

Electronic Supplementary Information

Supramolecular delivery of fluorescent probes in developing embryos

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Experimental procedures

Materials and methods. Chemicals were purchased from commercial sources and used as received. CH₂Cl₂ and MeCN were distilled over CaH₂. THF was distilled over Na and benzophenone. H₂O (18.2 MΩ cm) was purified with a Barnstead International NANOpure Diamond Analytical system. Compounds **1–3**, **5** and **6** were prepared following literature procedures.^{S1–S4} Matrix-assisted laser-desorption ionization (MALDI) mass spectra were recorded with a Bruker BioFlex IV spectrometer. NMR spectra were recorded with a Bruker Avance 400 spectrometer. DLS and SLS measurements were performed with a Malvern Zetasizer Nano-S apparatus. Average supramolecular weights were estimated from the concentration dependence of the SLS intensity, following a literature protocol.^{S5} Absorption spectra were recorded with a Varian Cary 100 Bio spectrometer in quartz cells with a path length of 1.0 cm. Emission spectra were recorded with a Varian Cary Eclipse spectrometer in aerated solutions. Fluorescence quantum yields were determined against either an EtOH solution of cresyl violet ($\phi = 0.54$)^{S6} or a THF solution of **5** ($\phi = 0.92$),^{S1b} following a literature protocol.^{S7} Fluorescence images were recorded with a Leica SP5 confocal laser-scanning microscope.

4. A solution of **6** (106 mg, 0.3 mmol), poly(ethylene glycol) methyl ether ($M_n = 2,000$, 600 mg, 0.3 mmol), and 4-(*N,N*-dimethylamino)pyridine (DMAP, 31 mg, 0.3 mmol) in CH₂Cl₂ (30 mL) was stirred for 10 min at 0 °C. *N,N*-Dicyclohexylcarbodiimide (DCC, 62 mg, 0.3 mmol) was added and the resulting solution was stirred for a further 30 min at 0 °C. The mixture was allowed to warm up to ambient temperature, stirred for 24 hours under these conditions and filtered. The filtrate was concentrated under reduced pressure and the residue was purified by column chromatography [SiO₂, CH₂Cl₂/ MeOH (95:5, v/v)] to give **4** (162 mg, 27%) as a red solid. MALDI: $m/z = 2404$ [M + H]⁺; ¹H NMR (CDCl₃): $\delta = 8.20$ (2H, d, 8 Hz), 7.42 (2H, d, 8 Hz), 4.00–3.51 (176H, m), 3.40 (3H, s), 2.53 (6H, s), 2.32 (4H, q, 8 Hz), 1.28 (6H, s), 1.00 (6H, t, 8 Hz).

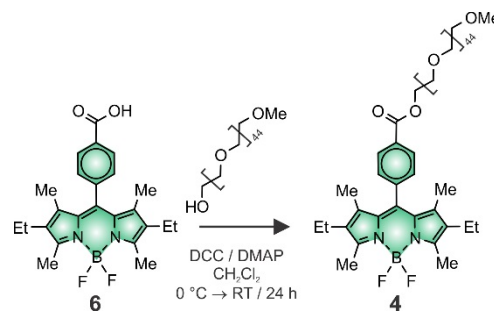


Fig. S1 Synthesis of **4**.

Doped polymer nanoparticles. CH₂Cl₂ solutions of either **1** (25 mg mL⁻¹, 200 μL for imaging; 0.1–1000 μg mL⁻¹, 1000 μL or 2.5 mg mL⁻¹, 200 μL for spectroscopy) or **3** (25 mg mL⁻¹, 200 μL for imaging; 0.1–1000 μg mL⁻¹, 1000 μL or 1.0 mg mL⁻¹, 200 μL for spectroscopy) were combined with CH₂Cl₂ solutions of **2** or **5** (1 mg mL⁻¹ for imaging or 0.1 mg mL⁻¹ for spectroscopy, 50 μL) and heated at 40 °C in an open vial. After the evaporation of the solvent, the residues were purged with air, dispersed in Dulbecco's PBS (pH = 7.2–7.6, 1.0 mL), sonicated for 5 min and flashed through a syringe filter with a pore size of 0.2 μm. The filtrates were used for the imaging and spectroscopic experiments without any further purification. A fraction of the water-insoluble fluorescent guests is lost in the filtration step. As a result, the concentrations of **2** and **5** in the filtrate were estimated from the absorbance at λ_{Ab} (Table S1) and their molar absorption

coefficients measured in THF ($123.6 \text{ mM}^{-1} \text{ cm}^{-1}$ for **2** and $89.8 \text{ mM}^{-1} \text{ cm}^{-1}$ for **5**). The loss of the water-soluble polymers is instead negligible in the filtration step. Thus, the concentrations of **1** and **3** in the filtrate can be estimated from the concentration of the polymer in the initial organic solution and the "supramolecular weight" (1,081 kDa for **1** and 64.5 kDa for **3**) of the nanoparticles determined by static light scattering measurements. The ratio between the concentrations of guest and host is the average number of fluorophores per nanoparticle.

***Drosophila melanogaster* embryos.** *Drosophila melanogaster* embryos expressing membrane-targeted GFP in all cells (Stock #30030, Bloomington *Drosophila* Stock Center NIH P40OD018537) were prepared for the imaging experiments adapting a literature protocol.⁵⁸ Specifically, fertilized egg cells were collected after 60–120 min from laying (stage 2–4), deposited on glass slides and maintained in a desiccator, together with CaSO_4 , at ambient temperature for 8 min. The slides were covered with a mixture (7:1, v/v) of Series 700 (Sigma H8898) and Series 27 (Sigma H8773) halocarbon oils and transferred individually on the stage of a Leica DMIL LED inverted microscope. Solutions of either the doped polymer nanoparticles or **4** (0.1 mM) in Dulbecco's PBS (pH = 7.2–7.6) were injected in the embryos with a MN151 Narishige micromanipulator. The slides were mounted on the stage of a Leica SP5 confocal laser-scanning microscope and imaged.

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- S1 Y. Gabe, Y. Urano, K. Kikuchi, H. Kojima and T. Nagano, *J. Am. Chem. Soc.*, 2004, **126**, 3357–3367.
S2 M. Tomasulo, E. Deniz, R. J. Alvarado and F. M. Raymo, *J. Phys. Chem. C*, 2008, **112**, 8038–8045.
S3 I. G. Moreno, D. Zhang, A. Costela, V. Martín, R. Sastre and Y. Xiao, *J. Appl. Phys.*, 2010, **107**, 073105-1–7.
S4 (a) Y. Zhang, S. Swaminathan, S. Tang, J. Garcia-Amorós, M. Boulina, B. Captain, J. D. Baker and F. M. Raymo, *J. Am. Chem. Soc.*, 2015, **137**, 4709–4719; (b) E. R. Thapaliya, C. Fowley, B. McCaughan, S. Tang, Y. Zhang, J. F. Callan and F. M. Raymo, *Langmuir*, 2015, **31**, 9557–9565
S5 D. Magde, J. H. Brannon, T. L. Cremers, J. Olmsted III, *J. Phys. Chem.*, 1979, **83**, 696–699.
S6 S. R. Sandler, W. Karo, J. Bonesteel, E. M. Pearce, *Polymer Synthesis and Characterization: A Laboratory Manual*, Academic Press, San Diego, 1998.
S7 J. R. Lakowicz, *Principles of Fluorescence Spectroscopy*, Springer, New York, 2006.
S8 A. C. Spradling and G. M. Rubin, *Science*, 1982, **218**, 341–347.

Spectroscopic data

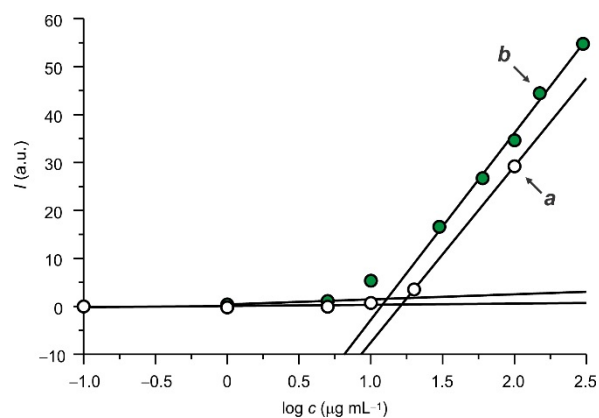


Fig. S2 Dependences of the emission intensity ($\lambda_{\text{EX}} = 580 \text{ nm}$, $\lambda_{\text{EM}} = 640 \text{ nm}$) of **2** on the concentrations of **1** (**a**) and **3** (**b**) in PBS at 25 °C.

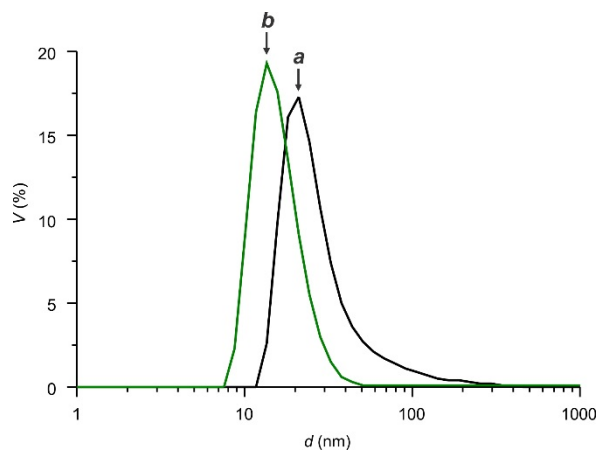


Fig. S3 Statistical distributions of the hydrodynamic diameters of **1** (**a**) and **3** (**b**) (0.5 mg mL^{-1}), determined by DLS in PBS at 25 °C.

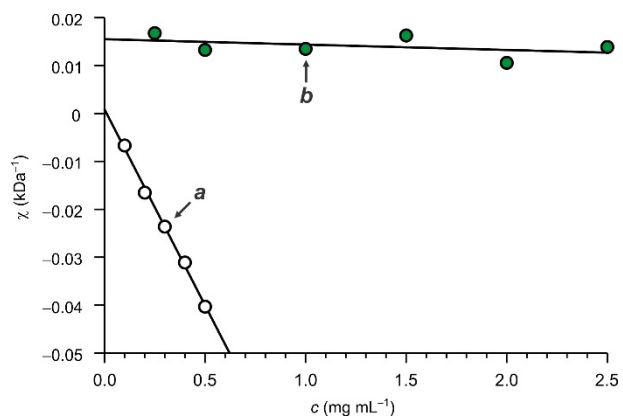
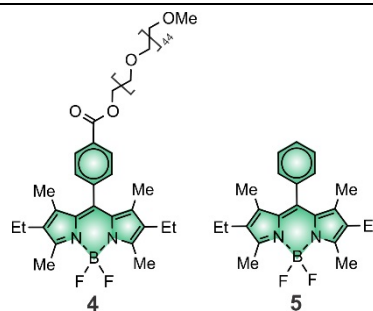


Fig. S4 Debye plots for **1** (**a**) and **3** (**b**), determined by SLS in PBS at 25 °C, where the intercepts on the vertical axis are the inverse of the corresponding supramolecular weights.

Table S1. Wavelengths at the absorption (λ_{Ab}) and emission (λ_{Em}) maxima and fluorescence quantum yield (ϕ) of **2–5** in PBS and THF at 25 °C [a].

	λ_{Ab} (nm)		λ_{Em} (nm)		ϕ [b]	
	PBS	THF	PBS	THF	PBS	THF
2	626	622	637	630	0.35	0.72
3	529	526	547	540	0.14	0.44
4	527	526	538	540	0.49	0.65
5	526	524	540	535	0.60	0.92

[a] The data listed for **2** and **5** in PBS were determined in the presence of **1** at a guest loading of 1.0% w/w, relative to the polymer host. [b] The values of ϕ listed for **2** were determined against an EtOH solution of cresyl violet ($\phi = 0.54$).⁵⁶ Those reported for **3–5** in PBS and for **3** and **4** in THF were determined against a THF solution of **5**. The ϕ listed for **5** in THF is a literature value.^{54b} The spectra of **5** are shown in Fig. S5.



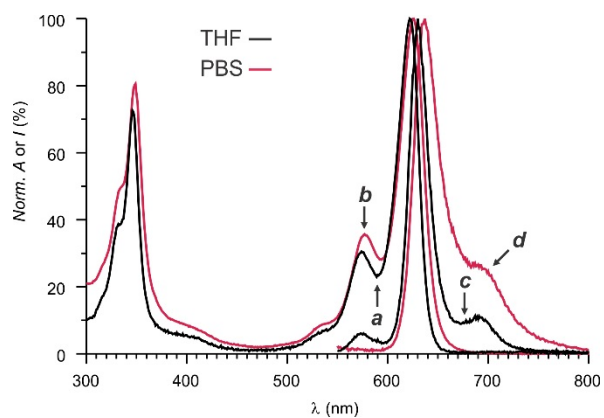


Fig. S5 Normalized absorption (*a* and *b*) and emission (*c* and *d*, $\lambda_{\text{Ex}} = 540$ nm) spectra of **2** in THF (*a* and *c*) and of nanoparticles of **1**, containing **2**, in PBS (*b* and *d*) at 25 °C.

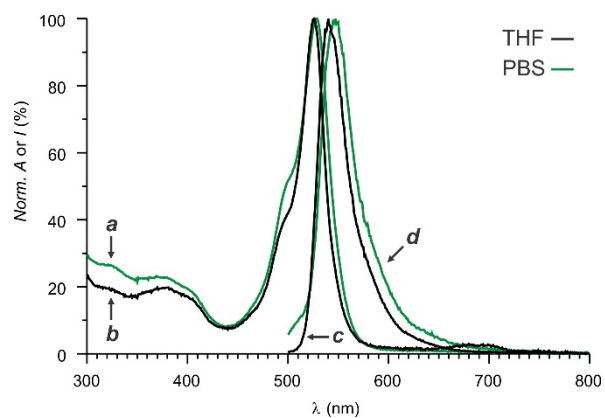


Fig. S6 Normalized absorption (*a* and *b*) and emission (*c* and *d*, $\lambda_{\text{Ex}} = 480$ nm) spectra of **3** in THF (*a* and *c*) and PBS (*b* and *d*) at 25 °C.

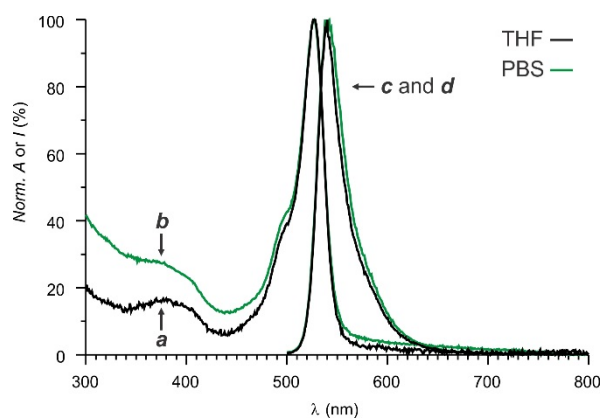


Fig. S7 Normalized absorption (*a* and *b*) and emission (*c* and *d*, $\lambda_{\text{Ex}} = 480$ nm) spectra of **4** in THF (*a* and *c*) and PBS (*b* and *d*) at 25 °C.

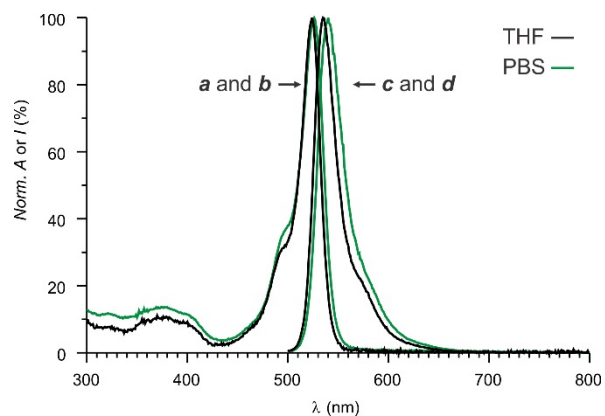


Fig. S8 Normalized absorption (*a* and *b*) and emission (*c* and *d*, $\lambda_{\text{Ex}} = 540$ nm) spectra of **5** in THF (*a* and *c*) and of nanoparticles of **1**, containing **5**, in PBS (*b* and *d*) at 25 °C.

Fluorescence images

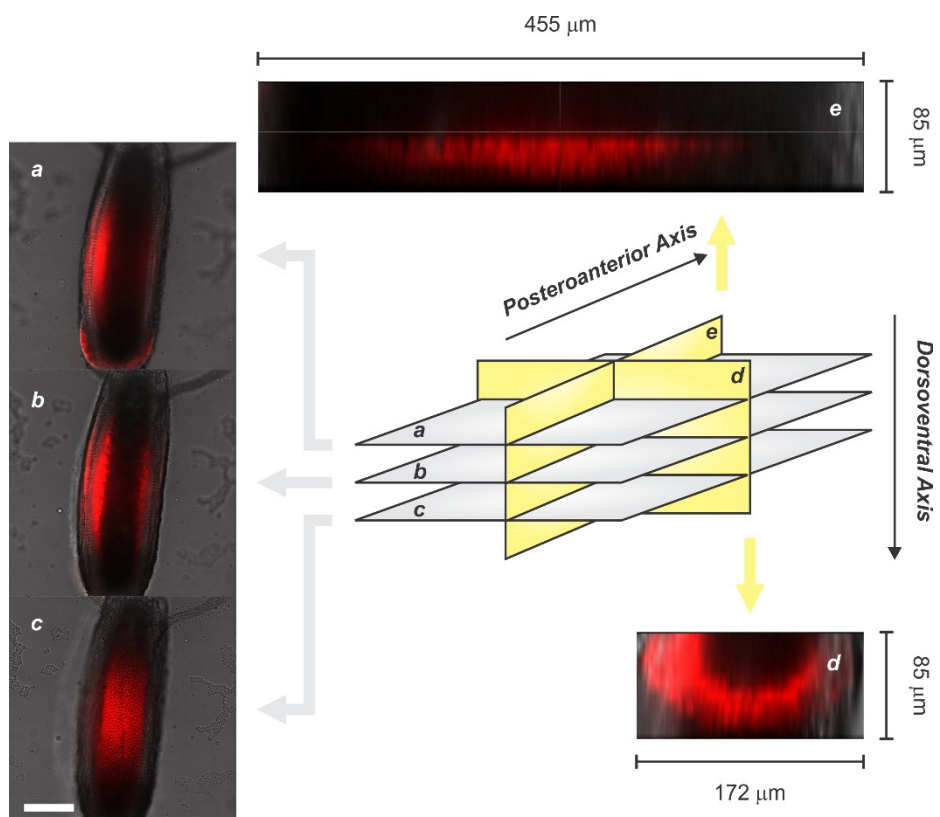


Fig. S9 Overlaid fluorescence and transmittance images (**a–c**, scale bar = 100 μm) of a *Drosophila melanogaster* embryo recorded upon injection of a PBS solution of nanoparticles of **1**, containing **2**, after 120 min from laying (stage 3–4) by scanning the focal plane in the dorsoventral direction (scan step = 30 μm) together with reconstructions of the spatial fluorescence distribution on two vertical planes (**d** and **e**). The fluorescence of **2** was recorded with a λ_{Ex} of 633 nm and a λ_{Em} of 640–750 nm.

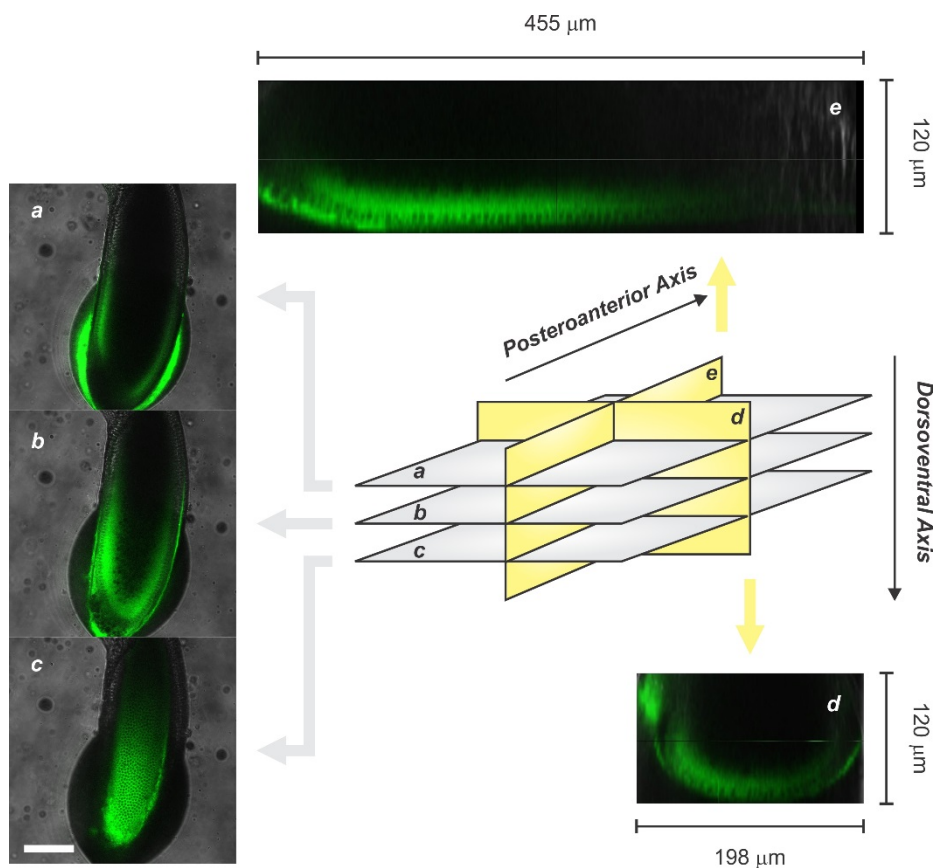


Fig. S10 Overlaid fluorescence and transmittance images (**a–c**, scale bar = 100 μm) of a *Drosophila melanogaster* embryo recorded upon injection of a PBS solution of **4** after 120 min from laying (stage 3–4) by scanning the focal plane in the dorsoventral direction (scan step = 30 μm) together with reconstructions of the spatial fluorescence distribution on two vertical planes (**d** and **e**). The fluorescence of **4** was recorded with a λ_{Ex} of 514 nm and a λ_{Em} of 525–600 nm.

Web Enhanced Object

Video S1 Sequence of overlaid fluorescence and transmittance images ($\lambda_{\text{Ex}} = 633$ nm, $\lambda_{\text{Em}} = 640$ –750 nm, frame time = 5 ms) of a *Drosophila melanogaster* embryo recorded over the course of 35 min upon injection of a PBS solution of nanoparticles of **1**, containing **2**, after 120 min from laying (stage 3–4).