Supporting Information

Visible-Light-Driven Antibacterial Activity of Poly(3-hexylthiophene-2,5-diyl) Nanoparticles

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Experimental Section

Materials

Poly(3-hexylthiophene-2,5-diyl) (**P3HT**) with 99% regioregularity (M_W = 48,600–81,000 and 20,000–45,000, unless otherwise specified, use M_W = 48,600–81,000) (**rrP3HT**) was obtained from Tokyo Chemical Industry Co., Ltd., **P3HT** with 50% regioregularity (M_W = 36,000) (**rraP3HT**) was obtained from Sigma–Aldrich. Tetrahydrofuran (THF, unstabilised), phosphate-buffered saline [PBS (−); without calcium and magnesium], and standard agar medium (Daigo) were purchased from Fujifilm Wako Pure Chemical Corporation. Luria–Bertani (LB) medium was purchased from Nacalai Tesque, Inc. The OxiSelect In Vitro ROS/RNS Assay Kit was obtained from Funakoshi Co., Ltd. The bacterial strain *Escherichia coli* (*E. coli;* ATCC 25922) was obtained from the Tissue Culture Laboratory, Katahira Campus, Tohoku University, Japan.

Measurements

Scanning electron microscope (SEM) images were observed using the Hitachi S-4800. Size distribution was measured using a Malvern Zetasizer NanoZS. The particle count was measured using a Malvern Zetasizer Advance Ultra Red. Static contact angle measurements were carried out using a contact angle goniometer DropMaster DMo-501 (Kyowa). The WAXS measurement was performed at BL08W in NanoTerasu (Miyagi,

Japan). Absorption spectra were measured using the UV-1900i Plus (SHIMADZU). A 300 W xenon light source "MAX-303" from ASAHI SPECTRA was used as the light source for analysing the antibacterial activity during the experiment.

DLS measurements

820 µL of the **NPs** dispersion (10–60 µM) was added to the capillary cell. Measurements were performed using a He-Ne laser (3.0 mW, 633 nm) at a controlled temperature of 25 °C. The scattered light was detected at 173°. The refractive index (1.33) and the viscosity (0.89 mPa·s) of ultrapure water at 25°C were used in data analysis.

SEM sampling

The **NPs** were fixed and dried onto Whatman® Polycarbonate Membrane filter (φ = 0.05 µm) using diaphragm pump and let dried overnight in desiccator. Dried sample was attached to specimen mount using carbon conductive adhesive tapes. The sample was sputtering with platinum prior to SEM measurements.

Contact angle measurements

Static contact angle measurements were performed at ambient temperature using the sessile drop method and image analysis of the drop profile. The samples were either

untreated quartz substrate or quartz substrate coated by drop-casting the **P3HT NPs** dispersion on a hot plate maintained at 80°C. For contact angle measurements, samples loaded onto the goniometer stage. The deionized water (milli-Q, Millipore, Molsheim, France) droplet volume was 2.0 μL, and the contact angle was measured 1 s after the drop was deposited on the sample, the reported value is the average of the results obtained on 3 droplets.

Antibacterial activity test procedure

The suspension of *E. coli* mixed with the sample was irradiated with visible light at an intensity of 30 mW/cm² and a wavelength of 385–740 nm for 6 h. Since the diffusion distance of reactive oxygen species (**ROS**) was within approximately 200 nm,[1] the *E. coli* suspension was stirred at 990 rpm to ensure effective **ROS** interaction with the *E. coli*, and antibacterial activity was evaluated. After 6 h, the samples were diluted 1,000,000-fold with PBS (−), and 1 mL of the diluted solution was mixed with 25 mL of agar medium. The agar medium was incubated in a 5% CO_2 incubator for 24 h. The number of colonies formed after 24 h was measured using ImageJ (NIH, Bethesda, MD, USA), and the sterilisation rate was calculated as:

$$\text{Sterilisation rate (\%)} = 100 - \left(\frac{\text{Number of target colonies}}{\text{Number of colonies in control}} \times 100\right) \quad (1)$$

Quantification of ROS generated from P3HT NPs

The total amount of **ROS** was quantified using the OxiSelect In Vitro ROS/RNS Assay Kit in accordance with the manufacturer's instructions. The assay kit employed the fluorescent probe 2',7'-dichlorodihydrofluorescein (**DCFH**) to generate the fluorescent substance 2',7'-dichlorodihydrofluorescein diacetate (**DCF**) in response to **ROS** and reactive nitrogen species (**RNS**) (**Fig. S1**). The fluorescence intensity of the generated **DCF** was measured, and the concentrations of **ROS** and **RNS** equivalent to H_2O_2 were quantified using an H_2O_2 standard curve. In this experimental system, because nitrogen-containing compounds were absent from the solution, we could quantify only **ROS**.[2] Therefore, the measured values were treated as the total amount of **ROS**. A standard curve was plotted from the fluorescence intensity of the H_2O_2 standard solutions measured using a fluorescence plate reader (**Fig. S4 (A)**). Using the standard curve and the fluorescence intensities of the samples (**Fig. S4 (B)**), the total amount of **ROS** was quantified for 2 mL of the **P3HT NPs** dispersion at a concentration of 10 μM. When calculating the total amount of **ROS**, the autofluorescence intensity of each sample was subtracted, and ultrapure water was used as the control.

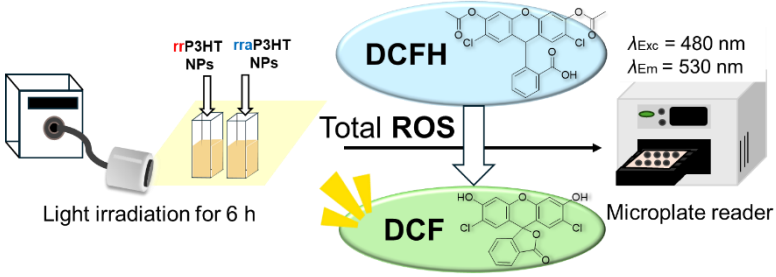


Fig. S1 Schematic representation of the OxiSelect In Vitro ROS/RNS Assay mechanism.

Evaluation of the effect of residual organic solvents on antibacterial activity

The effect of THF on antibacterial activity was investigated using **rrP3HT NPs** (diameter: Approximately 50 nm). After the **rrP3HT NPs** were prepared using the reprecipitation method, they were placed in a Smart Evaporator CEV1C-SU (Kanagawa, Japan) set at 23°C and operated for 12 min to remove residual THF. The **P3HT NPs** from which the THF had been removed were reanalysed by DLS to confirm the absence of aggregation, after which an antibacterial activity test was performed. In this experiment, the samples were irradiated with visible light (intensity: 30 mW/cm^2; wavelength: 385–740 nm) for 1 h.

Preparation of rrP3HT NPs and rraP3HT NPs

For **NP** fabrication by the reprecipitation method, **P3HT** solutions were prepared at 0.5, 1.0, and 3.0 mM. The molecular weight of **P3HT** used was 166 g/mol per repeating unit. The amounts of **P3HT** and THF used are listed in **Table S1**.

Table S1 The amount of **P3HT** and THF.

Code	**P3HT** (mg)	THF (mL)
rrP3HT-0.5 mM	0.24	2.88
rrP3HT-1.0 mM	0.44	2.63
rrP3HT-3.0 mM	0.68	1.36
rraP3HT-0.5 mM	0.55	6.23
rraP3HT-1.0 mM	0.65	3.93
rraP3HT-3.0 mM	2.45	4.92

P3HT was dissolved in THF and stirred at 70°C, 460 rpm for 10 min. The resulting **P3HT** solution (200 μL) was injected into ultrapure water (9.8 mL) under stirring at 25°C, 560 rpm.

Supplementary Figure

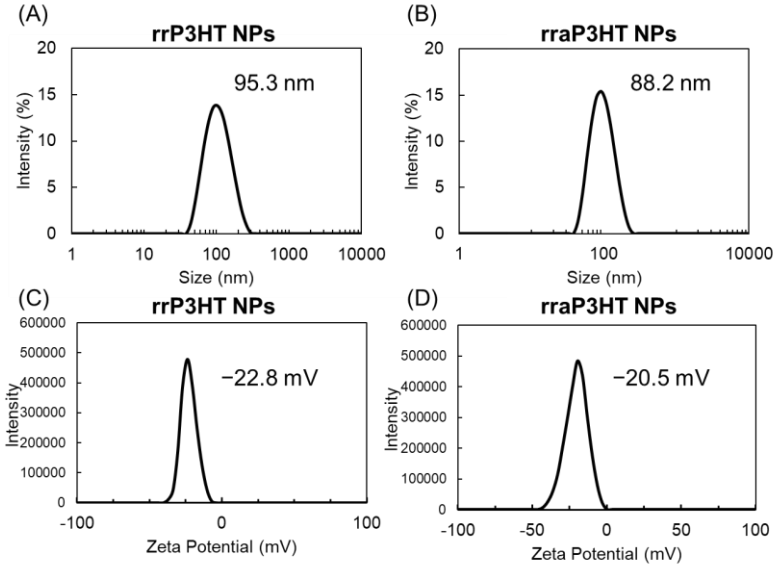


Fig. S2 (A) Dispersion stability and (C) Zeta potential of **rrP3HT NPs** and (B) Dispersion stability and (D) Zeta potential of **rraP3HT NPs**; DLS and zeta potential measurements immediately after preparation. These **NPs** were prepared by injecting a 3.0 mM **P3HT** solution (200 μL) into ultrapure water (9.8 mL) stirred at 560 rpm.

Table S2 Particle size and PDI changes as a function of time after preparation for **rrP3HT NPs** and **rraP3HT NPs**.

	rrP3HT NPs		**rraP3HT NPs**	
Measurement Time (day)	0	7	0	7
Z-Average Size (nm)	95.3	95.4	88.2	90.7
PDI	0.13	0.12	0.12	0.05

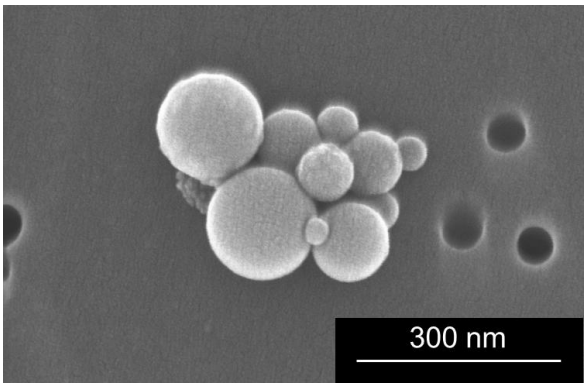


Fig. S3 Representative SEM image showing the spherical morphology of **P3HT NPs.**

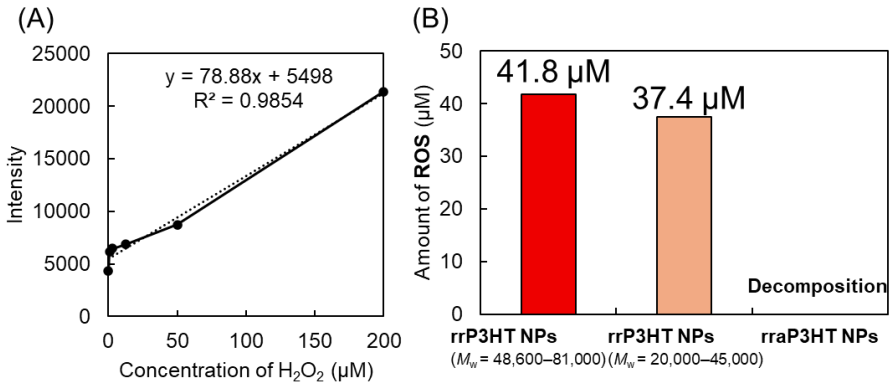


Fig. S4 (A) Hydrogen peroxide standard curve. (B) **ROS** production from **P3HT NPs** dispersion after visible light irradiation (385–740 nm, 30 mW/cm^2, 6h). The total amount of **ROS** generated from the **rraP3HT NPs** dispersion is labelled as "Decomposition" rather than **ROS** generation; this is because it was difficult to accurately quantify the **ROS** amount for the **rraP3HT NPs**, as prior research has reported that photo-degraded **rraP3HT** contain radical intermediates[3] that can also oxidize **DCFH**, and we also confirmed this degradation in our **rraP3HT NPs** through absorbance measurements before and after light irradiation.

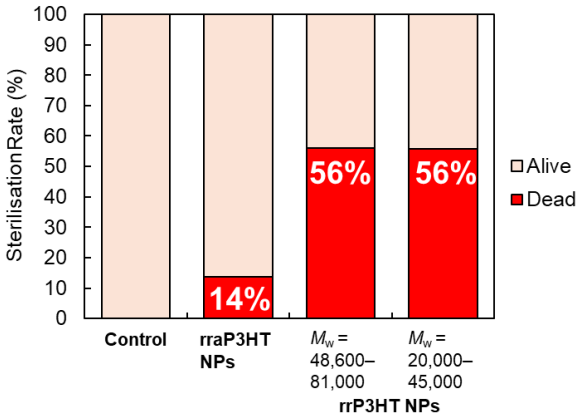


Fig. S5 The sterilisation rate of **rrP3HT NPs** (M_w = 48,600–81,000 or M_w = 20,000–45,000) dispersion and **rraP3HT NPs** (M_w = 36,000) dispersion after visible light irradiation (385–740 nm, 30 mW/cm^2, 1 h).

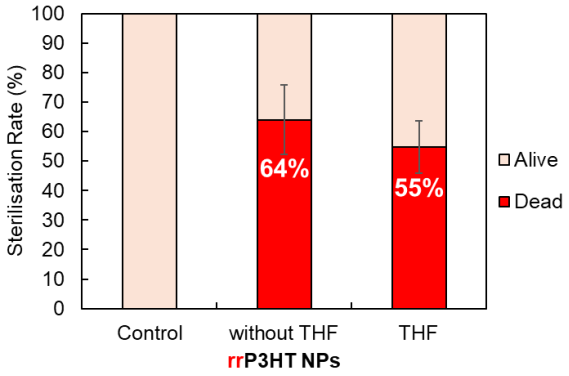


Fig. S6 Sterilisation rate of **rrP3HT NPs** (with THF or without THF) dispersion after visible light irradiation (385–740 nm, 30 mW/cm^2, 1h).

Table S3 Sterilisation rate of ultrapure water (with 2% (*v*/*v*) THF), **rrP3HT NPs** and **rraP3HT NPs** in the dark.

Sample	Water with 2%THF (*v*/*v*)	**rrP3HT NPs** in the dark	**rraP3HT NPs** in the dark
Sterilisation Rate (%)	0	0	0

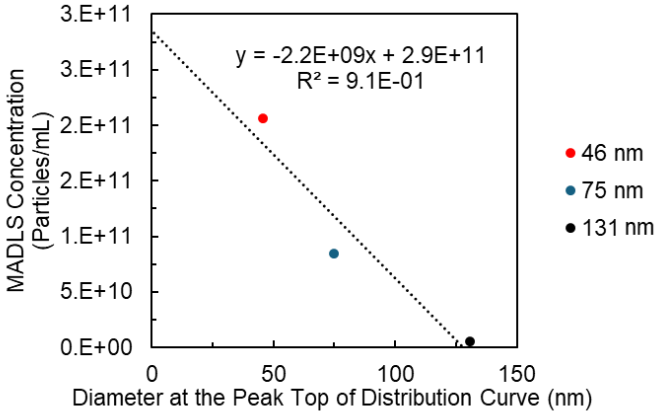


Fig. S7 Concentration values of various **rrP3HT NPs** (46, 75, 131 nm) dispersion measured by the MADLS method, plotted against the average particle size.

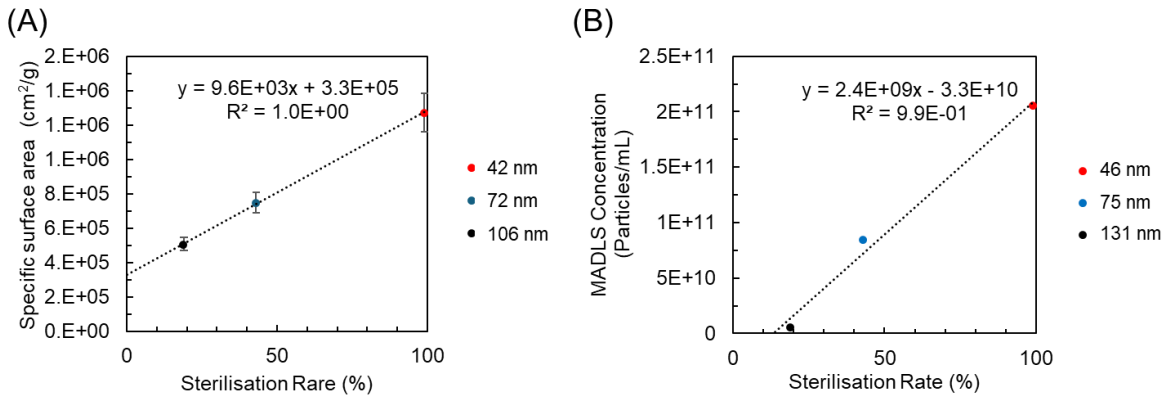


Fig. S8 (A) Graph plotting the sterilisation rate against the specific surface area calculated assuming **NPs** as ideal spheres.[4-6] The specific surface area (S_a) is calculated using the particle size (d) and density of **P3HT** (ρ = 1.13 g/cm^3) according to $S_a = 6/\rho d$. (B) Concentration values of various **rrP3HT NPs** dispersion measured by the MADLS method, plotted against the sterilisation rate.

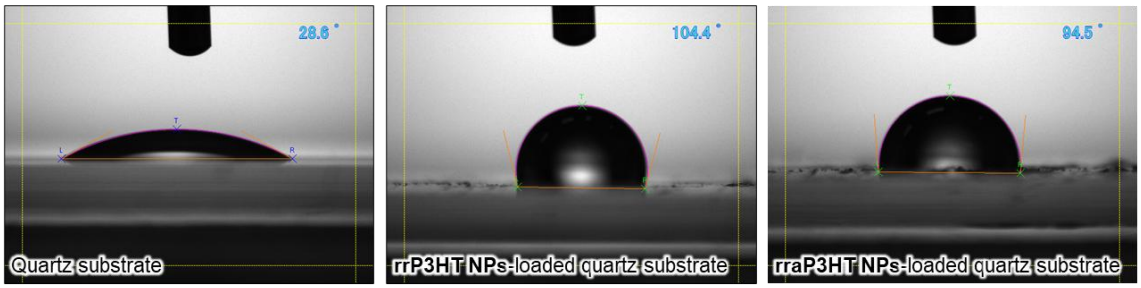
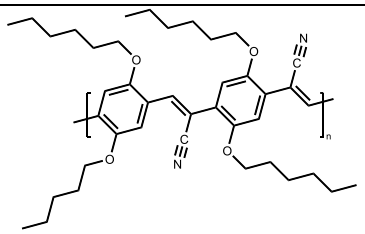
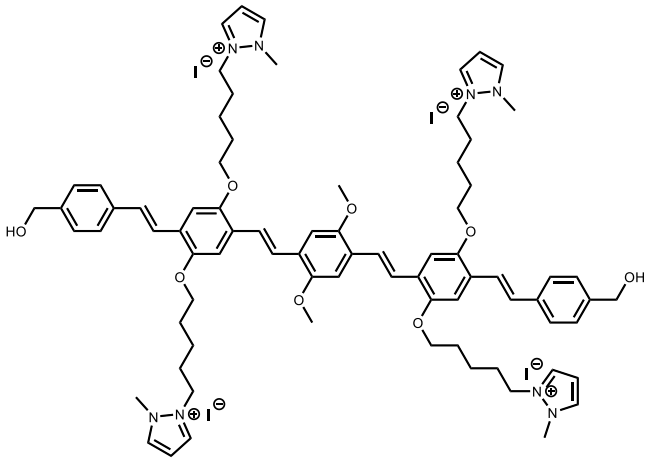
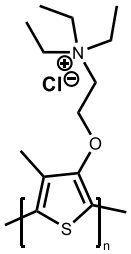
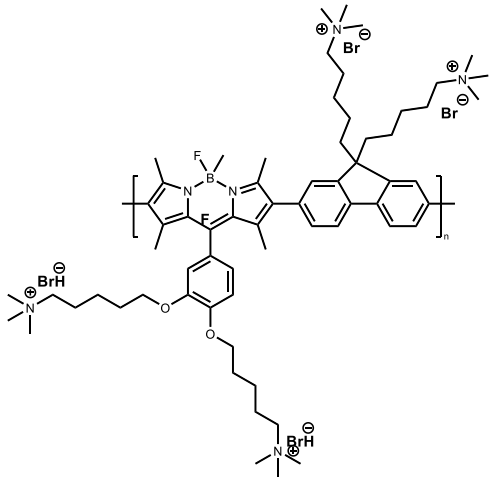


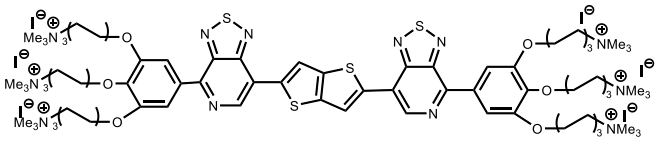
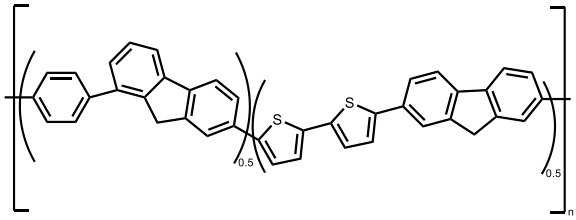
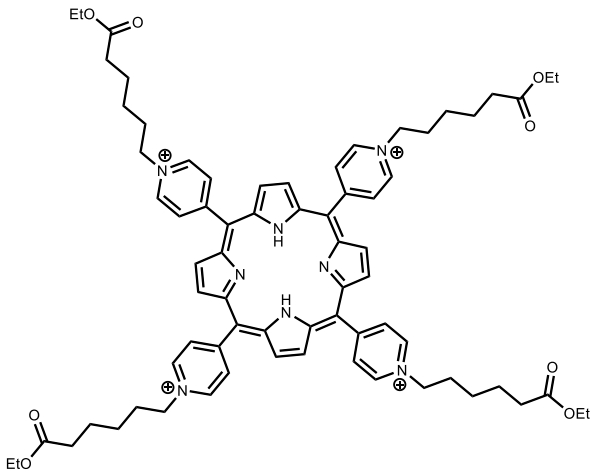
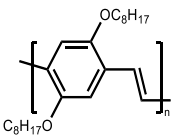
Fig. S9 Representative images of water contact angle measurements on a bare quartz substrate and **P3HT NPs**-loaded substrates. Average values (n = 3) are provided in the accompanying table.

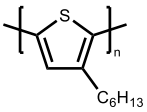
Table S4 Static contact angles of water on a quartz substrate and **P3HT NPs**-loaded quartz substrates.

Entry	Contact angle (°, 3 times average)
Quartz substrate	28.7
rrP3HT NPs-loaded quartz substrate	107.2
rraP3HT NPs-loaded quartz substrate	92.8

Table S5 Characteristics of photocatalytic sterilisation using π-conjugated polymers.

Ref. No.	π-conjugated polymer	Sterilisation rate	Light wavelength
22	 CN-PPV NPs	**99%**	450 nm
23	 OPV	**99%**	White light*
23	 PMNT	**98%**	White light*
23	 PBF	**95%**	White light*

24	Multifunctional PMB-modified CON (PMB-CON)	**98%**	White light*
25	 PTTP	**98%**	540 ± 60 nm
26	 “Fluorene-*co*-phenylene”-containing CPs	**97%**	White light*
8	Conjugated microporous polymer nanoparticles (CMP NPs)	**95%**	Visible light*
27	 Porphyrin TPyCP	**94%**	660 nm
28	 MEH-PPV	**93%**	White light*

This work	 **P3HT**	**99%**	Visible light (385–740 nm)

***** Wavelength of white and visible light is unknown.

References

1. Q. Tian, Y. Jiang, X. Duan, Q. Li, Y. Gao and X. Xu, *Water Res*, 2025, **268**, 122621.
2. K. Wang, C. Shang, R. Yin and Y. Xiang, *Environ Sci Technol*, 2023, **57**, 18735–18743.
3. N. Sai, K. Leung, J. Zador and G. Henkelman, *Phys Chem Chem Phys*, 2014, **16**, 8092–8099.
4. J. Hedberg, E. Blomberg and I. Odnevall Wallinder, *Environ Sci Technol*, 2019, **53**, 4030–4044.
5. M. Ciocca, M. Maver, C. Allara, D. Zanotelli, S. Krik, A. Orlando, T. Ruhle, S. Walz, T. Figueroa Gonzalez, G. Gentile, A. A. Lavdas, P. Ibba, F. Trevisan, Z. Milosz, M. Timpel, M. V. Nardi, A. Pedrielli, A. Gaiardo, P. Lugli, F. Cacialli, D. Leister, T. Mimmo and L. Petti, *Mater Horiz*, 2025, **12**, 7937–7950.
6. E. Zucchetti, M. Zangoli, I. Bargigia, C. Bossio, F. Di Maria, G. Barbarella, C. D'Andrea, G. Lanzani and M. R. Antognazza, *J Mater Chem B*, 2017, **5**, 565–574.