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Phosphorescent Pt(II) complexes spatially arrayed in micellar polymeric nanoparticles providing dual readout for multimodal imaging

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Abstract

In this paper we report phosphorescent Pt(II) complexes as monomers which can be directly incorporated into growing polymers. Due to the amphiphilic nature of the polymers they can self-assemble into micellar nanoparticles, where the phosphorescent Pt(II) complexes can arrange selectively in the core or shell of the nanoparticles. The complexes enable dual orthogonal imaging, made possible by the heavy metal, which enhances the contrast for these micelles in electron microscopy and facilitates spin-orbit coupling that turns on microsecond lifetime luminescence.

Re(I), Pt(II), and Ir(III), complexes have been explored extensively for bioimaging and phototherapy.^{1–6} In the case of Pt(II) complexes, phosphorescence can occur via spin-orbit coupling, which mixes singlet and triplet excited states. The electronic configuration of the metal center results in square-planar coordination compounds able to π -stack, which in turn

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favors metal-metal interactions involving the d_z^2 orbitals protruding out of the coordination plane. The splitting into doubly occupied bonding $d-d$ and empty antibonding $d-d^*$ orbitals shifts the absorption and the luminescence into the red: monomeric species emit from ligand-centered excited states with a sizeable triplet metal-to-ligand charge-transfer character (3MLCT), whereas the aggregates involve metal-metal-to-ligand charge-transfer states (3MMLCT). When the Pt-Pt distance is decreased, the red-shift is in turn enhanced and the excited state lifetimes shortened due to extended metal participation favoring radiative deactivation.⁷ The long-lived excited states of triplet emitters are usually quenched by molecular oxygen in solution, leading to highly reactive and therefore cytotoxic singlet oxygen. On the other hand, with relevance to bioimaging, the oxygen sensitivity can be used to monitor the concentration of oxygen as hypoxic areas light up with longer excited state lifetimes.⁸ We have shown that Pt(II) complexes can self-assemble into phosphorescent polymers, fibers and gels, leading to a $1D$ -confinement with significant Pt-Pt interactions and reduced diffusional quenching by molecular oxygen.^{9–11} At metallic and semiconducting interfaces, we monitored their $2D$ -confinement using optical as well as scanning tunneling microscopy and spectroscopy along with XPS and cyclic voltammetry to track hybridization with the substrate and intermolecular interactions.^{12–14} This class of Pt-based phosphorescent dye and their intramolecular interactions and spatial confinement¹⁵ provide avenues for multimodal imaging strategies. We reasoned that if one could gain control over that confinement within nanostructures, optical and electron microscopies could be coupled to track the complexes within nanoassemblies and in biological systems. This approach would take advantage of the unique photophysical properties of the Pt(II) complexes, and the high contrast offered by the metal centers, respectively. In this paper, we introduce such a strategy for spatially arraying photoactive Pt(II) complexes by selectively loading them in the core or shell of nanoparticles (*NPs*). The complexes are forced into the core or shell based on their distal or adjacent position with respect to the hydrophilic block of amphiphilic block copolymers that make up spherical micellar *NPs*. Spectrally- and time-resolved luminescence measurements, as well as Transmission Electron Microscopy (*TEM*) imaging techniques enabled the visualization of the metal complexes and gave information on their aggregation state resulting from Pt-Pt interactions within the *NPs*.

Pt(II)-complexes with luminescent properties were introduced into polymers as part of the main chain or as side and end groups utilizing post polymerization methods.^{16–18} We designed a Pt(II) complex as the first example of a Pt(II)-based phosphorescent dye capable of being directly incorporated into a growing polymer as a side chain (Fig. 1). The Pt(II)-based luminophore bearing a cyclometallating tridentate luminophore and an ancillary ligand bearing a norbornene moiety was directly polymerized via Ring Opening Metathesis Polymerization (*ROMP*) utilizing a functional-group tolerant, modified 2nd generation Grubbs' catalyst.^{19–21} The ligand precursors as well as the Pt(II) complex were synthesized as previously described.²² The Pt-containing monomer (**M_{Pt}**) was obtained by a ligand exchange reaction in which the chlorido moiety was replaced by a norbornene-substituted pyridine (Scheme S1 and S2). Time-dependent 1H NMR was used to confirm complete monomer polymerization (Fig. S1–S3). To obtain polymer amphiphiles capable of self-assembling into particles, **M_{Pt}** was copolymerized with a hydrophobic aminoethyl-diisopropylamine substituted norbornene monomer (**M_{am}**) and a hydrophilic oligoethylene-

glycol (*OEG*) modified monomer (**M_{OEG}**). Two polymers were synthesized aiming for conserved block lengths but with different monomer sequences. Polymer **P_{shell}** (**M_{am}-b-M_{OEG}-b-M_{Pt}**) placed the Pt-containing block adjacent to the hydrophilic block, whereas **P_{core}** (**M_{OEG}-b-M_{am}-b-M_{Pt}**) placed the metal distal. Both polymers showed monomer incorporation as expected with a narrow molecular weight distribution (Fig. S4 and Table S1). The two micellar *NPs* (**NP_{core}** and **NP_{shell}**) were prepared by dialysis of these two polymers from *DMF* into a 50:50 mixture of *DMF* and 10% *PBS*.

The two classes of soft nanoparticles, **NP_{core}** and **NP_{shell}**, were characterized by Dynamic Light Scattering (*DLS*, Fig. S5) and dry-state unstained *TEM* (Fig. S6, S7 and S8), where similar morphologies and diameters (30 nm) were observed (Fig. 1). Background-corrected Energy Filtered *TEM* (*EFTEM*) acquired using the energy loss of a Pt-edge along with high-angle annular dark-field (*HAADF*) imaging confirmed differential Pt-localization within the two different particles (Fig. 1, S9 and S10). Despite the hydrophobic character of **M_{Pt}**, **NP_{shell}** displayed a specific enrichment of Pt-atoms in the shell, probably due to the length ($n = 93$) and brush-like character of the hydrophilic block which forces the Pt-based luminophore to locate in the shell. *EFTEM* on **NP_{core}** showed that their contrast is homogenous across the particle, indicating a dispersed distribution of Pt within the hydrophobic domain probably due to the lack of phase separation between the two hydrophobic blocks. It should be noted that although **NP_{shell}** and **NP_{core}** appeared similar in size by *DLS* and dry-state *TEM* (30 nm), we also observed by *EFTEM* and *HAADF* that **NP_{shell}** appears slightly larger than **NP_{core}**, suggesting that the hydrophilic shell is visualizable because of shell-localized Pt-atoms. Furthermore, the thickness of the shell of **NP_{shell}** roughly reflects the difference in size between **NP_{core}** and **NP_{shell}** observed in *TEM* measurements. Diffraction contrast imaging (*TEM* bright-field and dark-field) shows that the shell of **NP_{shell}** is strongly coherently diffracting compared to the core. However, the electron diffraction pattern does not show pronounced single reflections. This confirms a partially nanocrystalline or locally ordered shell in the case of **NP_{shell}**. By contrast, no indication for coherently scattering domains can be observed for **NP_{core}**. The data demonstrate that metal-based luminophores can be loaded as *TEM*-contrast agents into micellar nanoparticles and that their localization within the resulting nanoparticle can be set by polymer design.

To study the photophysical properties of the materials, **M_{Pt}** was characterized by means of steady-state and time-resolved luminescence spectroscopy, showing the characteristic metal-perturbed ligand-centered character of the emitting triplet state (³*MP-LC*) with an excited state lifetime in the microsecond range that is quenched by molecular dioxygen (Fig. S11 and S14-S15). At 77K, the frozen matrix yields a clearly resolved vibrational progression and a longer lifetime (Fig. S12 and S16), which confirms the previous assignment. These results agree with our report on this class of complexes. In the solid state, the complex shows a red-shifted, unstructured emission band, which indicates aggregation and emission from ³*MMLCT* states. In the excitation spectrum, the band around 375 – 475 nm is extended up to 550 nm, as aggregation is already present in the ground state (Fig. S13). Such phenomena have been previously described for this type of complex in the solid state when small ancillary ligands are introduced.²² The amplitude-weighted average lifetimes are

shortened from 9 μ s in solution at room temperature (13 μ s at 77K) to 1.2 μ s in the solid (Fig. S17). In accordance, both classes of micelles show a characteristic monomeric emission profile peaking at 500 nm (Fig. 2). If compared with **M_{Pt}**, the excited state lifetimes at 500 nm are significantly shortened (3.0–3.5 μ s, Fig. S18–S21) most likely due to intermolecular quenching. Interestingly, the luminescence of the micelles is not significantly quenched by dissolved molecular dioxygen, showing the compact nature of the assemblies. There is a prominent feature around 650 nm upon confinement in the shell, which is indicative of aggregation-related Pt-Pt interactions as previously observed in the solid state for **M_{Pt}** and also confirmed by the red-shifted excitation band and shortened lifetime (1.4 μ s) when monitoring the luminescence at 600 nm. When distributed in the core, these additional excitation and emission bands were not observed (see Fig. 2). These results demonstrate that Pt(II) complexes that are forced to accumulate in the shell (**NP_{shell}**) are 2D-confined in a smaller volume than the ones distributed within **NP_{core}**. This leads to shorter average Pt-Pt distances for **NP_{shell}**, interaction of d_z^2 -orbitals, and the consequent population of 3MMLCT states. At the same time, the confinement is soft enough to allow the simultaneous emission from monomeric ^3MP-LC states. In contrast, Pt(II) complexes from **NP_{core}** show only monomeric ^3MP-LC luminescence and no measurable interactions between the metal-centers in accordance with a larger 3D-distribution volume. Only the excited state lifetimes for **NP_{core}** at 600 nm (1.4 μ s) suggest that part of the emission might arise from aggregated species.

As a proof-of-concept demonstration of the ability of Pt(II)-based luminophores to serve as multimodal markers in dynamic soft assemblies including biological environments, **NP_{shell}** were incubated with *HeLa* cells and uptake was monitored with two orthogonal readouts, namely *TEM* and Two-Photon Laser Scanning Microscopy (*TPLSM*). For *TEM*, ultrathin slices were prepared from *HeLa* cells incubated for 30 min either with **NP_{shell}** (Fig. 3, top left panels) or the vehicle control (*PBS*) (Fig 3. top right panels). *TEM* images show conserved morphological structures, such as cellular and nuclear membranes and organelles. However, cells incubated with **NP_{shell}** show high-contrast aggregates at distinct stages of the uptake process. One set of aggregates seems to be attached to the cellular membrane, prior to uptake (bottom yellow arrow in Fig. 3). In comparison to untreated cells, high-magnification *TEM* images taken from cells after incubation with **NP_{shell}** show high-contrast aggregates in the intracellular space as evidence for uptake.

Taking advantage of the Pt(II)-based luminophores, cellular uptake of **NP_{shell}** was tracked in live cells using *TPLSM*. Two-photon excitation at 750 nm was used, as the maximum single-photon absorbance of the luminophores lies in the *UV* (Fig. 2 and S22). *HeLa* cells were incubated with **NP_{shell}** for ca. 60 min and imaged subsequently without fixation (Fig. 3, left bottom panels). *NPs* were observed in the intracellular space as bright spots, which were visualized without any additional luminescent staining. In control samples incubated with *PBS*, only background signals from cellular structures were observed (Fig 3, bottom right panels). This background signal was used in both cases (**NP_{shell}** treated and control cells) to define the cell outline and an intra and extracellular space. *NPs* appear to have different sizes when comparing both imaging techniques. *TPLSM* is an optical modality and has a

diffraction-limited spatial resolution of about 0.3 μm , which also may account for the observed *NP* size in comparison to *TEM*.

The proof-of-concept study presented herein demonstrates that Pt(II) complexes can be incorporated into self-organizing copolymers that yield water-soluble micelles with site-specific confinement of the heavy atom. The confinement within the soft nanostructures and the resulting intermetallic interactions can be traced by monitoring a dual readout. The compact nature of the packing avoids quenching of the luminescence by molecular dioxygen, which in turn prevents the formation of cytotoxic reactive oxygen species. Furthermore, we demonstrated that Pt(II) complexes can be used as multimodal molecular imaging agents with orthogonal readouts, as the heavy atoms can be tracked in complex yet dynamic environments including living entities, both by electron as well as luminescence microscopy.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1.

Synthetic route towards polymeric *NPs* and their characterization. Left: The particles were prepared by assembly of copolymer amphiphiles incorporating the Pt(II)-containing luminophore. Polymerization of **M_{am}** as the hydrophobic block, followed by **M_{OEG}** as the hydrophilic block and the addition as **M_{Pt}** as the last monomer, yielded polymer **P_{shell}** (top). Polymerization of **M_{OEG}** as a first hydrophilic block, followed by **M_{am}** as the hydrophobic block and the addition as **M_{Pt}** as the last monomer, yielded polymer **P_{core}** (bottom). Dialysis into 10% *PBS* formed **NP_{shell}** or **NP_{core}** (top and bottom, respectively). Center: dry state and unstained *TEM* images of **NP_{shell}** (top) and **NP_{core}** (bottom). Both micelle populations show a diameter of approximately 30 nm, confirmed by *DLS* measurements as well (insets). Right: Energy Filtered *TEM* (*EFTEM*) analysis of the *NPs* using a three-window-method of the Pt-O-edge shows a fairly homogenous distribution of Pt atoms in **NP_{core}** while **NP_{shell}** shows an enrichment of Pt at preferentially in the shell.

Figure 2. Normalized excitation (dashed lines, $\lambda_{\text{em}} = 520$ nm) and emission (solid lines, $\lambda_{\text{ex}} = 320$ nm) spectra of NP_{shell} (top) and NP_{core} (bottom) in diluted PBS at room temperature (0.5 mg/mL). The dotted excitation spectrum was obtained by monitoring the emission at 600 nm, the ellipses highlight the bands corresponding to the excitation into $^1\text{MMLCT}$ and emission from $^3\text{MMLCT}$ states, whereas the arrows indicate the wavelengths at which the given amplitude-weighted average lifetimes have been measured (see also ESI).

Figure 3.

Top: TEM images of *HeLa* cells pretreated with *PBS* (right) or **NP_{shell}** (left) at a concentration of 0.1 mg/mL for 30 min and imaged at different magnifications. **NP_{shell}** attached to the cell membrane or in the intracellular space are shown with yellow arrows.

Bottom: Two-photon laser scanning microscopy (not time-gated) of *HeLa* cells pretreated with *PBS* (right) or **NP_{shell}** (left) at a concentration of 0.1 mg/mL polymer in *PBS* for 60–75 min. Cells were imaged in modified Tyrode's solution under two-photon excitation at 750 nm and fluorescence was collected through a 490–560 nm bandpass filter. **NP_{shell}**-pretreated cells show bright luminescent spots, indicated by yellow arrows.