# Electronic Supporting Information

Photo-tunable multicolour fluorescence imaging based on self-assembled fluorogenic nanoparticles.

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1. Solvents and reagents.

Perylene-3,4,9,10-tetracarboxylic dianhydride (PTCDA, mw=392.32 gmol-1) was purchased by Sigma Aldrich. The polyethertriamine Jeffamine T-403 (**J**, scheme SI_1) was a gift by Hunstmann Corporation.
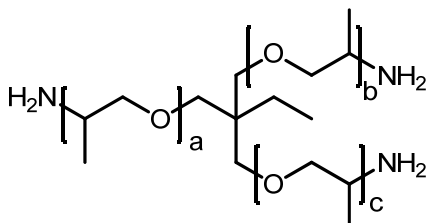
**Scheme SI_1.** Chemical formula of Jeffamine T-403 (**J**).

2. Characterization of Jeffamine T-403 (J).

The polyethertriamine **J** mass distribution was investigated by ESI-mass (1mg/1ml in MeOH). The resulting mass spectrum is shown in figure SI_01; it presents three groups of peaks in different colors corresponding to the mono-, di-, and tri-protonated **J** indicated as JH^+ (red) , JH_2^{2+} (green) and JH_3^{3+} (cyan) respectively. The different constitutional isomers are labeled by n, being n=a+b+c. The ESI-mass spectrum clearly show that isomers with n=5 and =6 are

the predominant species, being the sum of their molar fraction about 0.60. The sum of the molar fraction of the two isomer n=4 and n=7 is about 0.30. Lastly the sum of the molar fraction of the isomers n=3 and n=8 is about 0.05 and the molar fraction of isomers with n<3 or n>8 is less than 0.05. The molar mass calculated from the mass distribution data was 460 g/mol with a polydispersity index PdI=1.02.
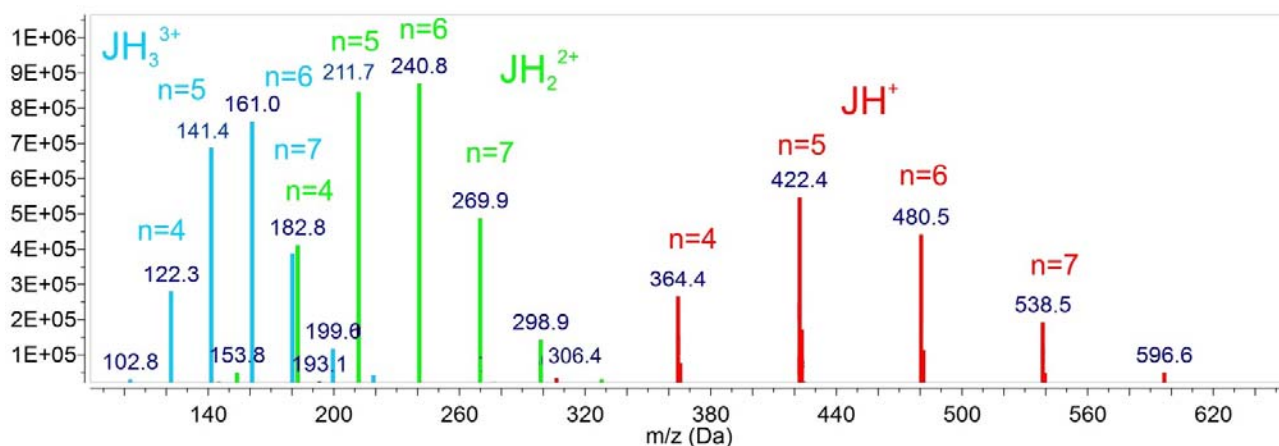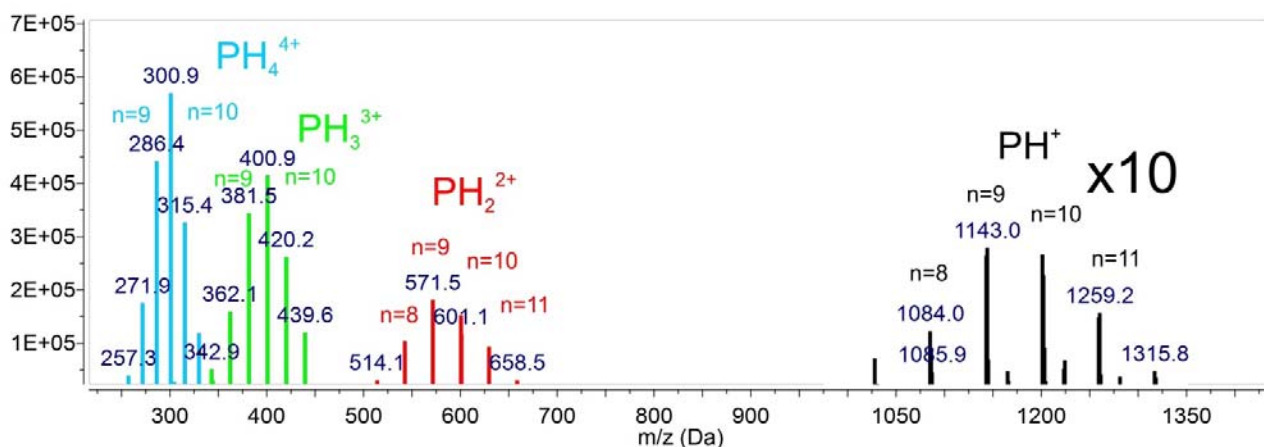

FIGURE SI_1. ESI mass spectrum of **J**.

^{1}H NMR (CD$_3$OD, 400 MHz): δ (ppm) 3.60 (m, 3H, CH$_2$C**H**(CH$_3$)O; 3.10-3.50 (m, 18H, OC**H$_2$**); 3.06-2.92 (m, 3H, C**H**(CH$_3$)NH$_2$); 1.40 (s, 2H, CH$_3$C**H$_2$**); 1.11 (d, 9H,CH(C**H$_3$**)O, J=6.2 Hz); 1.02 (d, 9H, CH(C**H$_3$**)N J=6.5 Hz); 0.81-0.89 (m, 3H, CH$_2$C**H$_3$**).

3. Synthesis and characterization of P.

For the synthesis of the tetramine **P** 100 mg (MW=392.32 g/mol, 0.25 mmol) of PTCDA and 2500 mg (5.4 mmol) of Jeffamine **J** were sonicated for 1 minute in 15 ml of Ethylene glycol in a 250 ml Erlenmeyer flask. The jeffamine perfectly dissolved in the solvent while the PTCDA was only suspended after the sonication. The flask was heated in a commercial microwave oven at 900 W five times for one minute manually stirring the reaction mixture every time.

After the heating the solution became deep purple colored and perfectly clear. The product **P** was isolated as a sticky solid on the surface of the flask by adding 150 ml of water to the reaction mixture and by stirring and heating at 80 °C for 10 minutes. The reaction yield was 90%.

[1H] NMR (CD$_3$OD, 400 MHz): δ (ppm) 8.71-8.61 (m, 8H, arom **CH**); 4.40-4.33 (m, 2H, NCH**CH$_3$**); 3.65-2.95 (m, 46H, CH$_2$**CH**(CH$_3$)O+O**CH$_2$**+**CH**(CH$_3$)NH$_2$); 1.59 (d, 6H, NCH(**CH$_3$**), J=6.7 Hz); 1.40 (s, 4H, CH$_3$**CH$_2$**); 1.2-0.85 (m, 36H,C(**CH$_3$**)HO+C(**CH$_3$**)HNH$_2$+CH$_2$**CH$_3$**).

FIGURE SI_2. ESI mass spectrum of **P**. The signals of **PH$^+$** have been multiplied by 10.

Mass distribution of P. The mass distribution of **P** was investigated by ESI-mass (1mg/1ml in MeOH). The resulting mass spectrum is shown in figure SI_01; it presents four groups of peaks in different colors corresponding to the mono-, di-, tri- and tetra-protonated **P** labeled as **PH$^+$** (black), **PH$_2$$^{2+}$** (red) and **PH$_3$$^{3+}$** (green) and **PH$_4$$^{4+}$** (cyan) respectively. The different constitutional isomers are labeled by n, **being** n=a+b+c+d+e+f. The ESI-mass spectrum clearly show that isomers with n=9, 10 and 11 are the predominant species, being the sum of their molar fraction about 0.75. The sum of the molar fraction of the two isomer n=8 and n=12 is about 0.20. Lastly the sum of the molar fraction of the isomers with n<8 or n>12 is less than

0.05. The molar mass calculated from the mass distribution data was 1190 g/mol with a polydispersity index PdI=1.01.

4. Determination of the degree of conversion of PTCDA into P after microwaves irradiation.

In order to measure the concentration of **P** in the reaction mixture after microwaves irradiation 0.010 ml of such homogeneous mixture has been diluted in 10 ml of ethanol and the absorption spectrum has been recorded. The resulting spectrum shows the typical structured band of PDI with a maximum at 526 nm where the absorbance is 1.33. Being the molar absorption coefficient of **P** at that wavelength 8×10^{-4} M^{-1} cm^{-1} we calculated the concentration of **P** (c=0.017 M) to be compatible with a complete conversion of PDATC in PDI.
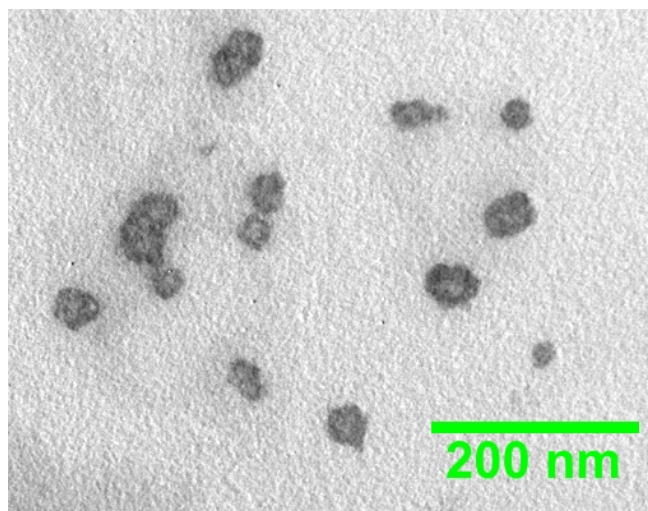
5. DLS Experiments

The determination of the nanoparticles hydrodynamic diameter distributions was carried out through Dynamic Light Scattering measurements employing a Malvern Nano ZS instrument equipped with a 633 nm laser diode. Samples were housed in disposable polystyrene cuvettes of 1 cm optical path length, using water as solvent. The width of DLS hydrodynamic diameter distribution is indicated by PdI (Polydispersion Index). In case of a mono-modal distribution (gaussian) calculated by means of cumulant analysis, $PdI=(\sigma/Z_{avg})^2$, where σ is the width of the distribution and Z_{avg} is average diameter of the particles population respectively.

6. ESI-Mass

ESI-mass experiments were carried out with an Agilent Technologies-Bruker LC-MSD Ion-Trap 1100 series ESI and APCI sources, m/z 50-4000 mass range.

7. TEM Experiments

A Philips CM 100 transmission electron microscope operating at 80 kV was used. For TEM investigations a standard 3.05 mm copper grid (400 mesh) covered by a Formvar support film was dried up under vacuum after deposition of a drop of nanoparticles suspension

Figure SI_3. TEM image of the P NPS.

8. Photophysical measurements.

UV-Vis absorption spectra were recorded with a Perkin Elmer P40 Spectrometer.

The fluorescence spectra were recorded with an Edinburgh FLS920 equipped with a photomultiplier Hamamatsu R928P. The same instrument connected to a PCS900 PC card was used for the Time Correlated Single Photon Counting (TCSPC) experiments. Luminescence quantum yields (uncertainty, ± 15%) were determined using N,N′-Bis(2,5-di-tert-butylphenyl)-3,4,9,10-perylenedicarboximide (Aldrich) in $CHCl_3$ solution (Φ = 0.99). Fluorescence intensities were corrected for inner filter effects according to standard methods (*Handbook of Photochemistry*, 3rd ed., Eds. M. Montalti, A. Credi, L. Prodi, M. T. Gandolfi. CRC Taylor & Francis, Boca Raton, 2006).

9. Determination of the fraction of P adsorbed by the yeast cells

A suspension of cells of commercial Saccharomyces Cerevisiae was prepared by Vortex stirring 100 mg of yeast in 10 ml of PBS solution. 0.25 ml of the resulting suspension were added to 2.25 ml of the PBS dispersions of P having a concentration of P ranging from 1×10^{-6} to 1.2×10^{-4} M. The resulting suspensions were vortex stirred for one minute in Eppendorf test tubes and incubated at room temperature for 30 minutes. Lastly the incubated suspension were centrifuged for 5 minutes at 1000 rpm. The surnatant has been transferred to spectrophotometric cuvettes in order to measure the absorbance spectrum. The fraction adsorbed χ at the different concentrations was determined by comparing the final absorbance at 498 nm (A) to the one measured before the yeast addition (A_0) according to equation SI_1.

$$\text{Eq. SI\_1} \qquad \chi = (A_0 - A/0.9)/A_0$$

10. Fluorescence microscopy measurements.

The samples for fluorescence microscopy were prepared by incubating the yeast cells with the NPs dispersions as reported in the previous section. In this case the cells were not centrifuged and 0.1 ml of the suspension were directly dropped (without any washing or post-treatment) on the top of a 0.13 mm thick cover slide and observed using an Olympus IX 71 inverted microscope equipped with a Xenon lamp (450 W) for fluorescence excitation and a Basler Scout scA640-70gc CCD camera for images acquisition. The lamp was attenuated with an absorptive filter Thorlabs NE30B and coupled to a fluorescence cube mounting the filters set Chroma 11001v2 blue. In this attenuated excitation conditions no significant changes were observe in the fluorescence images of the yeasts within the time scale of few

minutes. Images with 10X magnification were taken using the objective Olympus UPLFLN10X2 while the objective UPLFLN10X2 was used for 100X magnification.

11. Fluorescence photo-tuning experiments.

The photo-tuning experiments were carried out using the experimental setup and the sampled described in the previous section. The sample was first focused attenuating the excitation with the absorptive filter Thorlabs NE30B. Then the filter was removed and the acquisition immediately started.

12. Cytotoxicity experiments.

Saccharomyces Cerevisiae (2.5×10^6 cells/ml) were suspended either in PBS (samples **PBS0**, **PBS1** and **PBS2**) or in an SD culture medium (samples **C0**, **C1** and **C2**) either in the absence of **P** (samples **PBS0** and **C0**) or in the presence of **P** (10^{-6} M in the case of **PBS1** and **C1** and 10^{-5} M in the case of **PBS2** and **C2**). All the samples were incubated at 30 °C for 4 hours and stained with Trypan blue (0.4 %) in order to investigate cells viability. The total concentration of cells and the concentration of dead cells was determined using a hemocytometer.

13. Electrochemical experiments

The electrochemical experiments were carried out in argon purged water solution at 298 K. In the cyclic voltammetry (CV) the working electrode was a glassy carbon electrode (0.08 cm^2), the counter electrode was a Pt spiral. The potentials reported are referred to SCE. The concentration of **P** examined was of the order of 5×10^{-4} M; $LiClO_4$ 0.1 M was added as supporting electrolyte. Cyclic voltammograms were obtained with scan rates in the range

0.05–20 V s^{-1}. Measure reduction potential for **P** $E_{1/2}$ = -0.54 V (**P/P**$^-$). No oxidation of **P** was observed in the -1.00-0.00 V range.

14. pH dependent photophysical experiments.

The effect of pH on the absorption and fluorescence spectra of **P** was investigated in the 4.0-8.5 pH range in the case of a PBS solution 1x10^{-5} M of **P**. The pH of the PBS solution was decreased to 4.0 by adding CH$_3$COOH 1 M and then increased gradually by adding NaOH 0.1 M to give the pH value of figure SI_4 and SI_5.
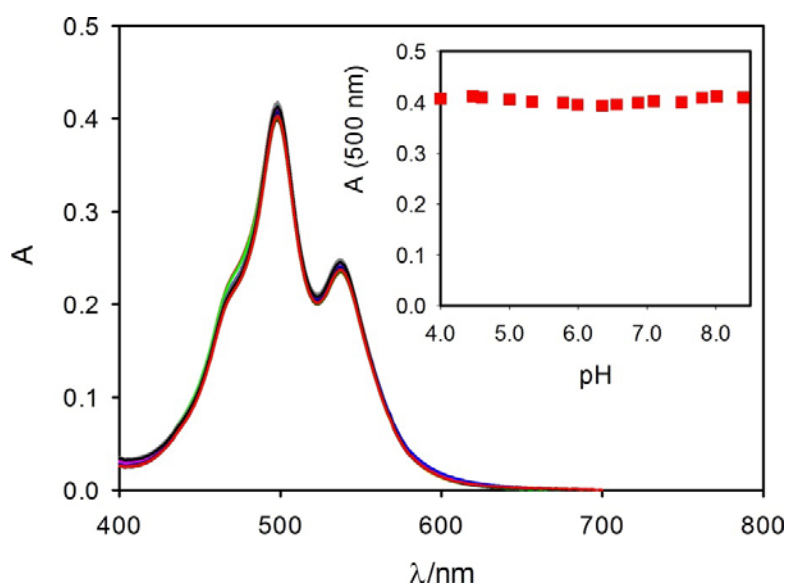
FIGURE SI_4. Absorption spectrum of **P** 1x10^{-5} M at different pH. Inset: absorption at 500 nm of the 1x10^{-5} M solution of **P** as a function of pH.
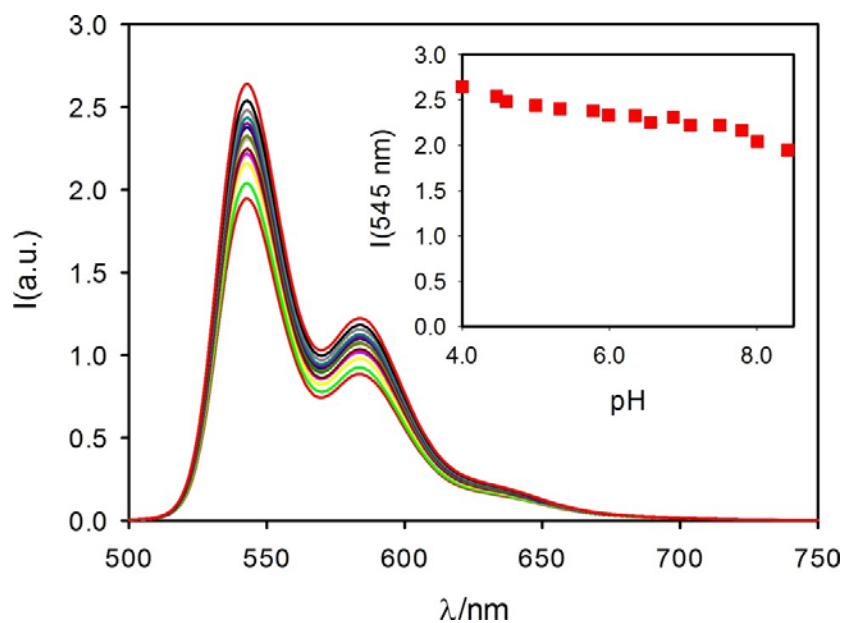
FIGURE SI_4. Fluorescence spectra of **P** 1×10^{-5} M at different pH. Inset: absorption at 500 nm of the 1×10^{-5} M solution of **P** as a function of pH.