Engineering thiophene-based nanoparticles to induce photodransduction in live cells under illumination

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I. Synthesis

**General:** organic solvents were dried by standard procedures. Thin-layer chromatographies (TLCs) were carried out on 0.2-mm thick plates of silica gel 60 F254 (Merck). Visualization was accomplished by UV light or phosphomolybdic acid solution. Silicagel chromatographies were performed on glass columns of different sizes hand packed with silica gel 60 (particle sizes 0.040-0.063 mm, Merck). Unless otherwise noted, all reactions were carried out under a dry, oxygen-free nitrogen atmosphere.

**Chemicals:** 3-Bromothiophene, n-butyllithium 2.5 M in hexane, 1,6-dibromohexane, Sodium ≥ 99 % pieces, Sephadex G-50 (Medium), Dialysis sack (Avg. flat width 35mm (1.4 in), MWCO 12000 Da) diethyl malonate, N-bromosuccinimide (NBS), N-hydroxysuccinimide (NHS), 4-(dimethylamino)pyridine (DMAP), N,N’-dicyclohexylcarbodiimide (DCC) bis(tributylstannyl)thiophene, tetrakis(triphenylphosphine)palladium(0) and 5,5’-bis (tributylstanny1)-2,2’-bithiophene were purchased from Sigma-Aldrich Co. Hydrochloric acid 37% RPE, potassium hydroxide pellets RPE and sodium bicarbonate RPE were purchased from Carlo Erba Reagents. CDCl3, THF-d8 and D2O were purchased from Spectra2000 Srl. All reagents and solvents were used as received. Melting points were determined on Kofler bank apparatus and are uncorrected.

**Characterizations:** 1H NMR and 13C NMR spectra were recorded on a Varian Mercury-400 spectrometer equipped with a 5-mm probe. Chemical shifts were calibrated using the internal CDCl3 resonance which was referenced to TMS. Mass spectra were collected on a Thermo Scientific TRACE 1300 gas chromatograph. UV-Vis spectra were recorded using an Agilent Technologies CARY 100 UV-Vis spectrophotometer. Photoluminescence spectra were collected on a Perkin Elmer LS50 spectrofluorometer. Fluorescence measurements were performed using an excitation wavelength corresponding to the maximum absorption wavelength. Dynamic light scattering (DLS) measurements were performed with a Nanobrook Omni Particle Size Analyzer, with a wavelength of 659 nm in backscattering mode. Nanoparticles were dispersed in deionized water during analysis and measurements were taken at 25°C. Zeta potential measurements were performed using Smoluchowski equation. Circular Dichroism (CD) spectra were recorded using a spectropolarimeter JASCO J-715 in water under ambient conditions. Scanning Electron Microscopy (SEM) images were collected with a SEM-FEG Zeiss LEO 1530 operating at V_{acc} = 5 keV. The nanoparticles were drop casted onto Si/SiO2 wafers and dried under vacuum with no further treatment.

**3-(6-bromohexyl)thiophene (2)** → A 100 mL two-necked round-bottom flask equipped with a stirrer bar was charged with 3-bromothiophene (4 g, 0.024 mol) and distilled hexane (30 mL) under N2 atmosphere. The flask was cooled to -40°C and the solution was stirred for 15 min. n-BuLi (10.8 mL, 0.027 mol) was added dropwise via syringe over 10 min. The mixture was stirred for 15 min, and anhydrous THF (3.5 mL) was added dropwise over 5 min. The solution was stirred for 1 h, the cooling bath was replaced with an ice-salt bath, and then the mixture was warmed to -10 °C.
1,6-Dibromohexane (15.1 mL, 0.098 mol) was added in one portion, and then the solution is left to return to room temperature and stirred 24 h. The mixture was concentrated under vacuum and extracted three times with CH₂Cl₂/H₂O. The resulting crude product was purified under vacuum distillation at 150°C for 1 h, to remove the starting material in excess (1,6-dibrohexane) and then filtered through silica gel (cyclohexane) to provide 2.10 g of product. Colorless oil; yield 50%. EI-MS m/z 248 (M⁺).

$^1$H NMR (400 MHz, CDCl₃, TMS/ppm): δ 7.23 (dd $^3$J=5.2 Hz, $^3$J=3.2, 1H), 6.93 (m, 2H), 3.40 (t, 2H), 2.65 (t, 2H), 1.91-1.84 (m, 2H), 1.67-1.56 (m, 2H), 1.52-1.48 (m, 2H), 1.47-1.36 (m, 2H).

$^{13}$C NMR (400 MHz, CDCl₃) δ 142.8, 128.2, 125.2, 119.9, 33.9, 32.7, 30.3, 30.1, 28.4, 27.9.

Diethyl 2-(6-(thiophen-3-yl)hexyl)malonate (3) → A 100 mL two-necked round bottom flask was cooled to -10 °C, charged with sodium (0.237 g, 0.0103 mol) and anhydrous ethanol (40 mL) under N₂ atmosphere. The solution was stirred until the complete dissolution of sodium (20 min) and then diethyl malonate (2.2 g, 0.0138 mol) was added dropwise over 15 min. After 30 min 3-bromohexylthiophene (1.7 g, 0.0069 mol) was added dropwise and the solution was stirred for 48 h. The reaction was quenched with HCl aqueous solution (3N, 10 mL) and extracted three times with CH₂Cl₂/H₂O. The crude product was purified by flash chromatography (Cyclohexane/CH₂Cl₂ 80/20) to provide 1.9 g of product. Colorless oil; yield 85%. EI-MS m/z 327 (M⁺).

$^1$H NMR (400 MHz, CDCl₃, TMS/ppm): δ 7.23 (dd $^3$J=4.8 Hz, $^3$J=2.8, 1H), 6.92 (m, 2H), 4.22-4.16 (m, 4H), 3.30 (t, 1H), 2.63 (t, 2H), 1.91-1.85 (m, 2H), 1.63-1.59 (m, 2H), 1.34-1.30 (m, 6H), 1.26 (t, 6H).

$^{13}$C NMR (400 MHz, CDCl₃) δ 169.5, 143.0, 128.2, 125.1, 119.8, 61.2, 52.0, 30.4, 30.2, 29.0, 28.9, 28.7, 27.2, 14.1.

2-(6-(thiophen-3-yl)hexyl)malonic acid (4) → Diethyl 2-(6-(thiophen-3-yl)hexyl)malonate (1.82 g, 0.0056 mol) was dissolved in a 20 % KOH aqueous solution (4g, 0.071 mol, in 20mL H₂O) and refluxed at 100 °C for 6 h. The reaction mixture was returned to room temperature and then HCl solution (6 N, 10 mL) was added in one portion. The resulting solution was extracted three times with (Et)₂O/H₂O, concentrated under reduce pressure and purified by gel permeation chromatography (CHCl₃), to provide 1.29 g of product. White powder; yield 90%. EI-MS m/z 270 (M⁺).

$^1$H NMR (400 MHz, CDCl₃, TMS/ppm): δ 7.23 (dd $^3$J=4.8 Hz, $^3$J=2.8, 1H), 6.92 (m, 2H), 3.43 (t, 1H), 3.61 (t, 2H), 1.96-1.92 (m, 2H), 1.63-1.59 (m, 2H), 1.42-1.30 (m, 6H).

$^{13}$C NMR (400 MHz, CDCl₃) δ 173.8, 142.9, 128.2, 125.1, 119.8, 51.0, 30.3, 30.1, 28.9, 28.8, 27.1.

8-(thiophen-3-yl)octanoic acid (5) → 2-(6-(thiophen-3-yl)hexyl)malonic acid (1.287 g, 0.0048 mol) was heated at 130-140 °C for 1 h, until evolution of carbon dioxide had practically ceased; then heated quickly to 170 °C and cooled. The crude product was extracted three times with (Et)₂O/H₂O, dried on Na₂SO₄ and purified by gel permeation chromatography (CHCl₃) to provide 1.05 g of product. Yellow oil; yield 95%. EI-MS m/z 228 (M⁺).

$^1$H NMR (400 MHz, CDCl₃, TMS/ppm): δ 7.23 (dd $^3$J=4.8 Hz, $^3$J=2.8, 1H), 6.92 (m, 2H), 3.43 (t, 1H), 3.61 (t, 2H), 1.96-1.92 (m, 2H), 1.63-1.59 (m, 2H), 1.42-1.30 (m, 6H).

$^{13}$C NMR (400 MHz, CDCl₃) δ 180.7, 143.1, 128.3, 125.1, 119.8, 51.0, 30.3, 30.1, 29.9, 28.8, 27.1.

8-(2,5-dibromothiophen-3-yl)octanoic acid (6) → To a solution of 8-(thiophen-3-yl)octanoic acid (1 g, 0.0044) dissolved in THF (30 mL) at 0 °C small portions of NBS (1.9 g, 0.0101) were added. The
reaction mixture was stirred overnight. The resulting product was filtered on silica gel and purified by gel permeation chromatography (CHCl₃), to provide 1.160g of product. White solid; yield 85 %. EI-MS m/z 386 (M⁺). ¹H NMR (400 MHz, CDCl₃, TMS/ppm): δ 6.77 (s, 1H), 2.50 (t, 2H), 2.35 (t, 2H), 1.65-1.61 (m, 2H), 1.56-1.51 (m, 2H), 1.34-1.32 (m, 6H). ¹³C NMR (400 MHz, CDCl₃) δ 180.5, 142.8, 130.9, 110.3, 107.9, 34.1, 29.5, 29.4, 28.96, 28.91, 28.8, 24.6.

2,5-dioxopyrrolidin-1-yl-8-(2,5-dibromothiophen-3-yl)octanoate (7) → A two-necked flask equipped with magnetic stirrer was charged with 8-(2,5-dibromothiophen-3-yl)octanoic acid (0.44 g, 0.0011 mol), anhydrous THF (10 mL), NHS (0.395 g, 0.0034 mol) and DMAP (0.110 g, 0.0009 mol). The solution was stirred and cooled in an ice bath to 0°C and then DCC (0.250 g, 0.0013 mol) was added over a 10 min period. After 30 min at 0°C the ice bath is removed and the reaction mixture was stirred overnight. The crude product was extracted three times in CH₂Cl₂ and NaHCO₃ aqueous solution (PH = 8), concentrated under vacuum and purified by flash chromatography (cyclohexane/CH₂Cl₂ 5/95), to provide 0.520 g of product. Colorless oil; yield >90 %. EI-MS m/z 480 (M⁺). ¹H NMR (400 MHz, CDCl₃, TMS/ppm): δ 6.76 (s, 1H), 2.81 (s broad, 4H), 2.58 (t, 2H), 2.49 (t, 2H), 1.76-1.69 (m, 2H), 1.57-1.50 (m, 2H), 1.39-1.32 (m, 6H). ¹³C NMR (400 MHz, CDCl₃) δ 169.2, 168.6, 142.8, 130.9, 110.3, 107.9, 30.9, 29.4, 29.3, 28.76, 28.74, 28.6, 25.6, 24.5.

Poly[3-(2,5-dioxopyrrolidin-1-yl 8-octanoate)-2,2'-bithiophene)] (8) → A mixture of 7 (0.14 g, 0.29 mmol), 2-5-Bis(tributylstannyl)thiophene (0.192 g, 0.29 mmol) and tetrakis(triphenylphosphine)palladium(0) (0.025 g, 0.023 mmol) in toluene (10 mL) was refluxed for 12 h. The crude product was extracted three times in CHCl₃/H₂O, filtered to remove the insoluble fraction and then precipitated for three times by addition of methanol to a solution of the polymers in chloroform to provide 72 mg of product. Dark red solid, mp 103-105°C. Yield 61%. ¹H NMR (400 MHz, CDCl₃, TMS/ppm): δ 7.12-7.01 (m), 2.80 (s, broad), 2.62-2.58 (m), 1.76-1.58 (m), 1.41-1.27 (m), 1.39-1.32 (m), 0.93-0.89 (m). GPC: Mw 4227 Mn 2810, PDI = 1.50. UV/Vis: λmax 454 nm, λem 568 in CHCl₃.

Poly[3-(2,5-dioxopyrrolidin-1-yl 8-octanoate)-2,2'-5'-2''-terthiophene)] (9) → A mixture of 7 (0.15 g, 0.311 mmol), 5-5'-bis(tributylstanny)-2,2'-bithiophene (0.231 g, 0.311 mmol) and tetrakis(triphenylphosphine)palladium(0) (0.029 g, 0.025 mmol) in toluene (10 mL) was refluxed for 12 h. The crude product was extracted three times in CHCl₃/H₂O, filtered to remove the insoluble fraction and then precipitated for three times by addition of methanol to a solution of the polymers in chloroform to provide 98 mg of product. Dark red solid, mp 213-215°C. Yield 65%. ¹H NMR (400 MHz, CDCl₃, TMS/ppm): δ 7.07-7.00 (m), 2.81 (s, broad), 2.63-2.59 (m), 2.38-2.36 (m), 1.76-1.61 (m), 1.42-1.28 (m), 1.39-1.32 (m), 0.94-0.89 (m). GPC: Mw 3634 Mn 2570, PDI = 1.41. UV/Vis: λmax 466 nm, λem 570 in CHCl₃.
Synthesis and Characterization of NPs-NHS

Functionalized nanoparticles, NPs-NHS, were prepared using reprecipitation method. Polymer 8 or 9 (8 mg) was dissolved in 400 µL of THF and the resulting solution was added dropwise via Hamilton syringe to 8 mL of milliQ-H₂O under magnetic stirring. The obtained suspension was filtered through a filter paper to remove aggregates, and then subjected to dialysis (dialysis sacks: molecular weight cut off 12000 g/mol) against 3 L of milliQ-water for 3 days to remove residual THF (see Scheme S1). DLS: NPs-NHS (8) → $Z_{\text{pot}} = -33.06$ mV, Diameter = 343 nm, PDI = 0.16; NPs-NHS (9) → $Z_{\text{pot}} = -41.10$ mV, Diameter = 317 nm, PDI = 0.16.

Scheme S1

Post-functionalization of NPs-NHS with D and L-tryptophan

To a solution of D or L-tryptophan (2.5 mg) dissolved in 0.1 M PBS (5 mL, pH 7.4), a suspension of NPs-NHS (8) (500 µg/mL) in 1 mL of milliQ water was slowly added. Then the resulting mixture was agitated on a rotating plate mixer (20 rpm) for 2 h (see Scheme S2).

Scheme S2

After centrifugation (5 minutes, 5000 rpm), the supernatant was removed and the precipitated nanoparticles were passed through a Sephadex column (G-50, mediumgrade) using milliQ water as eluent in order to remove the unreacted tryptophan. IR: $v_{\text{max}}$ (NaCl) 3402, 2956, 2870, 1739, 1645, 1602, 1431, 1360, 1314, 1231, 1214, 1157, 1120, 1068, 860, 741 cm⁻¹.
Synthesis and post-functionalization of 2,5-dioxopyrroolidin-1-yl 8-{[2,2':5',2''-terthiophen]-3'-yl}octanoate with L-tryptophan

**Scheme S3.** Synthesis of 2,5-dioxopyrroolidin-1-yl 8-{[2,2':5',2''-terthiophen]-3'-yl}octanoate (10)

A mixture of 7 (0.05 g, 0.13 mmol), 2(tributylstannyl)thiophene (0.085 g, 0.31 mmol) and tetrakis(triphenylphosphine)palladium(0) (0.010 g, 0.008 mmol) in toluene (5 mL) was refluxed for 12 h. The crude product was extracted three times in CH₂Cl₂/H₂O. The organic solution was concentrated under vacuum and purified by flash chromatography (cyclohexane/CH₂Cl₂/AcOEt 70/20/10), to provide 30 mg of product. Brown oil; yield 60%. EI-MS m/z 372 (M⁺).

**1H NMR (400 MHz, CDCl₃, TMS/ppm):** δ 7.31 (d, ³J=5.2 Hz, ⁴J=1.2 Hz, 1H), 7.19 (d, ³J=5.2 Hz, ⁴J=1.2 Hz, 1H), 7.15 (d, ³J=3.2 Hz, ⁴J=1.2 Hz, 1H), 7.12 (d, ³J=3.2 Hz, ⁴J=1.2 Hz, 1H), 7.06 (dd, ³J=5.2 Hz, ³J=3.2 Hz, 1H), 7.02-6.99 (m, 2), 2.83 (s broad, 4H), 2.72 (t, 2H), 2.58 (m, 2H), 1.75-1.58 (m, 4H), 1.42-1.28 (m, 6H).

**13C NMR (400 MHz, CDCl₃):** δ 169.2, 168.6, 140.1, 137.2, 135.8, 135.2, 127.8, 127.4, 126.5, 125.9, 125.4, 124.3, 123.6, 30.9, 30.4, 29.2, 29.1, 28.8, 28.6, 25.5, 24.5. UV/Vis: λmax 340 nm, λem 435 in CH₂Cl₂. IR: νmax (NaCl) 2924, 2853, 1707, 1508, 1458, 1412, 1375, 1260, 1078, 798, 694 cm⁻¹.

**Scheme S4.** Synthesis of 2-{8-{[2,2':5',2''-terthiophen]-3'-yl}octanamido}-3-(1H-indol-3-yl)propanoic acid (11)

(i) L-trypthophan, H₂O, room temperature, 5h, >90 %
To an aqueous solution of 10 (0.02 g, 0.05 mm), L-tryptophan (0.055 g, 0.27 mmol) was added and the solution was stirred for 5 h. The crude product was extracted three times in CH₂Cl₂/H₂O. Brown oil; yield >90 %. The product was characterized without further purification. EI-MS m/z 576 (M⁺).

$^1$H NMR (400 MHz, CDCl₃, TMS/ppm): δ 8.47 (s broad, 1H), 7.43 (d, J=7.2 Hz, 1H), 7.21 (d, J=4.4 Hz, 1H), 7.15 (d, J=4.4 Hz, 1H), 7.12-6.87 (m, 8H), 6.31 (s broad, 1H), 4.63 (s broad, 2H), 3.18 (m), 2.60 (t), 2.31 (m), 1.78 (m), 1.60 (m), 1.45 (m), 1.28 (m). UV/Vis: $\lambda$ max 342 nm, $\lambda$ em 435 in CH₂Cl₂, $\lambda$ max 362 nm, $\lambda$ em 445 in H₂O. IR: $\nu$ max (NaCl) 3374, 2923, 2852, 1719, 1654, 1522, 1459, 1413, 1376, 1260, 1095, 1020, 800, 743, 697 cm⁻¹.
II. $^1$H and $^{13}$C NMR spectra

Figure S1. $^1$H and $^{13}$C NMR spectra of compound 2.
Figure S2. $^1$H and $^{13}$C NMR spectra of compound 3.
Figure S3. $^1$H and $^{13}$C NMR spectra of compound 4.
Figure S4. $^1$H and $^{13}$C NMR spectra of compound 5.
Figure S5. $^1$H and $^{13}$C NMR spectra of compound 6.
Figure S6. $^1$H and $^{13}$C NMR spectra of compound 7.
Figure S7. $^1$H NMR spectrum of compound 8.

Figure S8. $^1$H NMR spectrum of compound 9.
Figure S9. $^1$H and $^{13}$C NMR spectra of compound 10.
**Figure S10.** $^1$H NMR spectrum of compound 11.

**Figure S11.** $^1$H NMR spectrum of poly(3-hexylthiophene) nanoparticles in D$_2$O/THF-$d$8.
III. Optical Properties

**Figure S12.** Absorption and emission spectra of compound 10 in CH₂Cl₂.

**Figure S13.** Absorption and emission spectra of compound 11 in CH₂Cl₂.
Figure S14. Absorption and emission spectra of compound 11 in H₂O.

Figure S15. Emission spectra of D and L tryptophan in H₂O.
Figure S16. Circular Dichroism spectrum of compound 11 and the corresponding absorption spectrum in H$_2$O.
IV. Scanning Electron Microscopy of NPs-NHS

Figure S17. SEM images of NPs-NHS obtained from polymer 8.
Figure S18. SEM images of NPs-NHS obtained from polymer 9.
Figure S19. SEM images of P3HT NPs
V. Characteristics of the nanoparticles NPs-P3HT and NPs-NHs employed for the treatment of live HEK-293 cells

<table>
<thead>
<tr>
<th></th>
<th>Mean size (nm)</th>
<th>Polydispersity</th>
<th>Z-potential (mV)</th>
</tr>
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<tbody>
<tr>
<td>NPs-NHS</td>
<td>335</td>
<td>0.1</td>
<td>-40</td>
</tr>
<tr>
<td>NPs-P3HT</td>
<td>320</td>
<td>0.09</td>
<td>-37</td>
</tr>
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</table>
VI. MTT assay of HEK-293 cells treated with NPs-NHS

Cytotoxicity of NPs was evaluated by MTT assay [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] (Sigma Aldrich) on HEK-293 cells. Basically, this assay exploits the metabolism of living cells to transform a water-soluble tetrazolium salt into a purple colored formazan compound. Absorbance of the purple product is directly related to the proliferation of cells. Cells with a healthy metabolic activity are proliferating cells hence display higher absorbance values.

Cells were seeded in 48 well plates at a density of $2 \times 10^4$ cells/well, NPs were added directly during the plating phase with a dilution in order to work with a fixed optical density of 0.2. Cell proliferation was evaluated after 6 hours, 1 day, 2 days and 3 days of incubation with NPs. For each time point, the growing medium was replaced with RPMI without phenol red containing 0.5 mg/mL of MTT. The samples were incubated again for 2 h at 37 °C in dark. Formazan salt produced by cells through reduction of MTT was then solubilised with 200 μL of ethanol and the absorbance was read at 560 nm and 690 nm. The proliferation cell rate was calculated as the difference in absorbed intensity at 560 nm and 690 nm.

Figure S19 shows a viability assay conducted for cell cultures incubated with NPs-NHS, NPs-P3HT and untreated samples (N=8 for each condition). As expected, cell proliferation is in general increased as the incubation time increases. Cells treated with NPs-P3HT display good cell activity, while it is evident that exposure to NPs-NHS leads to decreased proliferation properties (J. Pecher, S. Mecking, Chem. Rev. 2010, 110, 6260-6279).

![MTT assay graph](image)

**Figure S20.** Normalized absorbance of MTT assay in vitro at four different time points for different NPs treatment and control cell samples. Data are reported as average over N=8 samples ± SEM (standard error mean).
VII. Laser Scanning Confocal Microscopy images

**Figure S21.** LSCM images of successive slices of HEK-293 cells incubated for 30 min with NPs-P3HT. Cells cytoskeleton was stained with phalloidin (in green), while the nanoparticles are seen in red channel.
Figure S22. Confocal optical sections of cells loaded with NPs-NHS (panels a) and NPs-P3HT (panels b), stained with phalloidin (green, actin). Emission from NPs is visible in the red channel.
Figure S23 highlights a portion of Figure 5 A of the manuscript helping to give evidence to the fact that NPs-NHS are not internalized. Together with video S1.gif, it is possible to better appreciate small changes in localization of NPs outside cell membrane. In particular, arrow 1 points out a group of particles which come into focus only when focal plane is $Z=0.5 \, \mu m$, and they go out-of-focus at $Z=1.5 \, \mu m$. On the other hand, arrow 2 show a portion of cell in which actin filaments are focused only at $Z=1.5 \, \mu m$, meaning that the higher border of the cell has been reached, and some NPs-NHS are visible only at that focal plane. The same can be said for NPs-NHS pointed out by arrow 3, which are better focused only when the highest portion of cell is focused.
VIII. Images taken with inverted microscope used for electrophysiology and area of NPs aggregates from particle analysis over single cells

Figure S24. Brightfield and fluorescence images taken with inverted microscope used for electrophysiology experiments with a 40x objective. Cell cultures were treated with NPs-NHS (upper panel) and NPs-P3HT (lower panel). Single cells analysed with ImageJ are outlined in green. Scale bar, 30 µm.
Table S2. Area of the aggregate with the maximum dimension obtained from particle analysis carried out over single cells outlined in Figure S24.

<table>
<thead>
<tr>
<th>CELL</th>
<th>AREA MAXIMUM AGGREGATE NPS-NHS (µm²)</th>
<th>AREA MAXIMUM AGGREGATE NPS-P3HT (µm²)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CELL1</td>
<td>11.38</td>
<td>4.98</td>
</tr>
<tr>
<td>CELL2</td>
<td>3.93</td>
<td>2.88</td>
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<tr>
<td>CELL3</td>
<td>8.44</td>
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<td>STANDARD DEVIATION</td>
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</tr>
</tbody>
</table>

Typically, it can be observed that the biggest aggregates formation prevails in case of samples treated with NPs-NHS (the maximum area of aggregates has an average of 8.11 µm²), against the case of NPs-P3HT, whose maximum aggregates cover on average 6.69 µm² of cells.

In order to check the possibility that the photoinduced signal upon illumination (Figure 7) is due to a thermal effect, we need to estimate what is the required change in temperature.

Depolarization of the membrane potential due to a change in membrane capacitance is described by equation 1:\[\Delta V \approx \frac{\Delta C_m}{C_m} (V_r - V_\sigma) = \alpha_c(V_r - V_\sigma)\Delta T.\] From the statistical analysis of the depolarization peaks observed with NPs-NHS, we obtain an average value of \[\Delta V \approx 0.07 \text{ mV}.\]

Considering typical values \[V_r = -30 \text{ mV}, \ V_\sigma = 120 \text{ mV}, \ \text{and} \ \alpha_c = 0.0031 K^{-1},\] as obtained for HEK-293 cells grown on flat substrate, we then work out a required increase in temperature of \[\Delta T \approx 0.16 K.\]

The temperature gradient with respect to the background (here considered unchanged) in a single nanoparticle after light exposure is given by equation 2:\[\Delta T = \frac{I_0 \pi R_{np}^2}{4\pi K r},\] where \(I_0\) is the intensity of the light (200 mW/mm²), \(R_{np}\) is the NP’s radius (we adopt the average value \(R_{np} = 175 \text{ nm}\)), \(K\) is the thermal conductivity of water (0.6 W/mK) and \(r\) is the distance from the nanoparticle centre. At the particle surface, \(r = R_{np}\) the expected \(\Delta T\) is thus \(\approx 0.015 K.\) Note that according to equation 2 this value is linearly dependent on the particle radius. This is one order of magnitude smaller than required for supporting the thermal mechanism. We have here two possible ways out. One is to consider aggregation. A large cluster can absorb more energy and give rise to a larger temperature gradient according to a simple linear law with the radius. In order to reach the temperature range required the radius should be one order of magnitude larger. This, in terms of a 3D spherical cluster, corresponds to a rather unlucky configuration where a thousand particles are connected.

However, due to the peculiar docking of the single NPs-NHS onto the cell membrane, a quasi 2D aggregation could be envisaged, where the NPs coagulate in a surface patch of more than 1 µm radius on top of the cell (Figure S22 and Table S1). This local modification of the cell outskirt could result into a photoactive site able to absorb visible light and convert it into local heating, thus affecting the membrane potential. This explanation is fully consistent with the thermal-like response of the cell, but it is difficult to be tested as imaging does not provide enough resolution.
Reference