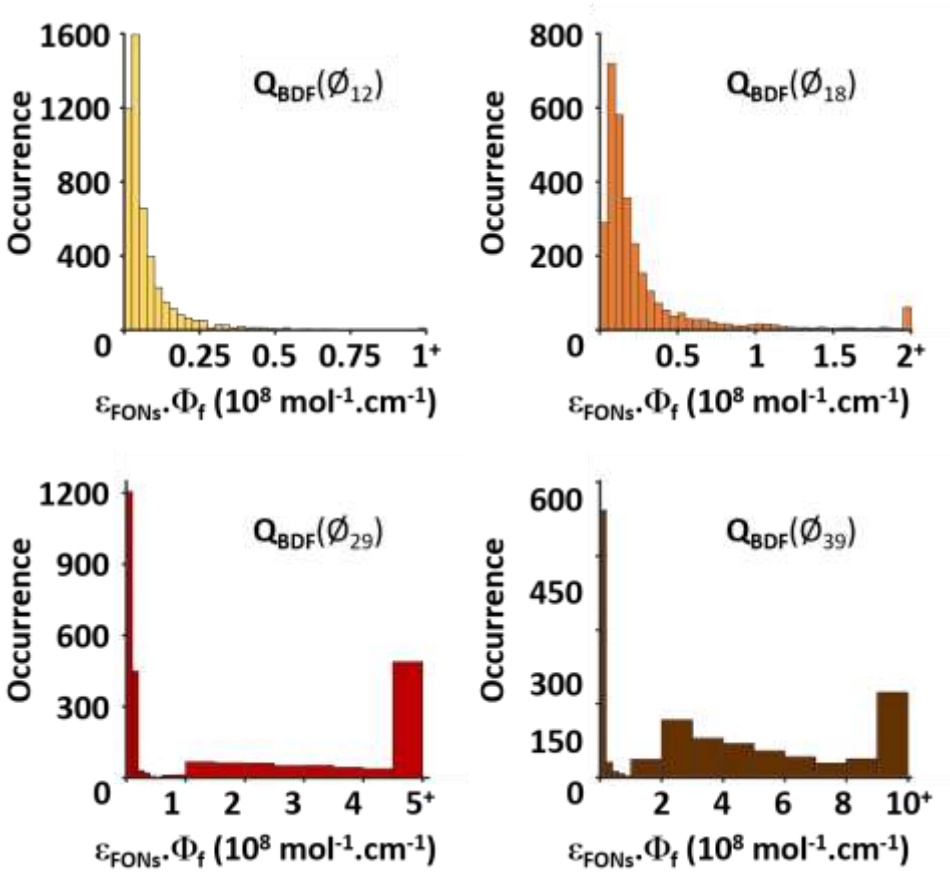
4. Supplementary Figures



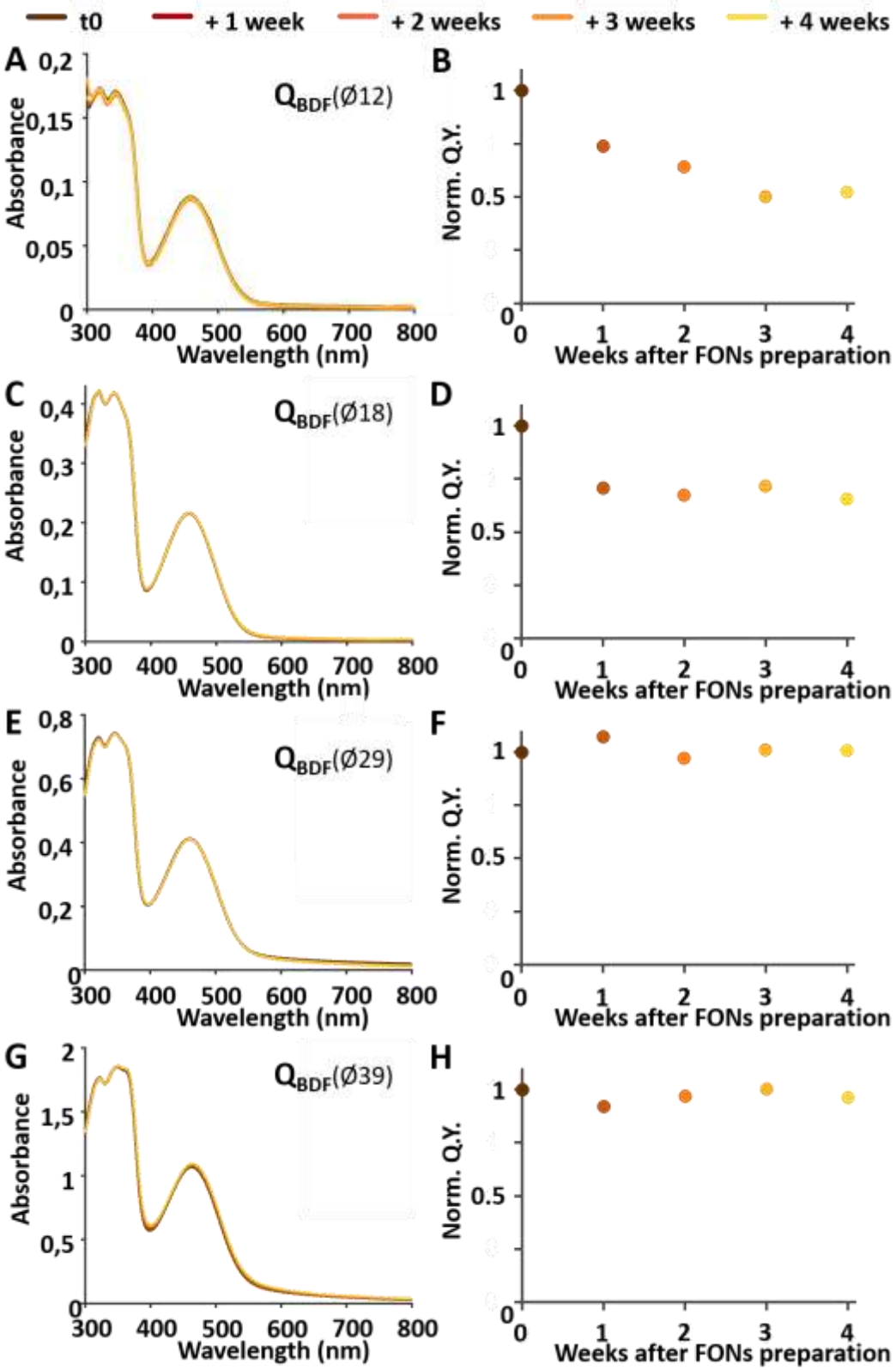

**Supplementary Figure S1**: Representative TEM images and size distributions (insets) of FONs obtained by nanoprecipitation of (Left) 1 mM, (Middle) 2 mM, (Right) 4 mM **$Q_{BDF}$** dye in THF into water (1 % v/v). **A** FONs were prepared and imaged on the same day. Analysis was performed on 20 to 40 fields of view per preparation on populations of 4801, 3004, 2624 and 1236 objects respectively coming from 2 to 3 different batches of FONs. **B** FONs imaged 6 months after preparation. Analysis was performed on 4 to 15 fields of view per preparation on populations of 946, 886, 958 and 465 objects respectively coming from 1 batch of FONs aged at room temperature. All FONs of diameters higher than 100 nm were pooled in a single bin indicated as 100+ on the abscissa of the inset histograms.



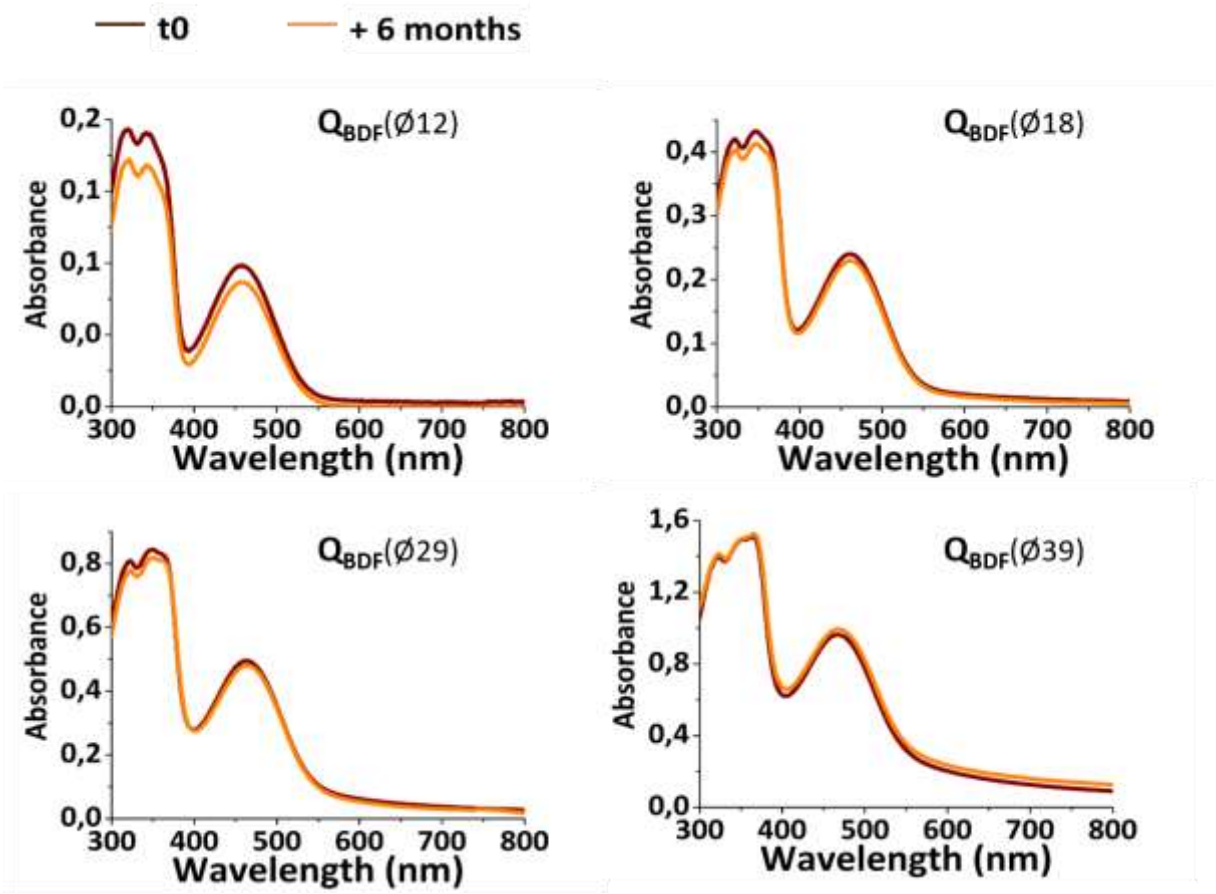

**Supplementary Figure S2. A** Superimposed raw absorbance spectra of **$Q_{BDF}$** FONs of different sizes. **B** Superimposed attenuation coefficients of **$Q_{BDF}$** moieties inside of FONs. Dye concentration inside the FONs was determined as described in the experimental section. **C** Attenuation coefficients of **$Q_{BDF}$** moieties inside of FONs of each size compared to the attenuation coefficient of **$Q_{BDF}$** dye in THF. **D** Normalized absorbance of **$Q_{BDF}$** FONs of each size compared to the normalized absorbance of **$Q_{BDF}$** dye in THF. Color code indicated in panel A is maintained throughout the figure.



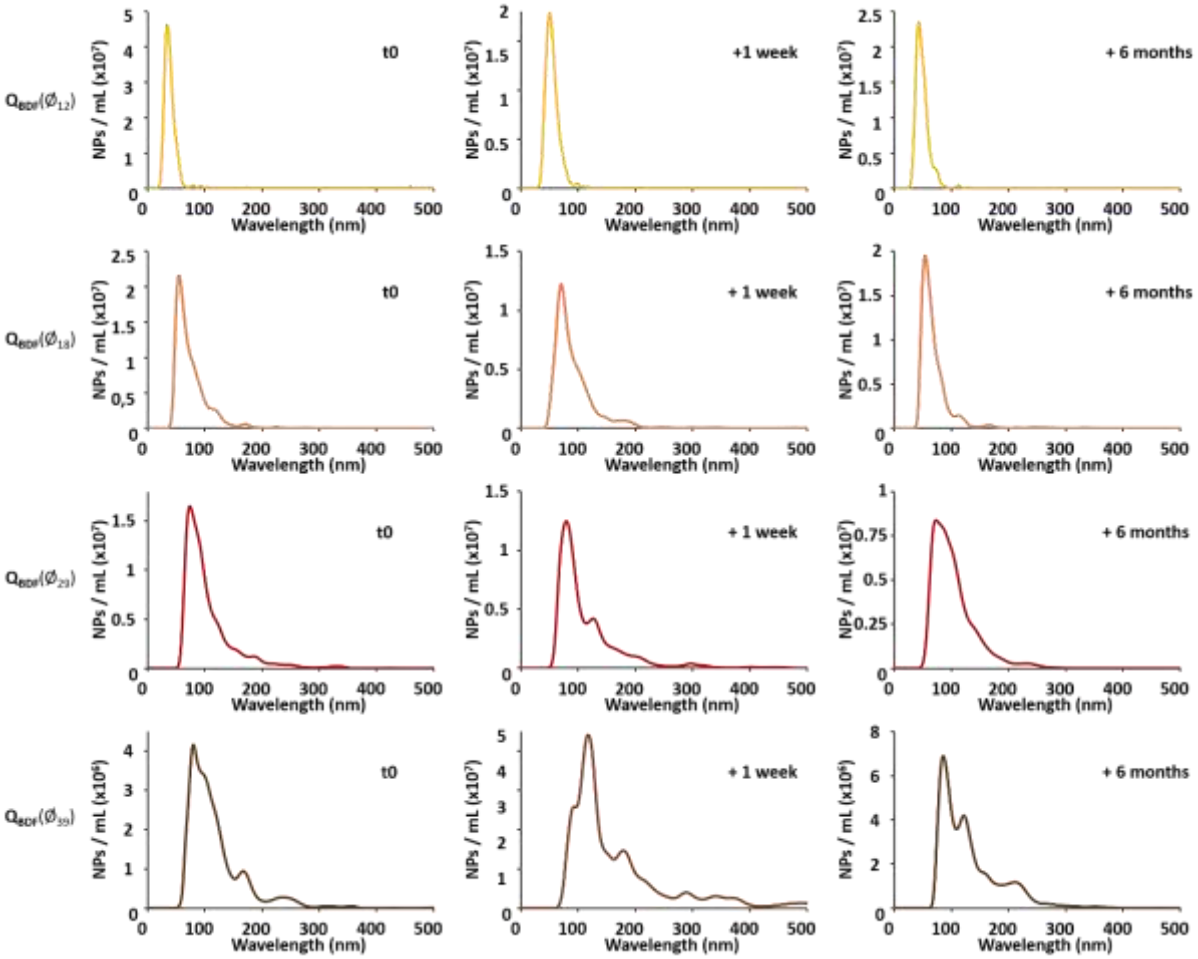

**Supplementary Figure S3.** Distribution of FONs brightness in relation with their size distribution. For comparison, the brightness of the molecular form in low-medium polarity organic solvent is $2.10^4$ $M^{-1}cm^{-1}$.



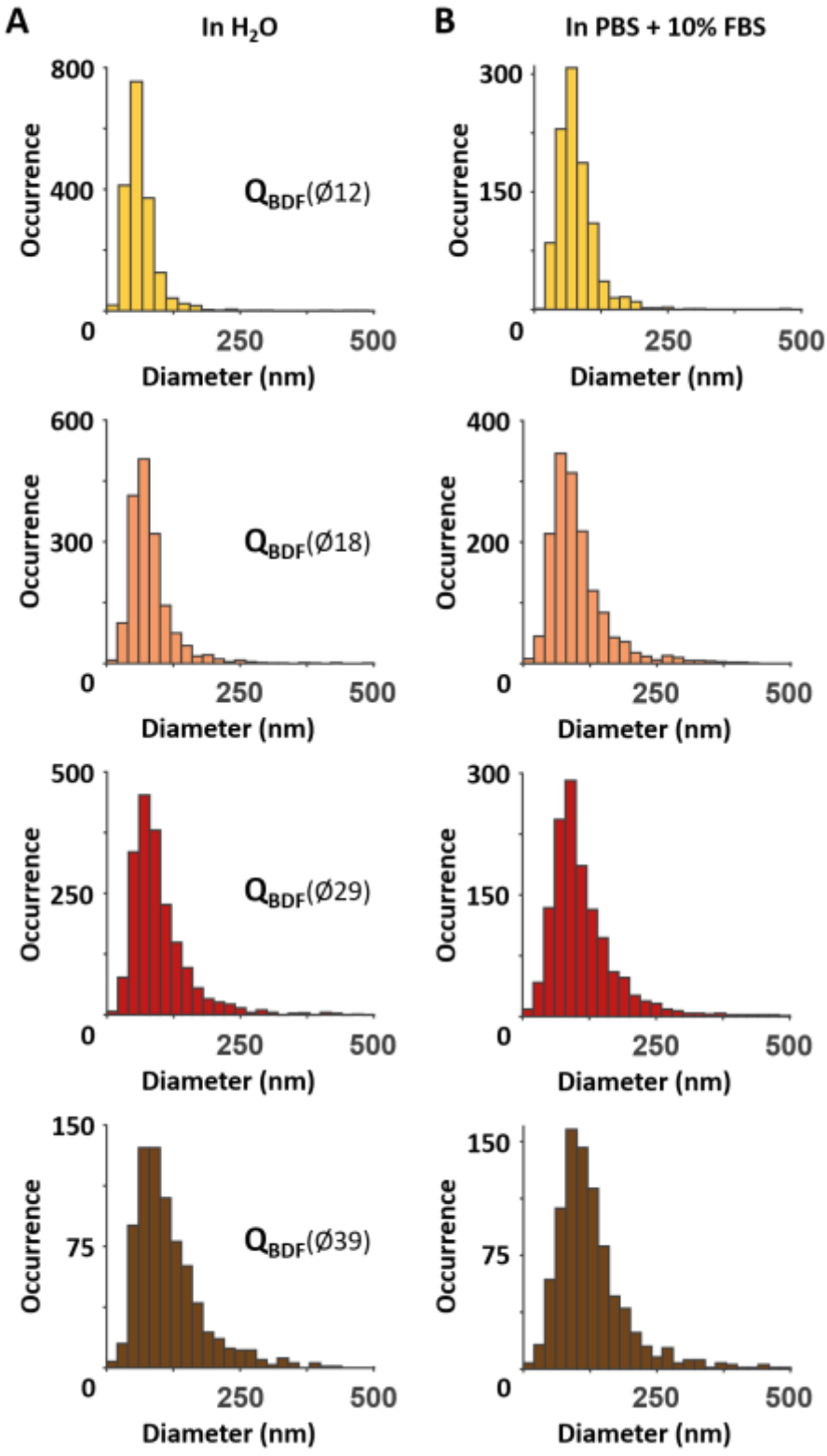

**Supplementary Figure S4. A,C,E,G** Overlapped absorbance spectra of FONs measured once a week for a month after FONs preparation. **B,D,F,H** Normalized quantum yields (average of 2 to 3 preparations) measured once a week for a month after FONs preparation. Color coding is the same for all panels and is indicated at the top of the figure.



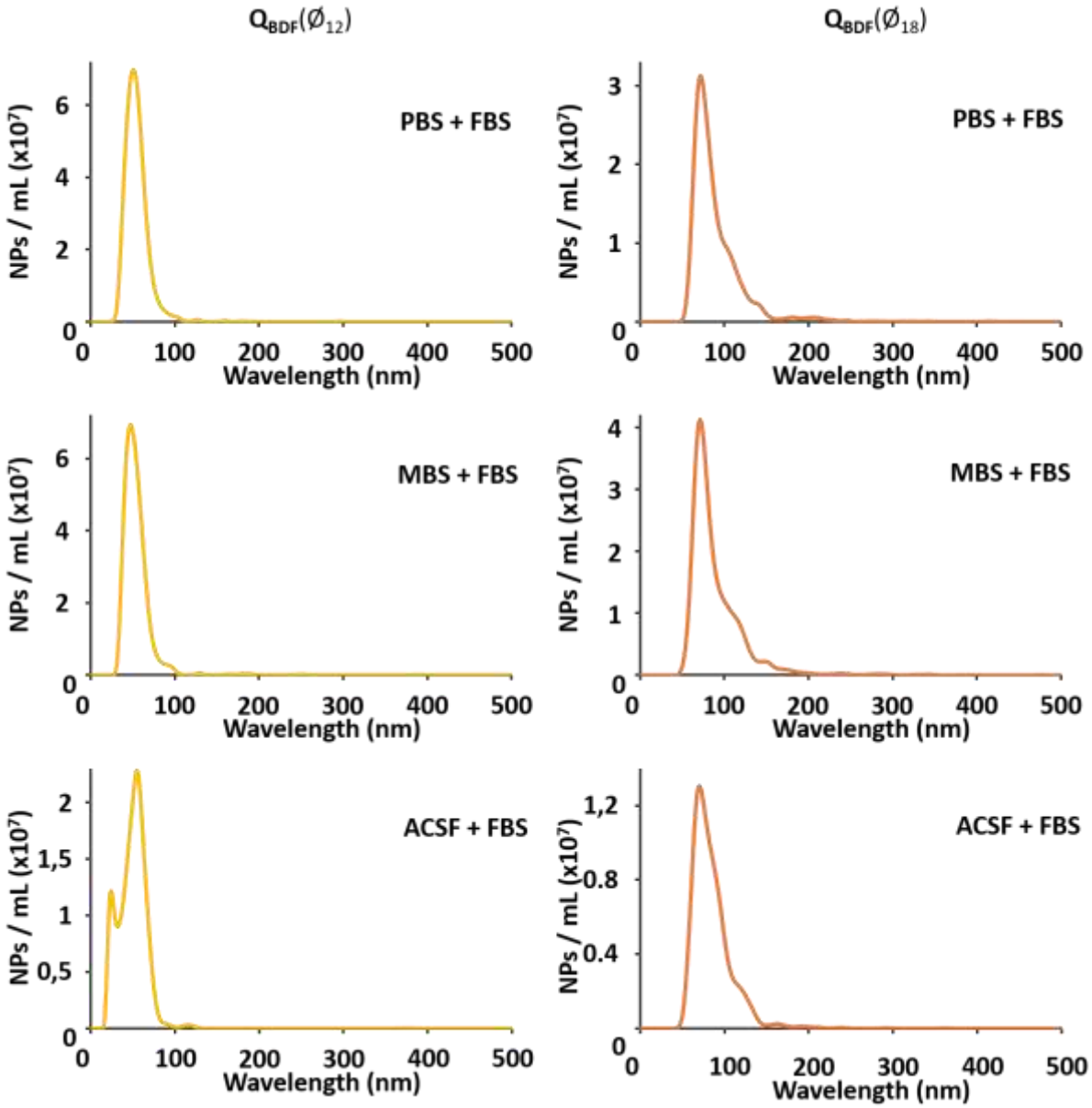

**Supplementary Figure S5.** Overlapped absorbance spectra of FONs measured 6 months after FONs preparation. Color coding is the same for all panels and is indicated at the top of the figure.



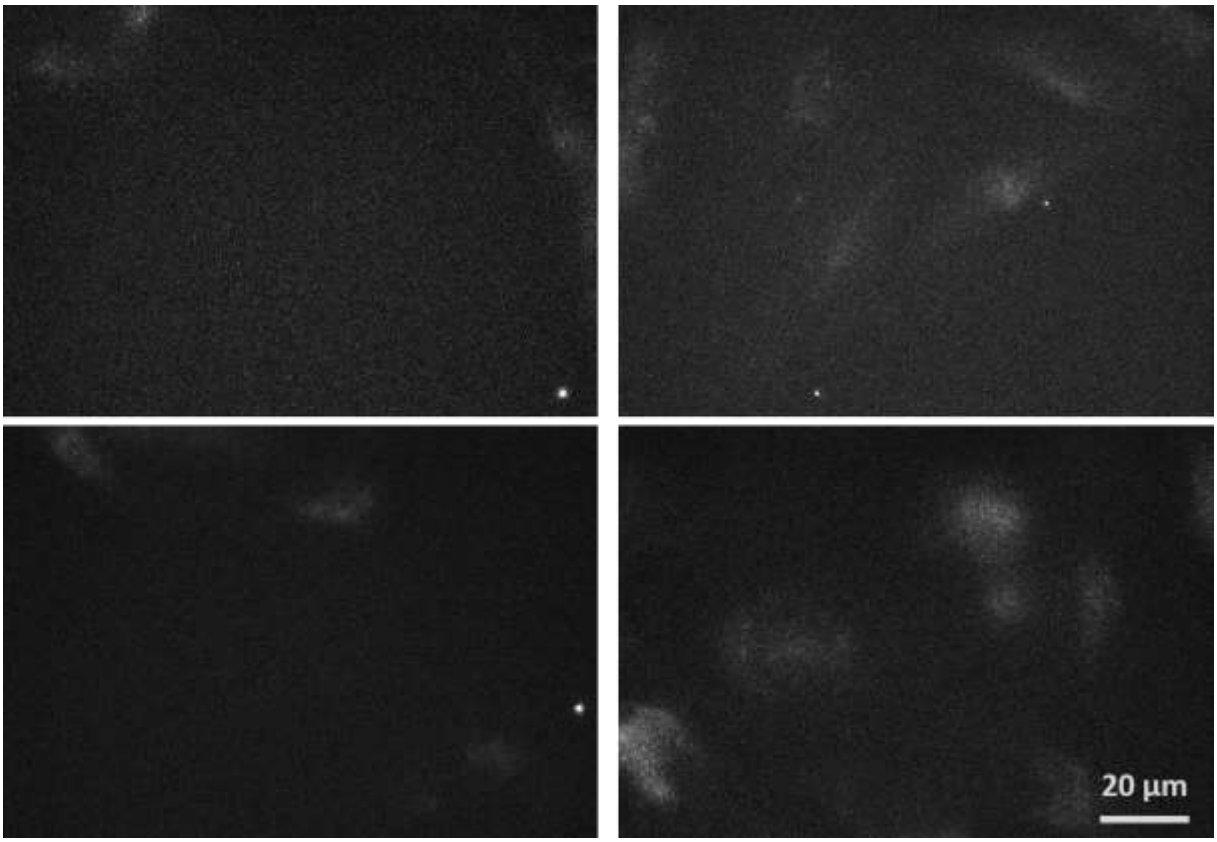

**Supplementary Figure S6**: Distributions of hydrodynamic diameters of **$Q_{BDF}$** FONs in water as measured using NTA. Diameters were measured within 48h of FONs preparation (Left), one week later (Middle) or 6 months later (Right)



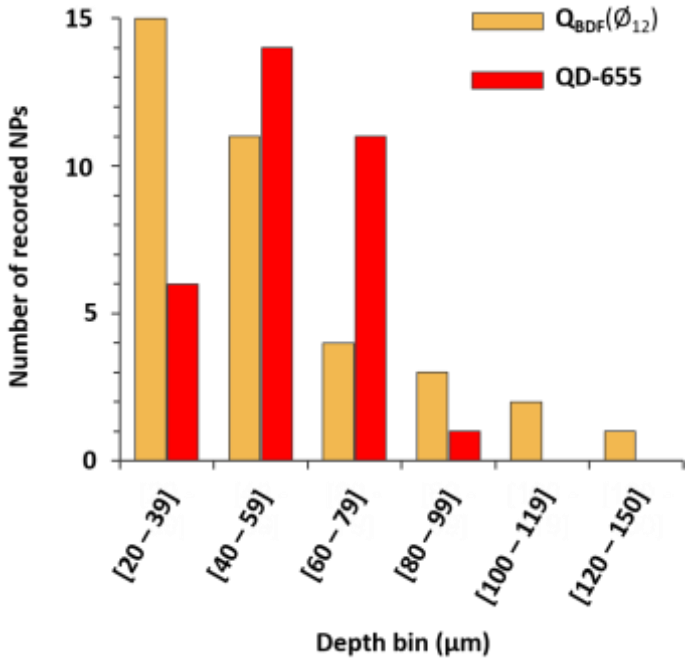

**Supplementary Figure S7.** Size distributions of $\mathbf{Q_{BDF}}$ FONs determined by Single Particle Tracking in **A** mQ grade water and **B** PBS supplemented with 10% FBS. Analysis was performed on 3 to 5 fields of view per preparation.




**Supplementary Figure S8**: Distributions of hydrodynamic diameters of **$Q_{BDF}$** FONs in buffered saline media of various pH and osmolarity measured using NTA. PBS = Phosphate Buffered Saline, pH7.4, ~300 mosm; MBS = MES Buffered Saline, pH 5.5, ~310 mosm; ACSF = Artificial CerebroSpinal Fluid, pH 7.4, ~280 mosm; FBS = Foetal Bovine Serum. Diameters were measured within a week of FONs preparation.




**Supplementary Figure S9.** Gallery of fluorescence images of $\mathbf{Q_{BDF}}(Ø_{18})$ FONs incubated for 24h with HeLa cells. Complementary data to Figure 2C.




**Supplementary Figure S10.** Number of $\mathbf{Q_{BDF}}(Ø_{12})$ (orange) and **QD-655** (red) nanoparticles recorded as singles objects diffusing in organotypic brain tissue per depth interval. Of note, emphasis was given to detecting NPs deep in tissue such that the number of recordings in the first bin ([20-39] µm) does not reflect the number of NPs that could be seen at this depth. Indeed, so close to the surface, a large number of NPs were visible and could have been recorded.

## 5. Supplementary references


[1] Z. H. Li, M. S. Wong, H. Fukutani, Y. Tao, *Chem. Mater.* **2005**, *17*, 5032.

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