

Electronic Supplementary Information for

Ultrasound-induced Transformation of Fluorescent Organic Nanoparticles from a Molecular Rotor into Rhomboidal Nanocrystals with Enhanced Emission

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Abbreviations

4-(diphenylamino)-phthalonitrile = **DPAP**; cryogenic transmission electron microscopy = Cryo-TEM; atomic force microscopy = AFM; dynamic light scattering = DLS; selected-area electron diffraction = SAED.

Materials and Methods

Preparation of DPAP dispersions

DPAP was synthesised following a reported procedure.¹

For the preparation of the **DPAP** dispersions in a 87/13 (v/v) water/methanol mixture, a solution of **DPAP** in methanol (286 µL) was added to deionized water (1914 µL) under vigorous stirring (final volume of the prepared solvents mixture = 2200 µL). A similar procedure, but using different ratio of the solution of **DPAP** in methanol and deionized water, has been used for the preparation of the other mixtures which present different water/methanol ratio. The water/methanol mixtures at different **DPAP** concentration (from 4.5×10^{-6} up to 2.5×10^{-4} M) were obtained using methanol solutions at different

DPAP concentration. The ultrasonicated dispersions were prepared sonicating the as-prepared **DPAP** dispersions for 1 minute (or 10 minutes) using an Elmasonic P ultrasound instrument in pulse mode (frequency 37 kHz, power 100 W) at 20 °C.

Cryo-TEM studies

For Cryo-TEM studies, a drop of the dispersion (87/13 (v/v) methanol/water mixture) was put on a lacey carbon-coated copper TEM grid (200 mesh, Science Services) where most of the liquid was removed with blotting paper, leaving a thin film stretched over the grid holes. The specimens were instantly shock vitrified by rapid immersion into liquid ethane and cooled to approximately 90 K by liquid nitrogen in a temperature-controlled freezing unit (Zeiss Cryobox, Zeiss SMT GmbH). The temperature was monitored and kept constant in the chamber during all the sample preparation steps. After the sample is frozen, it was inserted into a cryo-transfer holder (CT3500, Gatan) and transferred to a Zeiss EM922 Omega EF-TEM. Examinations were carried out at temperatures around 90 K at an acceleration voltage of 200 kV. Zero-loss filtered images ($\Delta E = 0$ eV) were taken under reduced dose conditions (100–1000 electrons/nm²). All images were registered digitally by a bottom-mounted CCD camera system (Ultrascan 1000, Gatan) combined and processed with a digital imaging processing system (Gatan Digital Micrograph 3.9 for GMS 1.82).

Fluorescence microscopy studies

A few μ l of the 87/13 (v/v) methanol/water dispersion was spread between two microscope glass slides. The probe was examined using a Leica TCS SP5 II confocal microscope, exciting at 405 nm, and a LAS AF software, version 2.6.0 build 7266.

AFM studies

AFM images were obtained with a Nanosurf Easyscan 2 instrument in tapping mode using Pointprobe-Plus Silicon SPM Sensors from Nanosensors as cantilevers. The samples were prepared by drop-casting a few μ l of a **DPAP** dispersion ($[\text{DPAP}] = 2.5 \times 10^{-4}$ M) on a silicon surface.

DLS studies

DLS data were collected with a Zetasizer Nano ZS in back scatter mode with a He-Ne laser ($\lambda_{\text{exc}} = 633 \text{ nm}$) using quartz cells with 10 mm optical path length at 25°C. All measurements were preceded by 1 min. equilibration time and for each probe, 5 measurements consisting of 5 runs each were applied. The DLS measurements imply spherical particles, which is true for unsonicated samples, but deviates significantly for sonicated samples, as confirmed by the microscopic techniques.

Photophysical studies

All solvents were spectroscopic grade and were purchased from various commercial suppliers (Sigma-Aldrich, Merck and Roth). UV/vis spectra were recorded with a Perkin Elmer Lambda 2 instrument and steady-state emission with a FluoroMax 3 fluorometer by HORIBA JobinYvon using a quartz cell with 10 mm optical path length. For emission below room temperature, a Haake KT40 thermostat/cryostat was installed. Fluorescence lifetimes were measured with a HORIBA JobinYvon Fluorolog instrument. For excitation, a NanoLED 295 nm and 403 nm light source was used.

Computational studies

Quantum mechanical (QM) calculations of the structural and optical properties of DPAP have been carried out by methods rooted into density functional theory (DFT), here B3LYP,² and its time-dependent extension (TD-DFT), here CAM- B3LYP.³ The modified triple-zeta SNST basis set⁴ has been used in all calculations. Solvent effects have been included by means of the conductor-like version of the Polarizable Continuum Model (C-PCM).⁵ Emission energies have been computed with a state-specific PCM calculation.⁶ Molecular structures corresponding to the first singlet excited state have been relaxed before emission calculations using analytical gradients.⁷ All quantum mechanical calculations have been carried out with the Gaussian09 software package.⁸

Table S1 Emission lifetimes and amplitudes for DPAP (4.5×10^{-5} M) in methanol and in 87/13 (v/v) methanol/water dispersions, $\lambda_{\text{exc}} = 403$ nm.

	τ_1 (ns)	τ_2 (ns)	A1 (%)	A2 (%)
methanol DPAP solution	0.56	-	-	-
unsonicated DPAP dispersion	8.6	1.7	65	35
ultrasonicated DPAP dispersion (1 min.)	12.5	4.4	70	30
frozen ultrasonicated DPAP dispersion (1 min.)	13.2	4.9	75	25

Table S2 Data collection and structure refinement for **DPAP**. CCDC 922344 contains the supplementary crystallographic data for this compound. These data can be obtained free of charge from The Cambridge Crystallographic Data Centre via www.ccdc.cam.ac.uk/data_request/cif.

Single crystals of **DPAP** suitable for X-ray diffraction studies were obtained by slow evaporation of a solution of **DPAP** in dichloromethane.

Empirical formula	C ₂₀ H ₁₃ ClN ₃	
Formula weight	336.79	
Temperature	100(2) K	
Wavelength	1.54178 Å	
Crystal system	Triclinic	
Space group	P-1	
Unit cell dimensions	a = 8.1851(4) Å	$\alpha = 94.129(5)^\circ$.
	b = 8.5344(5) Å	$\beta = 99.175(4)^\circ$.
	c = 13.5521(9) Å	$\gamma = 114.976(4)^\circ$.
Volume	836.84(8) Å ³	
Z	2	
Density (calculated)	1.337 Mg/m ³	
Absorption coefficient	2.058 mm ⁻¹	
F(000)	348	
Crystal size	0.25 x 0.15 x 0.08 mm ³	
Theta range for data collection	3.34 to 67.37°.	
Index ranges	-9 ≤ h ≤ 8, -9 ≤ k ≤ 10, -16 ≤ l ≤ 15	
Reflections collected	9154	

Independent reflections	2854 [R(int) = 0.0392]
Completeness to theta = 67.37°	94.8 %
Absorption correction	Semi-empirical from equivalents
Refinement method	Full-matrix least-squares on F ²
Data / restraints / parameters	2854 / 0 / 278
Goodness-of-fit on F ²	1.062
Final R indices [I>2σ(I)]	R1 = 0.0598, wR2 = 0.1613
R indices (all data)	R1 = 0.0663, wR2 = 0.1681
Largest diff. peak and hole	0.645 and -1.028 e·Å ⁻³

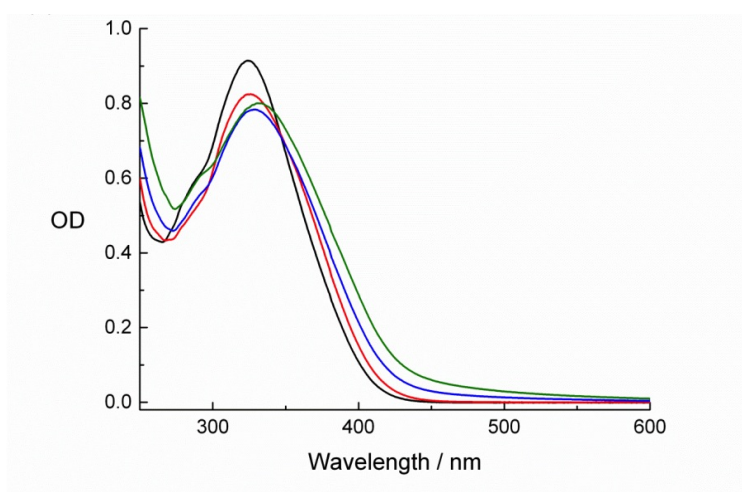


Fig. S1 Absorption spectra of **DPAP** in methanol (black) and in different water/methanol mixtures – 60/40 (v/v) (red), 77/23 (v/v) (blue), and 87/13 (v/v) (green).

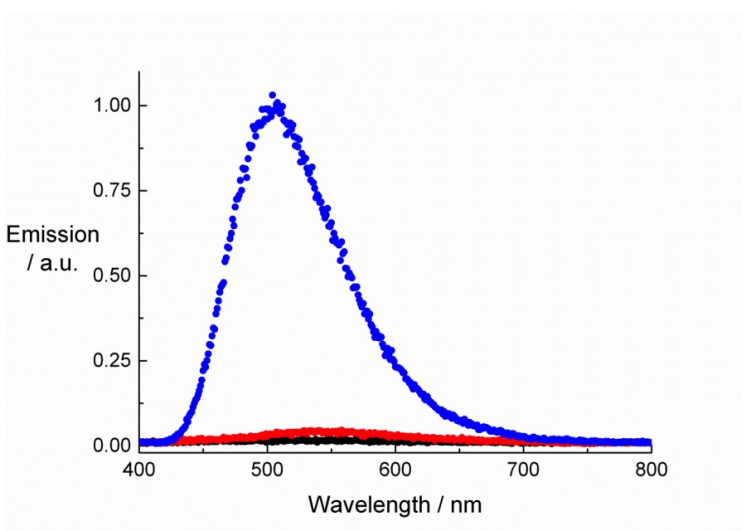


Fig. S2 Fluorescence spectra ($\lambda_{\text{exc}} = 324 \text{ nm}$) of 87/13 (v/v) water/methanol **DPAP** dispersions at different concentrations – $4.5 \times 10^{-6} \text{ M}$ (black), $1.1 \times 10^{-5} \text{ M}$ (red), and $2.0 \times 10^{-5} \text{ M}$ (blue).

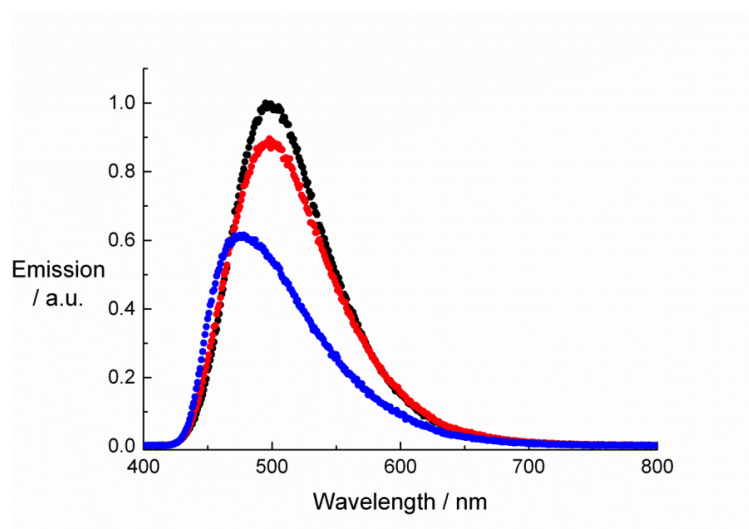


Fig. S3 Fluorescence spectra ($\lambda_{\text{exc}} = 324$ nm) of 87/13 (v/v) water/methanol **DPAP** dispersions at 4.5×10^{-5} M without ultrasonication treatment – as prepared (black), after 1 hour (red), and after 2.5 hours (blue).

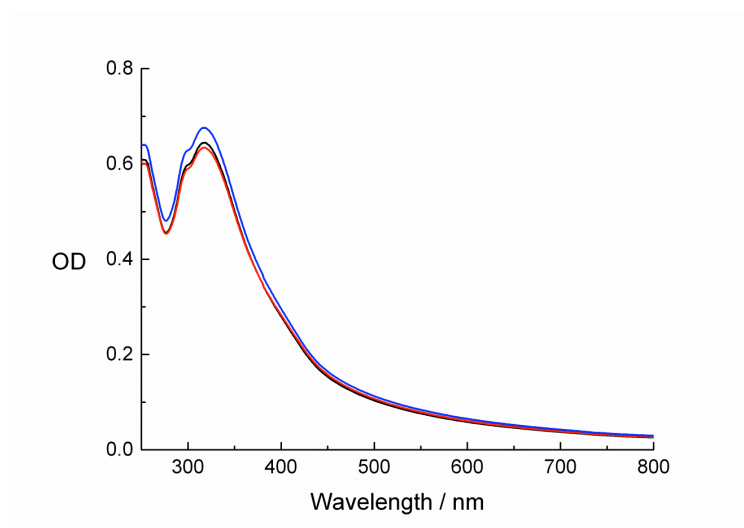


Fig. S4 Absorption spectra of an ultrasonicated 87/13 (v/v) water/methanol **DPAP** dispersion at 4.5×10^{-5} M – as prepared (black), after 2 days (red), and after 5 days (blue).

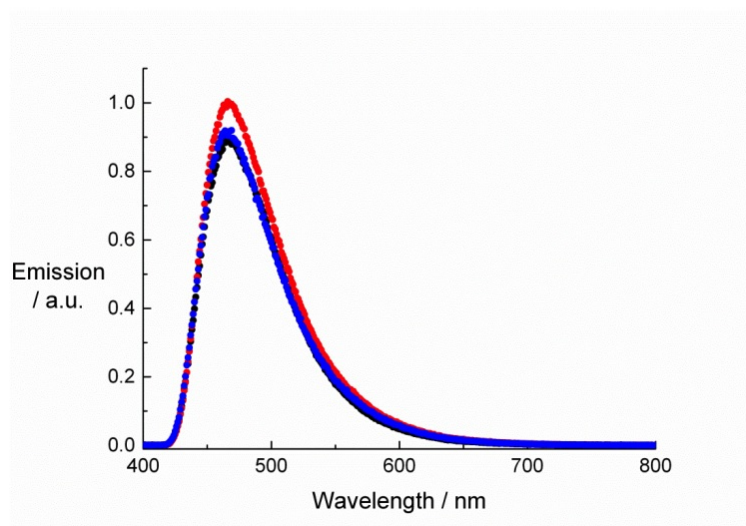


Fig. S5 Fluorescence spectra ($\lambda_{\text{exc}} = 324 \text{ nm}$) of an ultrasonicated 87/13 (v/v) water/methanol **DPAP** dispersion at $4.5 \times 10^{-5} \text{ M}$ – as prepared (black), after 47 days (red), and after 81 days (blue).

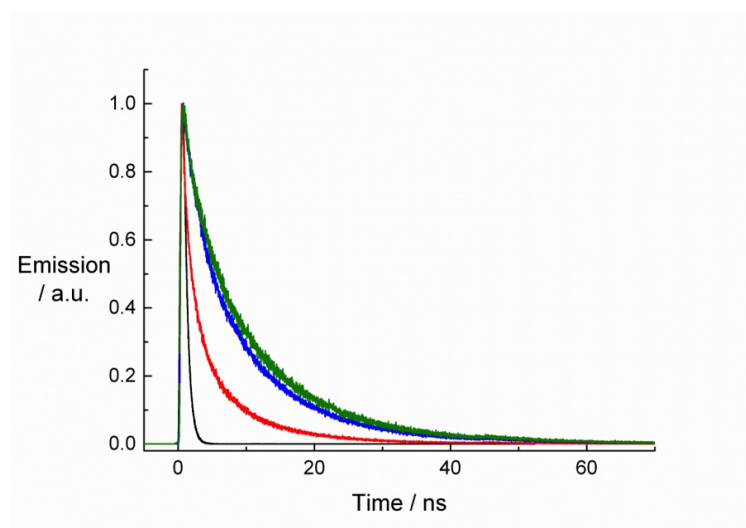


Fig. S6 Time fluorescence profiles of **DPAP** ($4.5 \times 10^{-5} \text{ M}$) in methanol (black) and in an 87/13 (v/v) water/methanol mixture without (red) and with ultrasonication treatment – at room temperature (blue) and at 258 K (green).

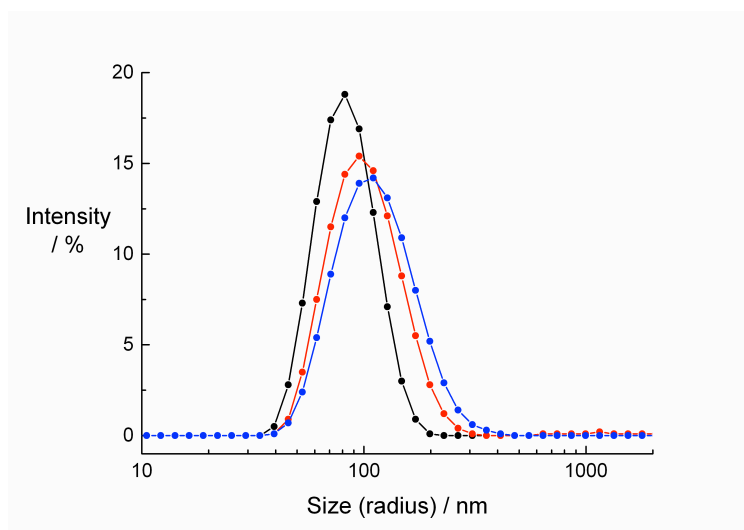


Fig. S7 DLS of 87/13 (v/v) water/methanol **DPAP** dispersions at 4.5×10^{-5} M –without (black) and with (red) ultrasonication treatment. The blue curve refers to the **DPAP** ultrasonicated dispersions at 2.5×10^{-4} M.

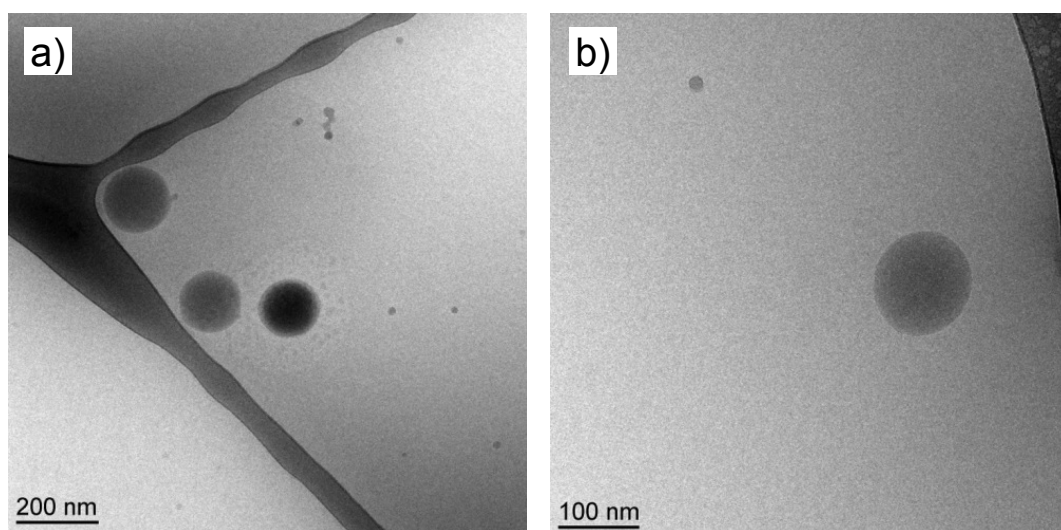


Fig. S8 a,b) Cryo-TEM images of 87/13 (v/v) water/methanol dispersions of **DPAP** at 2.5×10^{-4} M – without ultrasonication treatment.

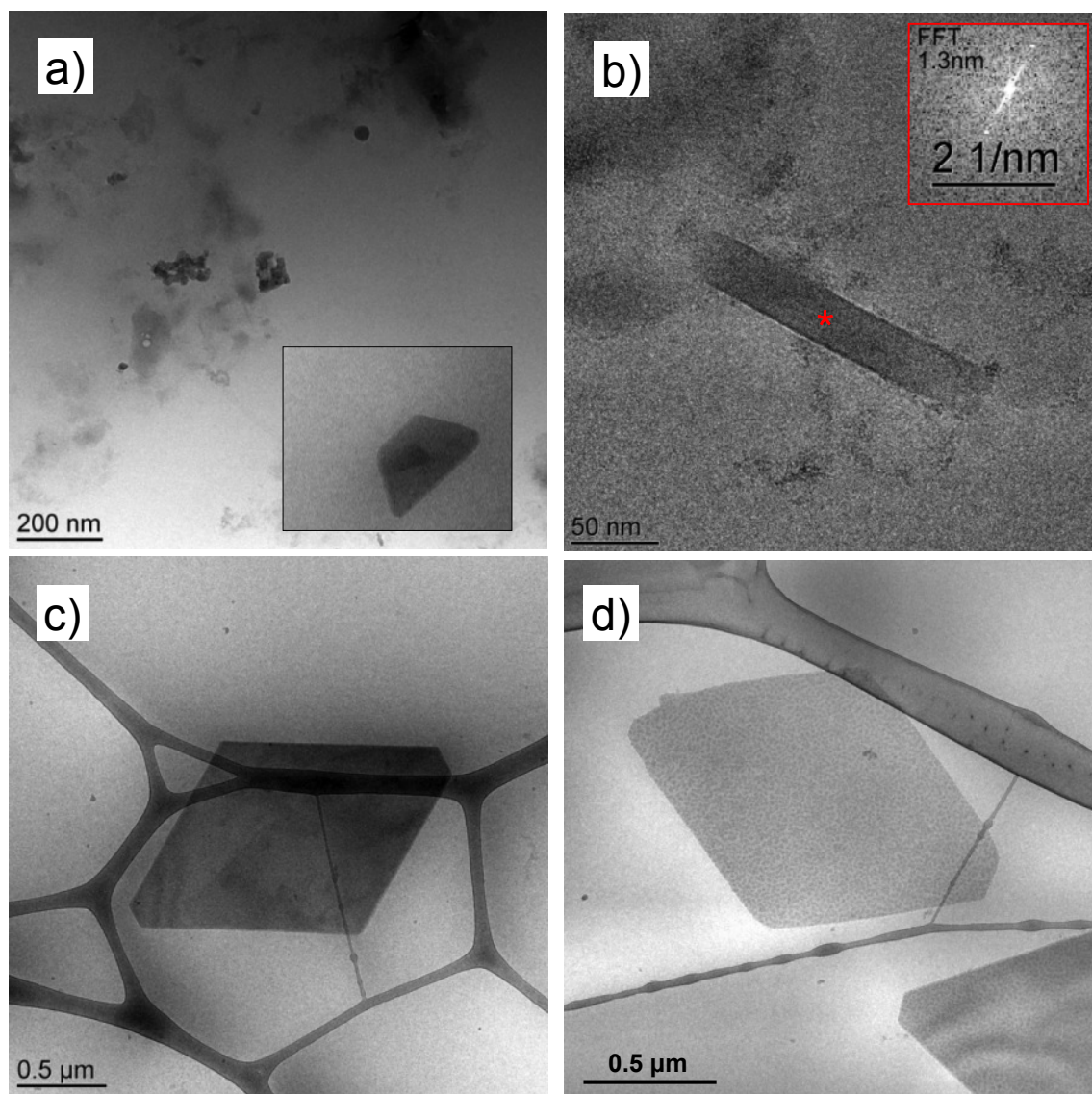


Fig. S9 a,b) Cryo-TEM images of ultrasonicated 87/13 (v/v) water/methanol dispersions of **DPAP** at a) 1.0×10^{-4} M and b) 2.5×10^{-4} M. Inset in a): a **DPAP** nanocrystal observed in another region of the same sample. Inset in b): SAED pattern of the laterally-positioned nanocrystal in b). The five-point, red star in b) indicates the center of the radiation beam in the SAED experiment. c,d) Cryo-TEM images of ultrasonicated 87/13 (v/v) water/methanol dispersions of **DPAP** at 2.5×10^{-4} M after 10 minutes of ultrasonication.

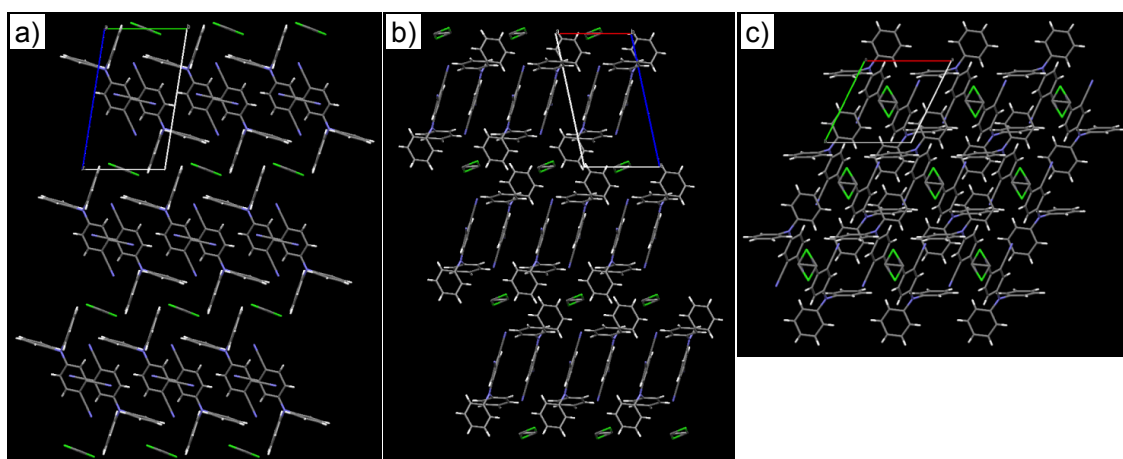


Fig. S10 View of a 3x3x3 unit cell of the **DPAP** crystal structure, co-crystallized with dichloromethane solvent molecules, along the three crystallographic axes *a* (100 plane) a), *b* (010 plane) b) and *c* (001 plane) c). Color code: carbon (grey), nitrogen (violet), hydrogen (white), chlorine (green). The crystallographic axes of the unit cell are colored in red (*a* axis), green (*b* axis) and dark blue (*c* axis).

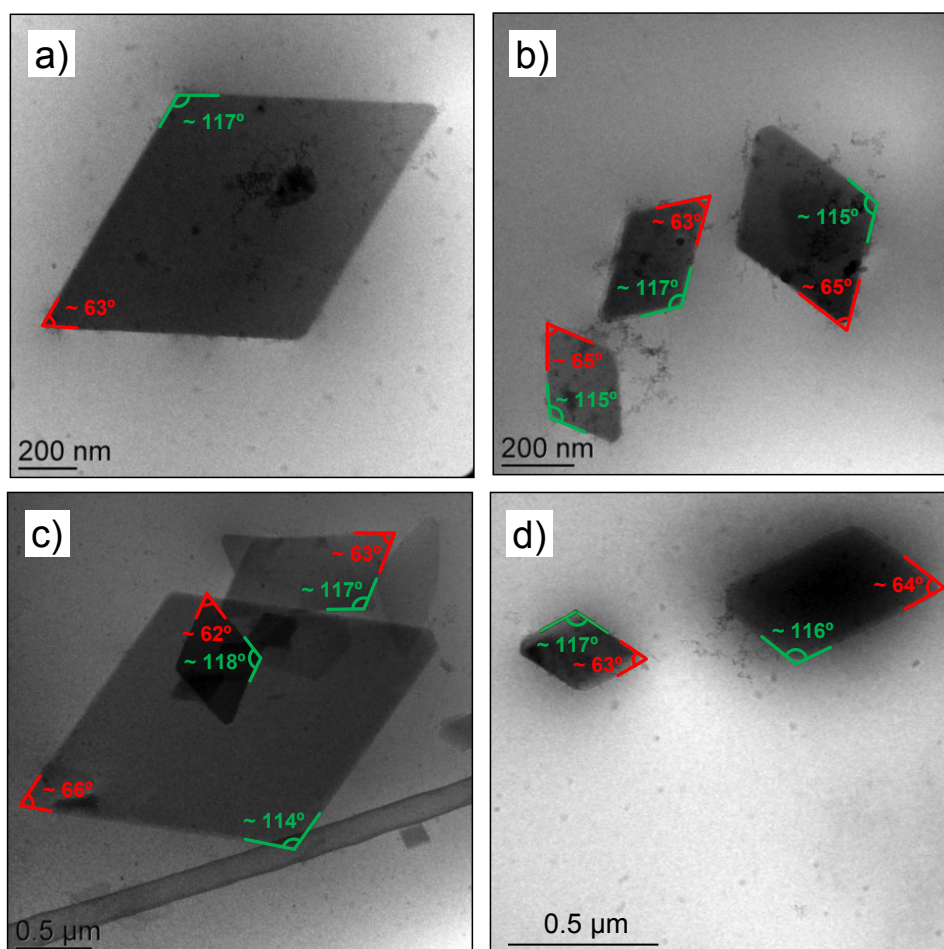


Fig. S11 a-d) Cryo-TEM images of ultrasonicated 87/13 (v/v) water/methanol dispersions of **DPAP** at 2.5×10^{-4} M. The acute (red) and obtuse (green) internal angles of some of the rhomboidal nanocrystals in the images have also been included with their respective angle values (in degree).

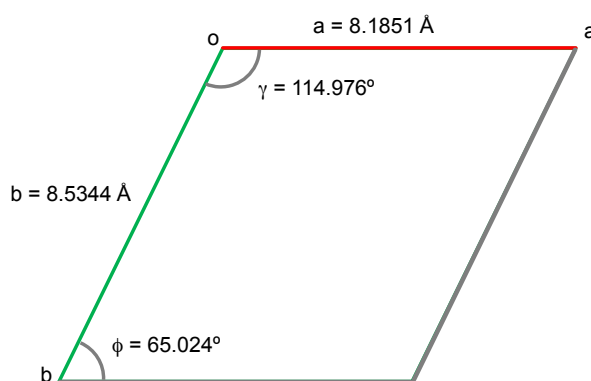


Fig. S12 Schematic representation of the unit cell in the single-crystal structure of **DPAP** along the c crystallographic axis. The length of the a and b axes as well as the internal angles of the resulting rhomboid γ (the angle between the a and b axes) and ϕ ($(360^\circ - 2\gamma)/2$). The a and b crystallographic axes of the unit cell are colored in red and green, respectively. o indicates the origin of the a , b , and c axes.

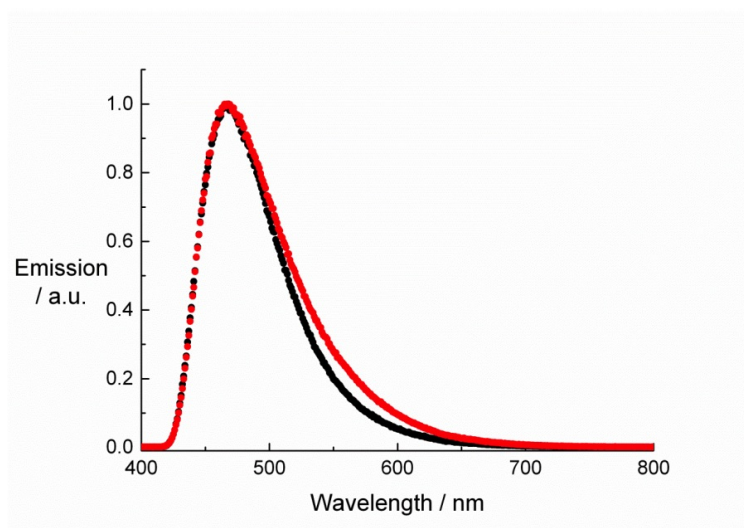


Fig. S13: Fluorescence spectra ($\lambda_{\text{exc}} = 324 \text{ nm}$) of ultrasonicated 87/13 (v/v) water/methanol **DPAP** dispersions at different concentrations – $4.5 \times 10^{-5} \text{ M}$ (black) and $2.5 \times 10^{-4} \text{ M}$ (red). Both spectra are normalized.

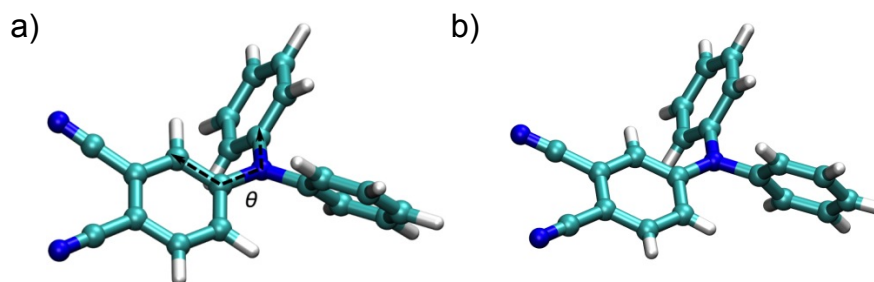


Fig. S14 a) Optimized geometry of **DPAP** in methanol, as issuing from DFT B3LYP calculations including solvent via PCM, and b) X-ray crystal structure of the **DPAP** monomer. The C–C–N–C dihedral angle, θ , is chosen to describe the orientation of the phthalonitrile unit with respect to the amino moiety. In methanol, the dihedral angle is around 25°, which corresponds to the minimum-energy configuration, while in the crystal $\theta=12.4^\circ$.

References

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