Development and Characterization of Conducting Polymer Nanoparticles for Photodynamic Therapy *In Vitro*

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ABSTRACT

Conducting polymer nanoparticles (CPNPs), composed of the conducting polymer poly[2-methoxy-5-(2-ethylhexyl-oxy)-p-phenylenevinylene] (MEH-PPV) were studied for applications in biophotonics and therapeutics. The extent of cellular uptake, cytotoxicity, and effectiveness of these nanoparticles in photodynamic therapy (PDT) was investigated for four cell lines, namely TE-71, MDA-MB-231, A549 and OVCAR3. Confocal fluorescence imaging and flow cytometry show that CPNPs are taken up only in limited quantities by TE-71, while they are taken up extensively by the cancer cell lines. The uptake among the cancer cell lines was observed to vary with cell line, with CPNPs uptake increasing from MDA-MB-231 to A549 to OVCAR3. Fluorescence imaging experiments show that the CPNPs have high brightness and appear stable

1 in the intracellular environment. No cytotoxicity of non-photoactivated CPNPs (in dark) was
2 observed from MTT assay. After completion of PDT, the quantitative data on cell viability
3 suggest that cell death scales across the cell lines with CPNP uptake, is light dose dependent, and
4 is complete for OVCAR3. In addition, for OVCAR3 apoptotic cell death is observed after PDT.
5 The reported work illustrates the potential of the intrinsically fluorescent and photoactivatable
6 CPNPs for application in biophotonics and therapeutics.

7
8 *KEYWORDS : ROS, MEH-PPV, conducting polymer, triplets, PDT, aggregation*

10 **1. Introduction**

11 Chemotherapy [1-5], surgery [6-8] and radiotherapy [9,10] are currently widely used in clinical
12 settings for treatment of cancer. Multimodal treatments such as chemotherapy combined with
13 immunotherapy [11,12] or with radiotherapy [13] are also used. Research on further treatment
14 development is ongoing. Surgery has recently seen improvements by the development of laser
15 surgery [14-18] and fluorescence imaging guided surgery [19,20]. Small molecules such as
16 doxorubicin [21-23] have been extensively studied, and others such as STAT3 inhibitors [24-26],
17 are currently under investigation. Nanocarrier versions of small molecule drugs and new
18 nanoparticle drugs are also under development with the aim to improve delivery and therapeutic
19 properties [27,28] In addition, photodynamic therapy (PDT) has been shown to be a promising
20 treatment scheme for cancer through clinical trials and preliminary application at treatment
21 centers [29-34].

22 In PDT a photosensitizing material is internalized by cancer cells, and subsequently these cells
23 are irradiated with light. The photosensitizer generates Reactive Oxygen Species (ROS) under

1 exposure to light that can induce oxidative stress and subsequent structural damage in cancer
2 cells, potentially leading to cell death [35-39].

3 For normal cells, ROS are essential in the regulation of cell functions such as cell signaling and
4 apoptosis. ROS levels are kept in check under ordinary circumstances by intrinsic antioxidants
5 present in cells. Studies on formation of cancerous cells have shown that when ROS levels
6 increase to an extent where they can no longer be removed by the intrinsic antioxidants present
7 in cells, this condition can lead to cancer [40-43]. As such, cancer cells need elevated amounts of
8 ROS for proliferation, but at the same time cancer cells do not have sufficient antioxidants to
9 scavenge ROS. Thus even while cancer cells thrive with elevated ROS levels, they are also very
10 susceptible to externally stimulated elevation of ROS levels [44-47].

11 Developments in nanotechnology inspired photosensitizers have brought new developments to
12 PDT. Various inorganic nanoparticle photosensitizers such as quantum dots[48,49], gold
13 nanoparticles [50-52], magnetic nanoparticles [53,54], silica nanoparticles [55-58], and titanium
14 oxide nanoparticles [59] have been developed and studied. A few examples of organic
15 nanoparticle photosensitizers such as methacrylate polymer nanoparticles doped with verteporfin
16 as the photosensitizers and polymeric micelles of block copolymer poly(ethylene glycol)-co-
17 poly(D,L-lactic acid) (PEG-PLA) loaded with 5,10,15,20-tetrakis(mesohydroxyphenyl)porphyrin
18 (mTHPP) as the photosensitizer have been reported [60,61]. Liposomes doped with various
19 photosensitizers have been studied for application in PDT [62]. For instance, aminolevulinic acid
20 encapsulate liposomes were recently reported for PDT application [63]. Phthalocyanine
21 conjugated polypropylenimine dendrimers, and porphyrine phthalocyanine decorated dendrimers
22 [64,65] have also been reported.

1 Recently, photosensitizer-doped conducting polymer nanoparticles have received attention as
2 potential photosensitizers for PDT. Photosensitizer doped conjugated polymer nanoparticles
3 were developed and characterized by Grimland *et al.* Singlet oxygen formation by
4 tetraphenylporphyrine (TPP) photosensitizer under one- and two-photon excitation was proven
5 by UV-vis spectroscopy, and DNA damage as proof-of-concept was shown by these authors.
6 [66]. Shen *et al.* concurrently proved the formation of singlet oxygen for tetraphenylporphyrine
7 (TPP) photosensitizer doped conjugated polymer nanoparticles by singlet oxygen
8 luminescence[67]. Zhang *et al.* more recently followed a similar nanoparticle design scheme
9 with meta-tetra-(hydroxyphenyl)-chlorin (m-THPC) as photosensitizer to produce ROS[68].
10 In this article, conducting polymer nanoparticles fabricated from the conducting polymer poly[2-
11 methoxy-5-(2-ethylhexyloxy)-1,4-phenylenevinylene] (MEH-PPV) were studied for application
12 in PDT. Photosensitization was found to be intrinsic to these conducting polymer nanoparticles
13 (CPNPs) without the use of photosensitizer dopants. These CPNPs were investigated *in vitro* for
14 their potential application in PDT for cancer treatment. The CPNPs show bright emission *in vitro*
15 that enables fluorescence imaging of internalized CPNPs. It was observed that these CPNPs have
16 a strong intrinsic bias towards the cancer cell lines studied here without surface modification.
17 The CPNPs were observed to be not intrinsically cytotoxic (no cytotoxicity observed in dark),
18 and show very promising PDT results. PDT was highly effective for lung and ovarian cancer-
19 derived cell lines at moderate intensities of visible light, without observation of phototoxicity.

20 21 **2. Materials and Methods**

22 **2.1. Fabrication of NPs**

Poly[2-methoxy-5-(2-ethylhexyloxy)-1,4-phenylenevinylene] (MEH-PPV) with molecular weight (average M_n) 150,000-250,000 g/mol was purchased from Sigma Aldrich and used without any further purification. THF (tetrahydrofuran) Drisolv was purchased from EMD Millipore. The CPNPs were fabricated following previously published protocol. [69-71]. In short, 1 mg MEH-PPV was dissolved in THF and was diluted to a 10^{-6} M concentration. 1 ml of this solution of MEH-PPV in THF was quickly injected into 4 ml of DI water under vigorous stirring. Stirring was stopped immediately after injection of the polymer solution in water.

2.2. Characterization

2.2.1. Dynamic light scattering

The size of the CPNPs was determined with a Precision Detector Dynamic Light Scattering (PD2000DLS) machine. Data was analyzed using the Precision Deconvolve software.

2.2.2. UV-visible spectroscopy

The absorption spectroscopy on the CPNPs suspension in water was completed in a 1 cm path length quartz cuvette with an Agilent 8453 spectrophotometer using the Chemstation software.

2.2.3. Fluorescence spectroscopy

The fluorescence spectroscopy on the CPNPs suspension in water was performed in a 1 cm path length quartz cuvette with a NanologTM HoribaJobin Yvon fluorimeter.

2.3. Cell culture

TE-71 (Mouse thymic epithelial cell line), MDA-MB-231 (Human breast cancer cell line), A549 (Human lung cancer cell line) and OVCAR3 (Ovarian tumor cell line) were purchased from ATCC and grown in DMEM media supplemented with 10% FBS in in humidified atmosphere of 95% air/ 5% CO₂ at 37 °C.

2.4. Incubation with CPNPs for fluorescence imaging

The TE 71, MDA-MB-231, A549 and OVCAR3 cell lines were grown in 96 well plates for 24 hours. Then the media was removed and after washing with PBS the cells were incubated with CPNPs at following concentrations: 0 mg/ml (vehicle control), 0.4×10^{-4} mg/ml, 2×10^{-4} mg/ml and 3.6×10^{-4} mg/ml in media. After 24 hours the CPNPs solution was removed and cells were washed with PBS and harvested with trypsin. These were then plated in different 35mm petri dishes and allowed to grow for 24 hours again. Then the cells were fixed with 4% paraformaldehyde, stained with DAPI and suspended in PBS to image.

2.5. Intrinsic cytotoxicity of CPNPs

The cells were grown in 96 well plates and were incubated with 0.4×10^{-4} mg/ml, 2×10^{-4} mg/ml and 3.6×10^{-4} mg/ml of CPNPs, together with the corresponding controls. Cells were incubated with CPNPs for up to 24 hours. Cell viability was determined 0, 24, 48, 72 and 96 hours after addition of CPNPs by MTT assay. For the 0 and 24 hour time point, MTT was added immediately after adding CPNPs and 24 hours after addition of CPNPs, respectively. For the 48, 72 and 96 hours readings, the CPNPs solution was removed after 24 hours and replaced with regular media. MTT was then added at the corresponding time points. MTT was allowed to incubate for 4 hours to allow formazan to form. Then the solubilization solution was added and

after 6 hours the 96 well plates were read. The readings were taken at 570nm on a Biotek ELx800 absorbance microplate reader.

2.6. Photodynamic Therapy PDT

Cells were grown in 96-well plates for 24 hours. Then the cells were incubated with different doses of CPNPs. After 24 hours the media was replaced with HBSS (Hank's Balanced Salt Solution) and the 96 well plates with the cells were exposed to visible light by using a solar simulator (Newport 67005 Oriel Instruments) and a UV filter (FSQ-GG400) to block the UV light. Three light doses (60 J/cm^2 , 120 J/cm^2 and 180 J/cm^2) and three CPNP doses ($0.4 \times 10^{-4} \text{ mg/ml}$, $2 \times 10^{-4} \text{ mg/ml}$ and $3.6 \times 10^{-4} \text{ mg/ml}$) along with one control dose (0 mg/ml) were used for PDT. For each light dose three post-PDT incubation periods (0 hours, 4 hours and 12 hours) were evaluated. Then MTT was added to the 96 well plates immediately after PDT, and 4 and 12 hours post-PDT incubation. The experiment was performed 3 times ($n=3$).

2.7. Detection of ROS

The A549 and OVCAR3 cells were incubated with $2 \times 10^{-4} \text{ mg/ml}$ CPNPs. After 24 hours the media was replaced with HBSS and the samples were exposed to a 180 J/cm^2 light. The CellROX green reagent was added to the media 1) immediately after the light treatment and 2) after 2 hours of post-PDT incubation. After 30 minutes the reagent was washed and the cells were fixed with 4% paraformaldehyde and stained with DAPI. The cells were kept in PBS for imaging. Three negative controls 1) No CPNPs and no light dose, 2) only light dose of 180 J/cm^2 , and 3) only CPNPs dose of $2 \times 10^{-4} \text{ mg/ml}$, and one positive control with $100 \mu\text{M H}_2\text{O}_2$ were also performed.

2.8. Flow cytometry

TE-71, MDA-MB-231, A549 and OVCAR3 at 1×10^6 cells/ml concentration were incubated with 2×10^{-4} mg/ml CPNPs for 24 hours. Then the cells were harvested, washed and fixed with 4% paraformaldehyde, and flow cytometry was performed on these samples with a BD FACS Canto II flow cytometer (BD Biosciences). The data were processed with FlowJo software (FlowJO). The experiment was performed 2 times (n=2).

2.9. Optical Microscopy

2.9.1. Confocal Imaging for CPNP Uptake

The fixed cells were imaged by spinning disc confocal fluorescence microscope (Zeiss Axioskop2) by using the 488nm wavelength from a Kr-Ar ion laser and FITC filters to detect the fluorescence from CPNPs. The DAPI images were taken by excitation with mercury lamp and DAPI filters. The CPNPs images and DAPI images were overlaid using ImageJ software. To confirm that the CPNPs were inside the cells, images at every 0.3 μm were taken along the Z axis. A selection of these slices was used representing a spacing of the slices by 1.5 μm .

2.9.2. Intracellular Spectroscopy

Single particle fluorescence images and spectra were collected by using a home-built sample scanning confocal microscope. The 488 nm laser line from an Ar ion laser was used as the excitation source. Samples were excited with $1\text{W}/\text{cm}^2$ laser light. The laser was focused using a Zeiss 100x Fluar objective lens (NA 1.3, WD 0.17 mm). The sample was raster scanned across the focused laser beam using a Mad City Labs piezoelectric stage (Nano-LP100) to obtain a fluorescence image of the sample. The fluorescence was detected by an avalanche photodiode

(PerkinElmer SPCM-AQR-14). Fluorescence spectra were obtained by a spectrograph (PI Acton SP-2156) with the grating (150 g/mm blaze: 500 nm) centered at 600 nm which was coupled to a thermoelectrically cooled electron multiplying charge coupled device (EM-CCD, Andor iXon EM+ DU-897 BI). Each fluorescence spectrum was collected with 10 seconds exposure time for 3 consecutive frames and then averaged. Each spectrum shown in the manuscript is an average built from 100 spectra, which were averaged into “ensemble spectra” in Matlab.

2.9.3. Epiluminescence Imaging for ROS detection

Imaging was completed with an Olympus IX71 microscope using a 60X objective and Andor Zyla sCMOS (DG-152V-C1E-FI) camera. The DAPI channel was used for the nucleus and FITC channel for CellROX green reagent imaging.

3. Results and Discussion

3.1. CPNP fabrication and characterization

The CPNPs were prepared by the reprecipitation method [69-71]. The resulting CPNPs are small quasi-spherical clusters of about 20 to 50 polymer chains [72]. DLS data show that the CPNPs have an average size of 34 ± 6 nm in diameter with a narrow size distribution (**Fig. 1B**), consistent with our previous reports [69]. The zeta potential of the CPNPs is -12.4 ± 3.7 mV, showing that the CPNPs surface has a negative charge. The absorption and emission spectra of CPNPs suspended in water shown in **Fig. 1C** depict a broad range of absorption in the visible spectrum having an absorption maximum at 494 nm and an emission maximum at 591 nm, respectively. The CPNPs were observed to be stable in cell culture media for 96 hrs (data not shown) as previously documented by Wu et al. [72].

3.2. *In-vitro* evaluation of CPNP uptake

Spherical nanoparticles of around 30 nm in diameter [73-75] and with positive zeta potential on their surfaces are typically uptaken easily by cells [76-82]. The size range is approximated well by the CPNPs discussed herein. While the surface charge is not ideal for cellular uptake, the cancer cells are observed to internalize the CPNPs (*vide infra*). **Fig. 2** shows the confocal fluorescence images and corresponding phase contrast images of the cell lines incubated with 4 concentrations of CPNPs (0 mg/ml, 0.4×10^{-4} mg/ml, 2×10^{-4} mg/ml and 3.6×10^{-4} mg/ml), revealing significant CPNP uptake. It can be seen that the different cell lines take up different amounts of CPNPs and that the uptake is concentration dependent. The cancer cell lines abundantly take up nanoparticles in comparison to the normal control cell line. It has been reported that the charge on normal cell lines is highly negative whereas the cancer cell lines have less negative charge on them [81,83]. Due to lesser negative charge on the cell surface of cancer cells the CPNPs have less repulsion from the cancer cell line surfaces as compared to the normal cell lines. Comparing the cancer cell lines, OVCAR3 has the highest uptake followed by A549 and lastly MDA-MB-231. The high uptake of CPNPs by OVCAR3 cells can be attributed to the aggressiveness of this cell line [84-87].

CPNP uptake efficiency was further quantified by flow cytometry. **Fig. 3A-D** shows representative normalized histograms indicating the distribution of fluorescence intensity detected for cells incubated with CPNPs. All cell lines appear to have taken up nanoparticles, although there is an apparent difference in extent of uptake. Cumulative analysis of the flow cytometry data from two independent experiments was performed and the results were plotted as bar graphs in **Fig. 3E-F**. The percent cell population that has internalized CPNPs is shown in

Fig. 3E. The data show that 68.2% of TE-71 cells internalized CPNPs, while for MDA-MB-231, A549, and OVCAR3 81.6%, 85.6%, and 90.05% of the respective cells internalized CPNPs. The average fluorescence intensity of CPNPs in each cell line is shown in **Fig. 3F**. The low average fluorescence intensity observed for TE 71 implies that even though there are 68.2 % cells which have internalized CPNPs, the amount of CPNPs in each cell is very small. The OVCAR3 and A549 cell lines exhibit comparable average fluorescence intensity of CPNPs followed by MDA-MB-231. The flow cytometry data thus confirm the qualitative observations made by fluorescence imaging (**Fig. 2**).

To confirm that the CPNPs were indeed internalized as opposed to being stuck to the outside cell membrane, 3D confocal imaging experiments were completed. CPNPs are detected in the intracellular environment throughout the 3D stack of images, as shown in **Fig. 4** using A549 as a representative example. Similar results were obtained for each cell line studied herein. These images show that the CPNPs are located mostly at the periphery of the nucleus.

The stability of the CPNPs inside the cells was evaluated by Single Particle Spectroscopy. The A549 cell line was selected for this experiment, since it internalizes CPNPs but not to the extent that individual particles or clusters cannot be identified. **Fig. 5A** and **5B** show the brightfield and fluorescence image of a single A549 cell that internalized CPNPs. The expanded image in **Fig. 5C** allows for the observation of single CPNPs and clusters of CPNPs. These were individually addressed by confocal laser and emission spectra collected for 100 CPNPs/CPNP clusters. The resulting single particle ensemble spectrum is shown in **Fig. 5D**. The peak emission at 588 nm is comparable to the peak emission at 591 nm observed for freshly prepared CPNPs measured in water. Conversely, well dissolved MEH-PPV in a good solvent emits around 553 nm [69]. These data confirm that the CPNPs are stable and intact inside the cell.

3.3. Intrinsic cytotoxicity of CPNPs

The intrinsic cytotoxicity of the CPNPs was evaluated quantitatively by MTT assays. **Fig. 6** shows MTT data for the four cell lines incubated with three doses of CPNPs (0.4×10^{-4} mg/ml, 2×10^{-4} mg/ml and 3.6×10^{-4} mg/ml) together with the control (0 mg/ml). At no point is intrinsic cytotoxicity of CPNPs observed. The cells proliferate normally in presence of CPNPs as compared to the control dose. These data suggest that no ROS are formed in dark (*vide infra*), in contrast to other sensitizers such as quantum dots, which even in the absence of light can still lead to elevated ROS levels in cells, and cause damage to cell organelles or lead to cell death [88,89].

3.4. PDT

Fig. 7 shows MTT data quantifying the viability of the four studied cell lines loaded with the CPNPs after PDT. The cell lines were incubated with three different concentrations (0.4×10^{-4} mg/ml, 2×10^{-4} mg/ml and 3.6×10^{-4} mg/ml) of CPNPs and then exposed to different light doses (60 J/cm^2 , 120 J/cm^2 or 180 J/cm^2). These moderate light doses are sufficient for an effective PDT treatment, [29,36,90,91] and are possible due to the large absorption cross section of MEH-PPV used herein in the visible spectrum ($> 10^6 \text{ L/mol cm}^{-1}$). No phototoxicity effects were observed in these studies (*vide infra*). Evaluation of treatment results was determined immediately after the PDT application (**Fig. 7A**), and after an incubation period of 4 hours (**Fig. 7B**), and 12 hours (**Fig. 7C**). In each panel (**Fig 7A, B and C**) the cell viability at three CPNP concentrations and three light doses are compared with the control for each cell line (0 mg/ml CPNPs).

The cell viability for each waiting period decreases from TE-71 to MDA-MB-231 to A549 to OVCAR3. In addition, for each cancer cell line, viability decreases with increasing CPNP dose, although the highest dose (3.6×10^{-4} mg/ml) does not show significant improvement. For TE71 there is no significant CPNP dose response due to the limited CPNP uptake. For A549 and OVCAR3 dependence on the light dose is also observed, where increasing light dose results in lower viability. The 180 J/cm^2 light dose still shows measurable improvement in reducing cell viability in those cases. This observation can again be attributed to the limited CPNP uptake by TE71 and MDA-MB-231 in comparison to A549 and OVCAR3. In contrast, the higher amount of CPNPs uptaken by cells lines such as A549 and OVCAR3 will lead to higher amounts of ROS being generated upon irradiation, which is reflected in the observation of low cell viability. It is, however, not possible to rule out the effects of differences in inherent ROS levels of different cell lines on susceptibility to undergo oxidative stress through PDT [92,93]. Overall, effectiveness of PDT scales with the extent of CPNP uptake and administered light dose. The 2×10^{-4} mg/ml dose of CPNPs under light dose of 180 J/cm^2 was found to be most effective. For the OVCAR3 cell line cell viability is near zero under these conditions.

3.5. CPNPs as source of ROS

It's been previously shown that conducting polymers are able to undergo charge- and energy transfer from their triplet excited state to the ground state of oxygen to form singlet oxygen [94-96]. The rapid photobleaching of conducting polymers, both reversible and irreversible, has been attributed to this mechanism [97]. It was therefore hypothesized that CPNPs could yield ROS upon photoirradiation without the need for photosensitizer dopants. Specifically, upon exposure to light the CPNPs are photoexcited and in the case of MEH-PPV have an approximately 1.25%

1 probability of intersystem crossing to the triplet excited state[98]. Oxygen can deactivate the
2 latter by energy transfer, which results in excitation of the oxygen triplet ground state to the
3 highly oxidative singlet oxygen excited state (Type II photosensitization) [35,36,38,99].
4 Alternatively, oxygen or other substrates such as solvent- and biomolecules can accept an
5 electron from the triplet excited state of the conducting polymer to form radicals (Type I
6 photosensitization) [35,36,38,99]. It has previously been shown that conducting polymers can
7 interact directly with oxygen to form superoxide in a reversible fashion [97,100,101]. If other
8 substrates are involved, then these typically act as intermediate radicals toward the formation of
9 superoxide. The superoxide anion may react further to form hydrogen peroxide, which in
10 presence of iron forms highly reactive hydroxyl radicals [42,102-104]. Regardless of whether
11 Type I or Type II photosensitization occurred, the resulting reactive oxygen then react with
12 biomolecules like lipids, nucleic acids etc. to form peroxides leading to a chain reaction in the
13 cell organelles causing damage to and potential death of the cell.

14 To confirm that the CPNPs are indeed the source of ROS formation in cells upon irradiation with
15 light, cells were stained with CellRox Green Reagent (CGR). This reagent is non-fluorescent or
16 very weakly fluorescent while in a reduced state and upon oxidation by ROS shows strong green
17 fluorescence. Formation of ROS was detected with CGR staining in A549 and OVCAR3 cell
18 lines that had taken up CPNPs and were exposed to light, both immediately after *in-vitro* PDT
19 (**Fig. 8D**) and 2 hours after PDT was completed (**Fig. 8E**). Further confirmation comes from the
20 control experiments with CGR staining. **Fig. 8A-C** shows three negative controls: in absence of
21 the treatment (no particles, no light), in absence of CPNPs with 180J/cm² light dose, and in
22 absence of photoirradiation with 2 x 10⁻⁴ mg/ml CPNPs dose, respectively. As CGR emission is
23 only detected when full PDT treatment is applied, it can be concluded that the CPNPs are the

1 source of ROS under PDT. The data in **Fig. 8B** (no particles, 180J/cm² light dose) suggest that
2 under the PDT conditions used here no acute phototoxicity occurs, since no elevated ROS levels
3 are detected and cell morphology looks normal. In addition, the data in **Fig. 8C** (particles, no
4 light) also support the MTT data that CPNP are not acutely cytotoxic, since no detectable ROS
5 response is observed in absence of light.

7 **4. Conclusions**

8 Conducting polymer poly[2-methoxy-5-(2-ethylhexyl-oxy)-p-phenylenevinylene] (MEH-PPV)
9 nanoparticles (CPNPs) were studied for application in biophotonics and therapeutics, specifically
10 for PDT. The nanoparticles are abundantly taken up by cancer cells without specific targeting of
11 surface receptors. Cells proliferate normally in the presence of CPNPs, indicating the absence of
12 intrinsic cytotoxicity. The bright intrinsic fluorescence of the CPNPs is attractive for localization
13 after administering the CPNPs, while the large absorption cross section allows for the use of low
14 to moderate light levels for ROS generation. It was observed that the effectiveness of PDT scales
15 with the extent of CPNP uptake and administered light dose. The 2x10⁻⁴ mg/ml dose of CPNPs
16 under light dose of 180 J/cm² was found to be the most effective treatment scheme *in-vitro*.
17 Promisingly, for the OVCAR3 cell line cell viability is near zero under these conditions.

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References

- [1] Dent S, Oyan B, Honig A, Mano M, Howell S. HER2-targeted therapy in breast cancer: A systematic review of neoadjuvant trials. *Cancer Treat Rev* 2013;39:622-631.
- [2] Heinemann V, Douillard JY, Ducreux M, Peeters M. Targeted therapy in metastatic colorectal cancer - An example of personalised medicine in action. *Cancer Treat Rev* 2013;39:592-601.
- [3] Mountzios G, Soultati A, Pectasides D, Pectasides E, Dimopoulos MA, Papadimitriou CA. Developments in the systemic treatment of metastatic cervical cancer. *Cancer Treat Rev* 2013;39:430-443.
- [4] Verbrugghe M, Verhaeghe S, Lauwaert K, Beeckman D, Van Hecke A. Determinants and associated factors influencing medication adherence and persistence to oral anticancer drugs: A systematic review. *Cancer Treat Rev* 2013;39:610-621.
- [5] Xie X, Wang SS, Wong TCS, Fung MC. Genistein promotes cell death of ethanol-stressed HeLa cells through the continuation of apoptosis or secondary necrosis. *Cancer Cell Int* 2013;13:1-15.
- [6] Fisher B, Anderson S, Bryant J, Margolese RG, Deutsch M, Fisher ER, et al. Twenty-Year Follow-up of a Randomized Trial Comparing Total Mastectomy, Lumpectomy, and Lumpectomy plus Irradiation for the Treatment of Invasive Breast Cancer. *N Engl J Med* 2002;347:1233-1241.
- [7] Fong Y, Fortner J, Sun RL, Brennan MF, Blumgart LH. Clinical Score for Predicting Recurrence After Hepatic Resection for Metastatic Colorectal Cancer: Analysis of 1001 Consecutive Cases. *Annals of Surgery* 1999;230:309.

- [8] Ishitobi M, Suzuki O, Komoike Y, Ohsumi S, Nakahara S, Yagi T, et al. Phase II study of neoadjuvant anastrozole and concurrent radiotherapy for postmenopausal breast cancer patients. *Breast Cancer* 2014;21:550-556.
- [9] Kalbasi A, June CH, Haas N, Vapiwala N. Radiation and immunotherapy: a synergistic combination. *J Clin Invest* 2013;123:2756-2763.
- [10] Suneja G, Poorvu PD, Hill-Kayser C, Lustig RA. Acute toxicity of proton beam radiation for pediatric central nervous system malignancies. *Pediatr Blood Cancer* 2013;60:1431-1436.
- [11] Aketa H, Tatsumi T, Kohga K, Tsunematsu H, Aono S, Shimizu S, et al. The combination therapy of alpha-galactosylceramide and 5-fluorouracil showed antitumor effect synergistically against liver tumor in mice. *Int J Cancer* 2013;133:1126-1134.
- [12] Hussain M, Javeed A, Ashraf M, Hou YZ, Mukhtar MM. Multilevel pharmacological manipulation of adenosine-prostaglandin E-2/cAMP nexus in the tumor microenvironment: A 'two hit' therapeutic opportunity. *Pharmacol Res* 2013;73:8-19.
- [13] Ghattass K, Assah R, El-Sabban M, Gali-Muhtasib H. Targeting Hypoxia for Sensitization of Tumors to Radio- and Chemotherapy. *Curr Cancer Drug Targets* 2013;13:670-685.
- [14] Kumar A, Cascarini L, McCaul JA, Kerauola CJ, Coombes D, Godden D, et al. How should we manage oral leukoplakia? *Br J Oral Maxillofac Surg* 2013;51:377-383.
- [15] Milovanovic J, Djukic V, Milovanovic A, Jotic A, Banko B, Jesic S, et al. Clinical outcome of early glottic carcinoma in Serbia. *Auris Nasus Larynx* 2013;40:394-399.
- [16] Moore EJ, Hinni ML. Critical Review: Transoral Laser Microsurgery and Robotic-Assisted Surgery for Oropharynx Cancer Including Human Papillomavirus-Related Cancer. *Int J Radiat Oncol Biol Phys* 2013;85:1163-1167.

- [17] No D, Osterberg EC, Otto B, Naftali I, Choi B. Evaluation of continence following 532nm laser prostatectomy for patients previously treated with radiation therapy or brachytherapy. *Lasers Surg Med* 2013;45:358-361.
- [18] Rosch T. Progress in endoscopy: areas of current interest and topics to watch out for. *Endoscopy* 2012;44:1148-1157.
- [19] Stummer W, Pichlmeier U, Meinel T, Wiestler OD, Zanella F, Reulen H-Jr. Fluorescence-guided surgery with 5-aminolevulinic acid for resection of malignant glioma: a randomised controlled multicentre phase III trial. *The Lancet Oncology* 2006;7:392-401.
- [20] Troyan S, Kianzad V, Gibbs-Strauss S, Gioux S, Matsui A, Oketokoun R, et al. The FLARE Intraoperative Near-Infrared Fluorescence Imaging System: A First-in-Human Clinical Trial in Breast Cancer Sentinel Lymph Node Mapping. *Annals of Surgical Oncology* 2009;16:2943-2952.
- [21] De Beer EL, Bottone AE, Voest EE. Doxorubicin and mechanical performance of cardiac trabeculae after acute and chronic treatment: a review. *European Journal of Pharmacology* 2001;415:1-11.
- [22] Joseph MMA, S. R. ;George, S. K. ;Pillai, K. R. ;Mini, S. ;Sreelekha, T. T. . Galactoxyloglucan-Modified Nanocarriers of Doxorubicin for Improved Tumor-Targeted Drug Delivery with Minimal Toxicity. *Journal of Biomedical Nanotechnology* 2014;10:3253-3268.
- [23] Keizer HG, Pinedo HM, Schuurhuis GJ, Joenje H. Doxorubicin (adriamycin): A critical review of free radical-dependent mechanisms of cytotoxicity. *Pharmacology & Therapeutics* 1990;47:219-231.
- [24] Fagard R, Metelev V, Souissi Is, Baran-Marszak F. STAT3 inhibitors for cancer therapy: Have all roads been explored? *JAK-STAT* 2013;2:e22882.

- [25] Turkson JJ, R. STAT proteins: novel molecular targets for cancer drug discovery. *Oncogene* 2000;19:6613-6626.
- [26] Zhang X, Yue P, Page BDG, Li T, Zhao W, Namanja AT, et al. Orally bioavailable small-molecule inhibitor of transcription factor Stat3 regresses human breast and lung cancer xenografts. *Proceedings of the National Academy of Sciences* 2012;109:9623-9628.
- [27] Davis ME, Chen Z, Shin DM. Nanoparticle therapeutics: an emerging treatment modality for cancer. *Nat Rev Drug Discov* 2008;7:771-782.
- [28] Vyas DC, P. ;Saadeh, Y. ;Vyas, A. . The Role of Nanotechnology in Gastrointestinal Cancer. *Journal of Biomedical Nanotechnology* 2014;10:3204-3218.
- [29] Brown SB, Brown EA, Walker I. The present and future role of photodynamic therapy in cancer treatment. *The Lancet Oncology* 2004;5:497-508.
- [30] Buinauskaite E, Maciulaitis R, Buinauskiene J, Valiukeviciene S. Topical photodynamic therapy of actinic keratoses with 5-aminolevulinic acid: Randomized controlled trial with six months follow-up. *Journal of Dermatological Treatment* 2014;25:519-522.
- [31] Casie Chetty N, Hemmant B, Skellett A-M. Periocular photodynamic therapy for squamous intra-epidermal carcinoma. *Journal of Dermatological Treatment* 2014;25:516-518.
- [32] Gupta A, Avci P, Sadasivam M, Chandran R, Parizotto N, Vecchio D, et al. Shining light on nanotechnology to help repair and regeneration. *Biotechnol Adv* 2012;31:607-631.
- [33] Hopper C. Photodynamic therapy: a clinical reality in the treatment of cancer. *The Lancet Oncology* 2000;1:212-219.
- [34] Kubler AC. Photodynamic therapy. *Medical Laser Application* 2005;20:37-45.
- [35] Dolmans D, Fukumura D, Jain RK. Photodynamic therapy for cancer. *Nat Rev Cancer* 2003;3:380-387.

1 [36] Dougherty TJ, Gomer CJ, Henderson BW, Jori G, Kessel D, Korbélik M, et al.
2 Photodynamic Therapy. *J Natl Cancer Inst* 1998;90:889-905.

3 [37] Ferrari M. Cancer nanotechnology: Opportunities and challenges. *Nat Rev Cancer*
4 2005;5:161-171.

5 [38] Oleinick NL, Morris RL, Belichenko T. The role of apoptosis in response to photodynamic
6 therapy: what, where, why, and how. *Photochem Photobiol Sci* 2002;1:1-21.

7 [39] Pass HI. Photodynamic Therapy in Oncology - Mechanisms and Clinical Use. *J Natl Cancer*
8 *Inst* 1993;85:443-456.

9 [40] Droge W. Free radicals in the physiological control of cell function. *Physiol Rev*
10 2002;82:47-95.

11 [41] Valko M, Leibfritz D, Moncol J, Cronin MTD, Mazur M, Telser J. Free radicals and
12 antioxidants in normal physiological functions and human disease. *International Journal of*
13 *Biochemistry & Cell Biology* 2007;39:44-84.

14 [42] Valko M, Rhodes CJ, Moncol J, Izakovic M, Mazur M. Free radicals, metals and
15 antioxidants in oxidative stress-induced cancer. *Chem-Biol Interact* 2006;160:1-40.

16 [43] Wallace DC. A mitochondrial paradigm of metabolic and degenerative diseases, aging, and
17 cancer: A dawn for evolutionary medicine. *Annual Review of Genetics*; 2005. p. 359-407.

18 [44] Huang P, Trachootham D, Alexandre J. Targeting cancer cells by ROS-mediated
19 mechanisms: a radical therapeutic approach? *Nat Rev Drug Discov* 2009;8:579-591.

20 [45] Huang P, Trachootham D, Zhou Y, Zhang H, Demizu Y, Chen Z, et al. Selective killing of
21 oncogenically transformed cells through a ROS-mediated mechanism by beta-phenylethyl
22 isothiocyanate. *Cancer Cell* 2006;10:241-252.

- [46] Nogueira V, Park Y, Chen C-C, Xu P-Z, Chen M-L, Tonic I, et al. Akt Determines Replicative Senescence and Oxidative or Oncogenic Premature Senescence and Sensitizes Cells to Oxidative Apoptosis. *Cancer Cell* 2008;14:458-470.
- [47] Schumacker PT. Reactive oxygen species in cancer cells: Live by the sword, die by the sword. *Cancer Cell* 2006;10:175-176.
- [48] Chen J-Y, Lee Y-M, Zhao D, Mak N-K, Wong RN-S, Chan W-H, et al. Quantum Dot-mediated Photoproduction of Reactive Oxygen Species for Cancer Cell Annihilation. *Photochemistry and Photobiology* 2010;86:431-437.
- [49] Chou KL, Meng H, Cen Y, Li L, Chen JY. Dopamine-quantum dot conjugate: a new kind of photosensitizers for photodynamic therapy of cancers. *J Nanopart Res* 2013;15:9.
- [50] Huang P, Lin J, Wang S, Zhou Z, Li Z, Wang Z, et al. Photosensitizer-conjugated silica-coated gold nanoclusters for fluorescence imaging-guided photodynamic therapy. *Biomaterials* 2013;34:4643-4654.
- [51] Ito S, Miyoshi N, Degraff WG, Nagashima K, Kirschenbaum LJ, Riesz P. Enhancement of 5-Aminolevulinic acid-induced oxidative stress on two cancer cell lines by gold nanoparticles. *Free Radic Res* 2009;43:1214-1224.
- [52] Vankayala R, Huang Y-K, Kalluru P, Chiang C-S, Hwang KC. First Demonstration of Gold Nanorods-Mediated Photodynamic Therapeutic Destruction of Tumors via Near Infra-Red Light Activation. *Small* 2013;10:1612-1622.
- [53] Perrier M, Gary-Bobo M, Lartigue L, Brevet D, Morere A, Garcia M, et al. Mannose-functionalized porous silica-coated magnetic nanoparticles for two-photon imaging or PDT of cancer cells. *J Nanopart Res* 2013;15:17.

- [54] Wang F, Chen XL, Zhao ZX, Tang SH, Huang XQ, Lin CH, et al. Synthesis of magnetic, fluorescent and mesoporous core-shell-structured nanoparticles for imaging, targeting and photodynamic therapy. *J Mater Chem* 2011;21:11244-11252.
- [55] Kim S, Ohulchanskyy TY, Pudavar HE, Pandey RK, Prasad PN. Organically modified silica nanoparticles co-encapsulating photosensitizing drug and aggregation-enhanced two-photon absorbing fluorescent dye aggregates for two-photon photodynamic therapy. *J Am Chem Soc* 2007;129:2669-2675.
- [56] Tao X, Yang Y-J, Liu S, Zheng Y-Z, Fu J, Chen J-F. Poly(amidoamine) dendrimer-grafted porous hollow silica nanoparticles for enhanced intracellular photodynamic therapy. *Acta Biomaterialia* 2013;9:6431-6438.
- [57] Zhao ZX, Huang YZ, Shi SG, Tang SH, Li DH, Chen XL. Cancer therapy improvement with mesoporous silica nanoparticles combining photodynamic and photothermal therapy. *Nanotechnology* 2014;25:285701.
- [58] Ohulchanskyy TY, Roy I, Goswami LN, Chen Y, Bergey EJ, Pandey RK, et al. Organically Modified Silica Nanoparticles with Covalently Incorporated Photosensitizer for Photodynamic Therapy of Cancer. *Nano Letters* 2007;7:2835-2842.
- [59] Xue C, Wu J, Lan F, Liu W, Yang X, Zeng F, et al. Nano Titanium Dioxide Induces the Generation of ROS and Potential Damage in HaCaT Cells Under UVA Irradiation. *Journal of Nanoscience and Nanotechnology* 2010;10:8500-8507.
- [60] Ding H, Mora R, Gao J, Sumer BD. Characterization and Optimization of mTHPP Nanoparticles for Photodynamic Therapy of Head and Neck Cancer. *Otolaryngology -- Head and Neck Surgery* 2011;145:612-617.

- [61] Kameyama NM, S. ;Itano, O. ;Ito, A. ;Konno, T. ;Arai, T. ;Ishihara, K. ;Ueda, M. ;Kitagawa, Y. Photodynamic Therapy Using an Anti-EGF Receptor Antibody Complexed with Verteporfin Nanoparticles: A Proof of Concept Study. Cancer Biotherapy and Radiopharmaceuticals 2011;26:697-704.
- [62] Derycke ASL, de Witte PAM. Liposomes for photodynamic therapy. Adv Drug Deliv Rev 2004;56:17-30.
- [63] Choi KHC, C. W. ;Kim, C. H. ;Kim, D. H. ;Jeong, Y. I. ;Kang, D. H. . Effect of 5-Aminolevulinic Acid-Encapsulate Liposomes on Photodynamic Therapy in Human Cholangiocarcinoma Cells. Journal of Nanoscience and Nanotechnology 2014;14:5628-5632.
- [64] Figueira Fv, M.R. Pereira P, Silva S, A.S. Cavaleiro J, P.C. Tome J. Porphyrins and Phthalocyanines Decorated with Dendrimers: Synthesis and Biomedical Applications. Current Organic Synthesis 2014;11:110-126.
- [65] Taratula O, Schumann C, Naleway MA, Pang AJ, Chon KJ, Taratula O. A Multifunctional Theranostic Platform Based on Phthalocyanine-Loaded Dendrimer for Image-Guided Drug Delivery and Photodynamic Therapy. Mol Pharm 2013;10:3946-3958.
- [66] Grimland JL, Wu C, Ramoutar RR, Brumaghim JL, McNeill J. Photosensitizer-doped conjugated polymer nanoparticles with high cross-sections for one- and two-photon excitation. Nanoscale3:1451-1455.
- [67] Shen X, Li L, Wu H, Yao SQ, Xu Q-H. Photosensitizer-doped conjugated polymer nanoparticles for simultaneous two-photon imaging and two-photon photodynamic therapy in living cells. Nanoscale3:5140-5146.

- [68] Zhang Y, Pang L, Ma C, Tu Q, Zhang R, Saeed E, et al. Small Molecule-Initiated Light-Activated Semiconducting Polymer Dots: An Integrated Nanoplatform for Targeted Photodynamic Therapy and Imaging of Cancer Cells. *Analytical Chemistry* 2014;86:3092-3099.
- [69] Tenery D, Worden JG, Hu Z, Gesquiere AJ. Single particle spectroscopy on composite MEH-PPV/PCBM nanoparticles. *Journal of Luminescence* 2009;129:423-429.
- [70] Wu C, Peng H, Jiang Y, McNeill J. Energy Transfer Mediated Fluorescence from Blended Conjugated Polymer Nanoparticles. *The Journal of Physical Chemistry B* 2006;110:14148-14154.
- [71] Kasai H, Nalwa HS, Oikawa H, Okada S, Matsuda H, Minami N, et al. A Novel Preparation Method of Organic Microcrystals. *Japanese Journal of Applied Physics Part 2-Letters* 1992;31:L1132-L1134.
- [72] Wu C, Bull B, Szymanski C, Christensen K, McNeill J. Multicolor Conjugated Polymer Dots for Biological Fluorescence Imaging. *ACS Nano* 2008;2:2415-2423.
- [73] Gao HJ, Shi WD, Freund LB. Mechanics of receptor-mediated endocytosis. *Proc Natl Acad Sci U S A* 2005;102:9469-9474.
- [74] Lerch S, Dass M, Musyanovych A, Landfester K, Mailander V. Polymeric nanoparticles of different sizes overcome the cell membrane barrier. *Eur J Pharm Biopharm* 2013;84:265-274.
- [75] Tang L, Gabrielson NP, Uckun FM, Fan TM, Cheng JJ. Size-Dependent Tumor Penetration and in Vivo Efficacy of Monodisperse Drug-Silica Nanoconjugates. *Mol Pharm* 2013;10:883-892.
- [76] Chung YI, Kim JC, Kim YH, Tae G, Lee SY, Kim K, et al. The effect of surface functionalization of PLGA nanoparticles by heparin- or chitosan-conjugated Pluronic on tumor targeting. *J Control Release* 143:374-382.

- [77] Clift MJD, Rothen-Rutishauser B, Brown DM, Duffin R, Donaldson K, Proudfoot L, et al. The impact of different nanoparticle surface chemistry and size on uptake and toxicity in a murine macrophage cell line. *Toxicol Appl Pharmacol* 2008;232:418-427.
- [78] Gratton SEA, Ropp PA, Pohlhaus PD, Luft JC, Madden VJ, Napier ME, et al. The effect of particle design on cellular internalization pathways. *Proc Natl Acad Sci U S A* 2008;105:11613-11618.
- [79] Xia T, Kovochich M, Liong M, Zink JI, Nel AE. Cationic polystyrene nanosphere toxicity depends on cell-specific endocytic and mitochondrial injury pathways. *ACS Nano* 2008;2:85-96.
- [80] Xu PS, Van Kirk EA, Zhan YH, Murdoch WJ, Radosz M, Shen YQ. Targeted charge-reversal nanoparticles for nuclear drug delivery. *Angew Chem-Int Edit* 2007;46:4999-5002.
- [81] Zhang Y, Yang M, Portney NG, Cui DX, Budak G, Ozbay E, et al. Zeta potential: a surface electrical characteristic to probe the interaction of nanoparticles with normal and cancer human breast epithelial cells. *Biomed Microdevices* 2008;10:321-328.
- [82] Huhn D, Kantner K, Geidel C, Brandholt S, De Cock I, Soenen SJH, et al. Polymer-Coated Nanoparticles Interacting with Proteins and Cells: Focusing on the Sign of the Net Charge. *ACS Nano* 2013;7:3253-3263.
- [83] Cook GM, Jacobson W. The electrophoretic mobility of normal and leukaemic cells of mice. *Biochem J* 1968;4:549-557.
- [84] Parker N, Turk MJ, Westrick E, Lewis JD, Low PS, Leamon CP. Folate receptor expression in carcinomas and normal tissues determined by a quantitative radioligand binding assay. *Analytical Biochemistry* 2005;338:284-293.

- 1 [85] Ross JF, Chaudhuri PK, Ratnam M. Differential regulation of folate receptor isoforms in
2 normal and malignant tissues in vivo and in established cell lines. Physiologic and clinical
3 implications. *Cancer* 1994;73:2432-2443.
- 4 [86] Toffoli G, Cernigoi C, Russo A, Gallo A, Bagnoli M, Boiocchi M. Overexpression of folate
5 binding protein in ovarian cancers. *Int J Cancer* 1997;74:193-198.
- 6 [87] Zhou H, Liu X, Liu L, Yang Z, Zhang S, Tang M, et al. Oxidative Stress and Apoptosis of
7 Human Brain Microvascular Endothelial Cells Induced by Free Fatty Acids. *J Int Med Res*
8 2009;37:1897-1903.
- 9 [88] Green M, Howman E. Semiconductor quantum dots and free radical induced DNA nicking.
10 *Chemical Communications* 2005;121-123.
- 11 [89] Lovric J, Cho SJ, Winnik FoM, Maysinger D. Unmodified Cadmium Telluride Quantum
12 Dots Induce Reactive Oxygen Species Formation Leading to Multiple Organelle Damage and
13 Cell Death. *Chemistry & Biology* 2005;12:1227-1234.
- 14 [90] Peng Q, Warloe T, Berg K, Moan J, Kongshaug M, Giercksky K-E, et al. 5-Aminolevulinic
15 acid-based photodynamic therapy. *Cancer* 1997;79:2282-2308.
- 16 [91] Kawase Y, Iseki H. Parameter-finding studies of photodynamic therapy for approval in
17 Japan and the USA. *Photodiagnosis and Photodynamic Therapy* 2013;10:434-445.
- 18 [92] Gao F, Yi J, Yuan JQ, Shi GY, Tang XM. The cell cycle related apoptotic susceptibility to
19 arsenic trioxide is associated with the level of reactive oxygen species. *Cell Res* 2004;14:81-85.
- 20 [93] Yi J, Gao F, Shi G, Li H, Wang Z, Shi X, et al. The inherent cellular level of reactive
21 oxygen species: One of the mechanisms determining apoptotic susceptibility of leukemic cells to
22 arsenic trioxide. *Apoptosis* 2002;7:209-215.

- [94] Gesquiere AJ, Park S-J, Barbara PF. Hole-Induced Quenching of Triplet and Singlet Excitons in Conjugated Polymers. *J Am Chem Soc* 2005;127:9556-9560.
- [95] Halas NJ, Hale GD, Oldenburg SJ. Dynamics of triplet excitons in MEH-PPV measured by two-photon photoemission. *Proc SPIE, Optical Probes of Conjugated Polymers* 1997;3145:229-239.
- [96] Monkman AP, Burrows HD, da G. Miguel M, Hamblett I, Navaratnam S. Measurement of the S₀-T₁ energy gap in poly(2-methoxy,5-(2'-ethyl-hexoxy)-*p*-phenylenevinylene) by triplet-triplet energy transfer. *Chemical Physics Letters* 1999;307:303-309.
- [97] Gesquiere AJ, Park S-J, Barbara PF. F-V/SMS: A New Technique for Studying the Structure and Dynamics of Single Molecules and Nanoparticles. *The Journal of Physical Chemistry B* 2004;108:10301-10308.
- [98] Burrows HD, Seixas de Melo J, Serpa C, Arnaut LG, Miguel MdG, Monkman AP, et al. Triplet state dynamics on isolated conjugated polymer chains. *Chemical Physics* 2002;285:3-11.
- [99] Henderson BW, Dougherty TJ. How Does Photodynamic Therapy Work? *Photochemistry and Photobiology* 1992;55:145-157.
- [100] Park S-J, Gesquiere AJ, Yu J, Barbara PF. Charge Injection and Photooxidation of Single Conjugated Polymer Molecules. *J Am Chem Soc* 2004;126:4116-4117.
- [101] Chambon S, Rivaton A, Gardette J-L, Firon M. Reactive intermediates in the initiation step of the photo-oxidation of MDMO-PPV. *Journal of Polymer Science Part A: Polymer Chemistry* 2009;47:6044-6052.
- [102] Fariss MW, Chan CB, Patel M, Van Houten B, Orrenius S. Defining mitochondrial targets to combat the pleiotropic effects of toxic oxidative stress. *Mol Interv* 2005;5:94-111.

1 [103] Halliwell B, Chirico S. Lipid-Peroxidation - Its Mechanism, Measurement, and
2 Significance. Am J Clin Nutr 1993;57:S715-S725.

3 [104] Halliwell BG, J. M. C. . Oxygen-Toxicity, Oxygen Radicals, Transition-Metals and
4 Disease. Biochemical Journal 1984;219:1-14.

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Figure Captions

Fig. 1. A) Chemical structure of the conducting polymer MEH-PPV. Size distribution of CPNPs obtained by dynamic light scattering. The average size of the CPNPs is 34 ± 6 nm. C) Absorbance (red line) and emission (black line) spectra of CPNPs in water.

Fig. 2. From top to bottom confocal fluorescence images and corresponding phase contrast images of TE-71, MDA-MB-231, A549 and OVCAR3 cells incubated with increasing doses of CPNPs. CPNP doses are indicated at the top of the figure and are expressed in units of 10^{-4} mg/ml. Green dots represent the fluorescence from CPNPs and the blue color indicates nuclei stained with DAPI.

Fig. 3. Flow cytometry data on uptake of CPNPs by each cell line. Representative data showing normalized cell count of A) TE 71, B) MDA-MB-231, C) A549 and D) OVCAR3 cell lines with respect to detected CPNP fluorescence. E) Percentage of the cell population for each cell line that has internalized CPNPs (green bars) together with controls (red bars, no CPNPs, $n=2$), F) Average fluorescence intensity detected from CPNPs for each cell line depicted by green bars, together with controls (red bars, no CPNPs, $n=2$). The geometric mean of the fluorescence intensity distribution was used to plot the bar graphs in (F).

Fig. 4. Confocal slices of an A549 cell with CPNPs (green dots) at the periphery of the nucleus (blue).

Fig. 5. Single particle imaging and spectroscopy on CPNPs inside A549 cells. A) Bright field image of an A549 cell. B) Raster-scanned fluorescence image of the corresponding A549 cell with CPNPs inside the cell. The CPNPs can be seen clearly as spherical bright spots in the magnified region of the image shown in panel C). D) The ensemble spectrum of CPNPs inside the cell (black line) has λ_{max} at 588nm. The spectral shape and emission maximum indicate that the CPNPs remain stable in the intracellular environment. The inset in D) shows the autofluorescence of the cell with λ_{max} 550nm. A shoulder at this wavelength can be seen in the spectrum of CPNPs.

Fig. 6. MTT viability assays to evaluate the intrinsic cytotoxicity of CPNPs. A) TE-71, B) MDA-MB-231, C) A549 and D) OVCAR3 with control dose (0 mg/ml) and three doses of CPNPs (0.4×10^{-4} mg/ml, 2×10^{-4} mg/ml and 3.6×10^{-4} mg/ml). The incubation periods are 0, 24, 48, 72 and 96 hours.

Fig. 7. MTT assay to evaluate the cell viability after application of PDT. Post-PDT incubation period A) 0 hours, B) 4 hours, and C) 12 hours. Each panel has four cell lines, TE-71, MDA-MB-231, A549 and OVCAR3. Each cell line is administered with three doses of CPNPs (0.4×10^{-4} mg/ml, 2×10^{-4} mg/ml and 3.6×10^{-4} mg/ml) and 0 mg/ml as the control dose. The colored bars represent dose of light (red: 60 J/cm^2 , green: 120 J/cm^2 , blue: 180 J/cm^2).

Fig. 8. Epiluminescence images with corresponding phase contrast images of A549 and OVCAR3 cell lines for detection of ROS in by CellRox green reagent. A) Control in absence of treatment (no CPNPs, no light). B) Negative control by means of light exposure in absence

- 1 CPNPs. C) Negative control by means of incubation with CPNPs in absence of light exposure.
- 2 CPNP emission can be detected. D) Fluorescence images taken immediately after PDT, and E) 2
- 3 hours post-PDT. F) Positive control with 100 μ M H₂O₂.

Figure 1

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