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Supplementary Information

Synthesis in living cells with the assistance of supramolecular nanocarriers

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Experimental Procedures

Materials and methods. Chemicals were purchased from commercial sources and used as received with the exception of MeCN, which was distilled over CaH_2, and H_2O, which was purified with a Barnstead International NANOpure DIamond Analytical system. Compounds **1** and **3** were prepared according to literature procedures.[S1,S2] EISMS was performed with a Bruker micrOTO-Q II spectrometer. NMR spectra were recorded with a Bruker Avance 400 spectrometer. Fourier transform infrared spectra (FTIR) spectra were recorded on neat samples with a Perkin Elmer Frontier spectrometer.

7-Nitro-*N*-(triphenylphosphoranylidene)benzo[c][1,2,5]oxadiazol-4-amine (2). Ph_3P (65 mg 0.4 mmol) was added to a solution of **1** (52 mg, 0.4 mmol) in MeCN (20 mL) and the mixture was stirred for 30 min at ambient temperature. The resulting precipitate was filtered off, washed with hexane (20 mL) and dried to afford **2** (88 mg, 80%) as a red solid. ESIMS: *m*/*z* = 441.1117 $[M + H]^+$ (*m*/*z* calcd. for $C_{24}H_{18}N_4O_3P$ = 441.1133); ^{1}H NMR [$(CD_3)_2SO$]: δ = 5.97 (1H, d, 8 Hz), 7.64–7.69 (6H, m), 7.79–7.74 (3H, m), 7.85–7.90 (6H, m), 8.25 (1H, d, 8 Hz) ppm; ^{13}C NMR ($CDCl_3$): δ = 110.1, 110.3, 123.1, 126.2, 127.2, 128.3, 129.3, 129.5, 132.6, 132.7, 133.3, 133.4, 135.7, 144.7, 144.8, 150.3, 150.5, 153.9 ppm; ^{31}P NMR [$(CD_3)_2SO$]: δ = 15.03 ppm; FTIR: $\bar{\nu}$ = 1610, 1512, 1479, 1437, 1294, 1249, 1098, 995, 916, 802 cm^{-1}.

Crystallographic analysis. Red single crystals suitable for X-ray diffraction analysis were obtained after vapor diffusion of Et_2O into a $CHCl_3$ solution **2**. The data crystal was glued onto the end of a thin glass fiber. X-Ray intensity data were measured with a Bruker SMART APEX2 CCD-based diffractometer, using Mo Kα radiation (λ = 0.71073 Å).[S3] The raw data frames were integrated with the SAINT+ program by using a narrow-frame integration algorithm. Corrections for Lorentz and polarization effects were also applied with SAINT+. An empirical absorption correction based on the multiple measurement of equivalent reflections was applied using the program SADABS. The structure was solved by a combination of direct methods and difference Fourier syntheses and refined by full-matrix least-squares on F^2 with the SHELXTL software package.[S4] Crystal data, data collection parameters and results of the analyses are listed in Table S1. The compound crystallized in the monoclinic crystal system and the space group $P2_1/n$ was chosen based on the systematic absences in the intensity data. All non-hydrogen atoms were refined with anisotropic displacement parameters. Hydrogen atoms were placed in geometrically idealized positions and included as standard riding atoms during the least-squares refinements.

Absorption and emission spectroscopies. CH_2Cl_2 solutions of **3** (2.5 mg mL^{-1}, 400 μL) and either **1** (83 μg mL^{-1}, 100 μL) or Ph_3P (104 μg mL^{-1}, 100 μL) were mixed and the solvent was distilled off under reduced pressure. The residue was dispersed in PBS (1.0 mL) and the resulting dispersions were sonicated for 5 min, stored for 10 min at ambient temperature, passed through a syringe filter (200 nm) and used for the spectroscopic measurements without further purification. 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. The fluorescence quantum yield of **2** was determined against a fluorescein standard, following a literature protocol.[S5]

Fluorescence imaging. *Drosophilla melanogaster* S2 cells were cultured in Shields and Sang M3 Insect Medium (39.4 gL^{-1}) with fetal bovine serum (15%, v/v), penicillin-streptomycin solution (1% v/v at a final concentration of 100 k units L^{-1} penicillin and 100 mg L^{-1} streptomycin) and $KHCO_3$ (0.5 g L^{-1}) and incubated at 22 °C. The cells were seeded in glass-bottom plates at a density of 5×10^4 cells mL^{-1} and incubated for 30 min at 22 °C.

CH_2Cl_2 solutions of **3** (16 mg mL^{-1}, 1.0 mL) and either **1** (2 mg mL^{-1}, 60 μL) or Ph_3P (2 mg mL^{-1}, 80 μL) were mixed and the solvent was distilled off under reduced pressure. The residue was dispersed in PBS (1.0 mL) and the resulting dispersions were sonicated for 5 min and incubated with the cultured cells without further purification.

The cultured cells were incubated with a PBS solution (5%, v/v) of **1** (120 μg mL^{-1}) and **3** (16 mg mL^{-1}) for 30 min washed twice with PBS (100 μL), imaged, incubated further with Ph_3P (160 μg mL^{-1}) and **3** (16 mg mL^{-1}) for

another 30 min, washed twice with PBS (100 μL) and imaged again. The same protocol was repeated inverting the order of the two incubation steps. Alternatively, the cultured cells were incubated with a PBS solution (5%, v/v) of **1** (120 μg mL^{-1}) for 30 min, washed twice with PBS (100 μL), incubated further with a PBS dispersion (5%, v/v) of Ph_3P (160 μg mL^{-1}) and **3** (16 mg mL^{-1}) for another 30 min and imaged. Once again, the same protocol was repeated inverting the order of the two incubation steps. All images were recorded with a Leica SP5 confocal laser-scanning microscope.

S1 S. J. Lord, H. D. Lee, R. Samuel, R. Weber, N. Liu, N. R. Conley, M. A. Thompson, R. J. Twieg, W. E. Moerner, *J. Phys. Chem. B*, 2010, **114**, 14157.

S2 I. Yildiz, S. Impellizzeri, E. Deniz, B. McCaughan, J. F. Callan, F. M. Raymo, *J. Am. Chem. Soc.*, 2011, **133**, 871.

S3 Apex2 Version 2.2-0 and SAINT+ Version 7.46A; Bruker Analytical X-Ray System, Inc., Madison, Wisconsin, USA, 2007.

S4 (a) G. M. Sheldrick, SHELXTL Version 6.1; Bruker Analytical X-Ray Systems, Inc., Madison, Wisconsin, USA, 2000. (b) G. M. Sheldrick, *Acta Cryst*., 2008, **A64**, 112.

S5 J. R. Lakowicz, *Principles of Fluorescence Spectroscopy*; Springer: New York, 2006.

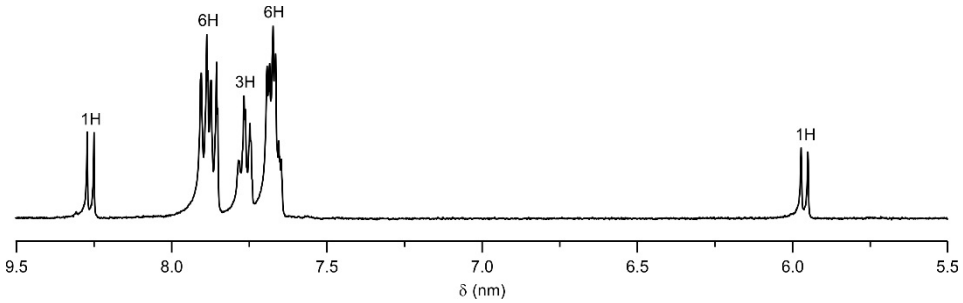

Fig. S1. ^{1}H NMR spectrum (400 MHz) of **2** in $(CD_3)_2SO$ at 25 °C.

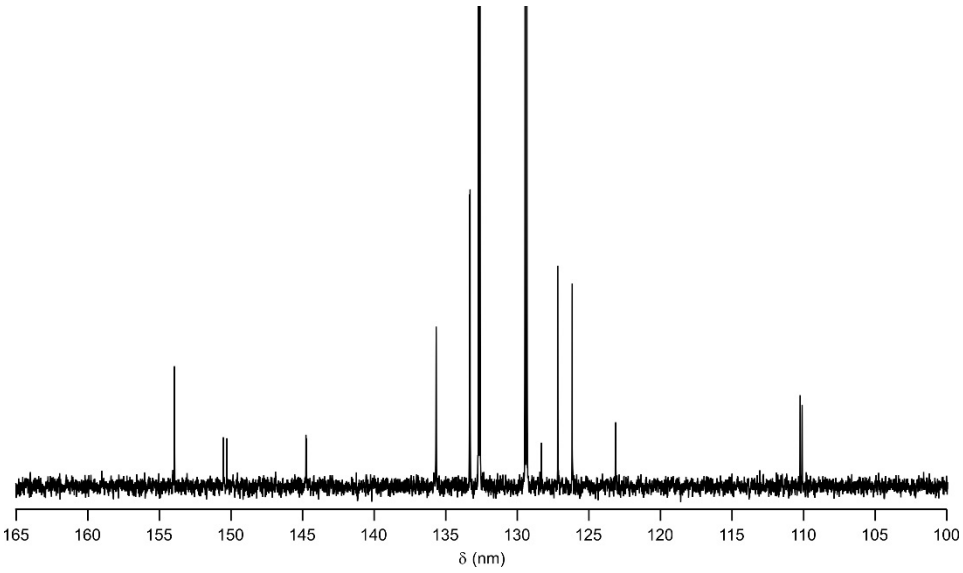

Fig. S2. ^{13}C NMR spectrum (400 MHz) of **2** in $CDCl_3$ at 25 °C.

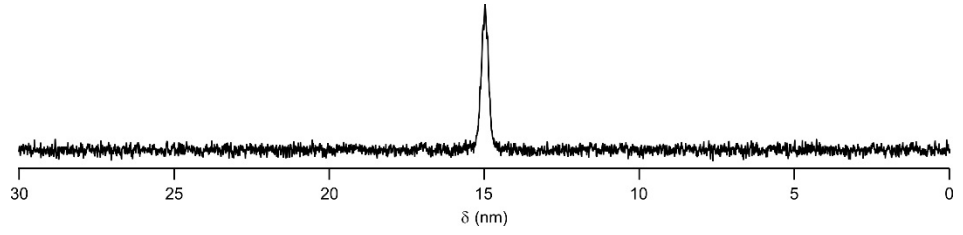

Fig. S3. ^{31}P NMR spectrum (400 MHz) of **2** in $(CD_3)_2SO$ at 25 °C.

Table S1. Crystallographic Data for **2**.

Empirical Formula	$C_{24}H_{17}N_4O_3P$
Formula Weight	440.39
Crystal System	Monoclinic
Lattice Parameters:	
a (Å)	11.9130(5)
b (Å)	8.4877(3)
c (Å)	21.1543(9)
β (°)	104.391(1)
V (Å^3)	2071.88(14)
Space Group	$P2_1/n$ (# 14)
Z Value	4
ρ_{calc} (g cm^{-3})	1.412
μ (Mo Kα) (mm^{-1})	0.168
T (K)	296
$2\Theta_{max}$ (°)	60.00
No. Obs. ($I > 2\sigma(I)$)	4975
No. Parameters	289
Goodness of Fit	1.035
Max. Shift in Cycle	0.001
*Residuals**: R1; wR2	0.0391; 0.1078
Absorption Correction, Max/min	Multi-Scan 0.9834/0.9388
Largest Peak in Final Diff. Map (e$^-$ Å^{-3})	0.300

* $R = \Sigma_{hkl}(\,|\,|F_{obs}|-|F_{calc}|\,|)/\Sigma_{hkl}|F_{obs}|$; $R_w = [\Sigma_{hkl}w(|F_{obs}|-|F_{calc}|)^2/\Sigma_{hkl}wF_{obs}^2]^{1/2}$, $w = 1/\sigma^2(F_{obs})$; $GOF = [\Sigma_{hkl}w(|F_{obs}|-|F_{calc}|)^2/(n_{data}-n_{vari})]^{1/2}$.

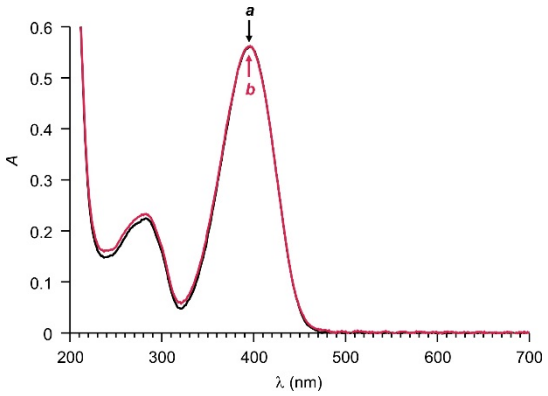

Fig. S4. Absorption spectra of a MeCN solution of **1** (40 μM) recorded before (***a***) and after (***b***) storage in the dark for 24 hours at 25 °C.

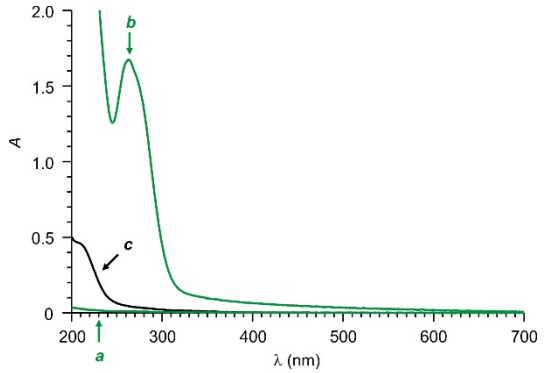

Fig. S5. Absorption spectra recorded at 25 °C after treating PPh_3 (10 μg) with PBS (1.0 mL) in the absence (***a***) or presence (***b***) of **3** (1.0 mg mL^{-1}). Absorption spectrum (***c***) of **3** (1.0 mg mL^{-1}) in PBS at 25 °C.

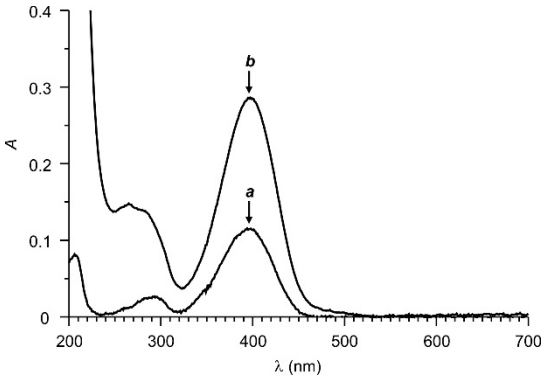

Fig. S6. Absorption spectra recorded at 25 °C after treating **1** (8 μg) with PBS (1.0 mL) in the absence (***a***) or presence (***b***) of **3** (1.0 mg mL^{-1}).

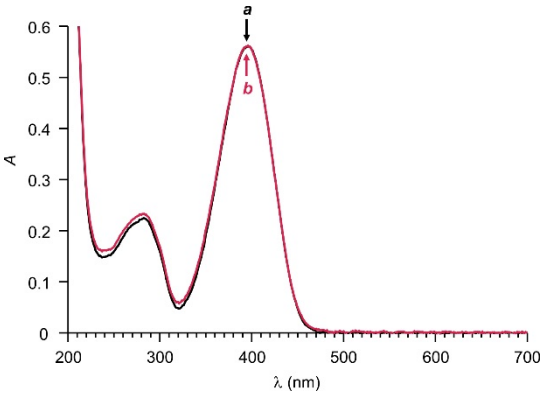

Fig. S7. Absorption spectra of a PBS solution of **1** (40 μg mL^{-1}) and **3** (1.0 mg mL^{-1}) recorded before (***a***) and after (***b***) storage in the dark for 3 hours at 25 °C.

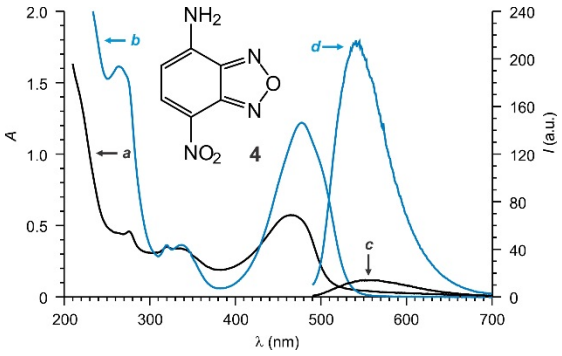

Fig. S8. Absorption and emission (λ_{Ex} = 480 nm) spectra of PBS solutions (25 °C) of **3** (1.0 mg mL^{-1}) and either **2** (8 μg mL^{-1}, ***a*** and ***c***) or **4** (8 μg mL^{-1}, ***b*** and ***d***).

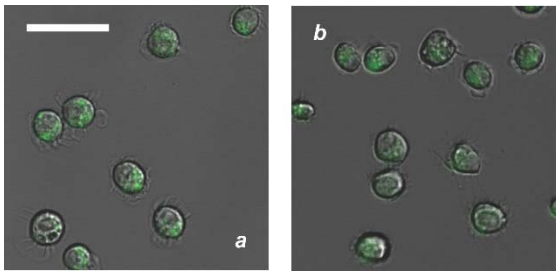

Fig. S9. Overlaid fluorescence and transmittance images (λ_{Ex} = 488 nm, λ_{Em} = 510–700 nm, scale bar = 25 μm) of S2 cells recorded after incubation with either a PBS solution (5%, v/v) of **1** (120 μg mL^{-1}) for 30 min, washing, further incubation with a PBS solution (5%, v/v) of Ph_3P (160 μg mL^{-1}) and **3** (16 mg mL^{-1}) for 30 min and washing (***a***) or a PBS solution (5%, v/v) of **3** (16 mg mL^{-1}) and Ph_3P (160 μg mL^{-1}) for 30 min, washing, further incubation with a PBS solution (5%, v/v) of **1** (120 μg mL^{-1}) for 30 min and washing (***b***).