Persistent Luminescence

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Semiconducting Polymer Nanoparticles with Persistent Near-Infrared Luminescence for In Vivo Optical Imaging

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Abstract: *Materials with persistent luminescence are attractive for in vivo optical imaging since they have a long lifetime that allows the separation of excitation of fluorophores and image acquisition for time-delay imaging, thus eliminating tissue autofluorescence associated with fluorescence imaging. Persistently luminescent nanoparticles have previously been fabricated from toxic rare-earth metals. This work reports that nanoparticles made of the conjugated polymer MEH-PPV can generate luminescence persisting for an hour upon single excitation. A near-infrared dye was encapsulated in the conjugated polymer nanoparticle to successfully generate persistent near-infrared luminescence through resonance energy transfer. This new persistent luminescence nanoparticles have been demonstrated for optical imaging applications in living mice.*

Persistent luminescence is the oldest known photo-luminescent phenomenon, with a history dating back to the discovery of the Bologne stone in the 17th century.[1,2] In spite of the long history, it has been rarely used for imaging applications in comparison to fluorescence. In vivo fluorescence imaging often suffers from high tissue autofluorescence. To better separate autofluorescence from target fluorescence signal,[3] extensive efforts have been made to develop fluorophores with larger Stokes shifts, and with emissions at near-infrared wavelengths. Alternatively, dyes with long excited-state lifetimes can enable time-resolved fluorescence measurements since the lifetime of autofluorescence is often very short (typically at nanoseconds). Phosphorescence emits through triplet states with a lifetime of the order of milliseconds—long enough to enable the time-resolved detection, but its intensity is generally weak and sophisticated instrumentation is required. Persistent luminescence can persist for minutes or even hours after initial light absorbance.[4] This extremely long lifetime thus allows for easy implementation of time-resolved luminescence imaging without additional instrumentation. Several persistent luminescent nanoparticles have been reported for in vivo optical imaging, all of which are based on rare-earth heavy metals like europium[5] or praseodymium,[6] and are poorly biocompatible because of potential toxicity.[7–9] In addition, their inorganic nature presents challenges for conjugation with organic groups or targeting ligands.[10]

Semiconducting polymer nanoparticles emerge as a new class of fluorescent nanomaterials with excellent optical properties.[11] They have been demonstrated for fluorescence, chemiluminescence, and photoacoustic imaging in living cells and small animals.[12] Here, we report the first example of semiconducting conjugated polymer nanoparticles that can emit persistent luminescence with a lifetime of nearly one hour after a single excitation exposure to white light, and show potential for in vivo optical imaging.

MEH-PPV is a fluorescent semiconducting polymer that has been widely used in solar panels,[13] as non-metallic conducting materials.[14,15] We used nano-precipitation of MEH-PPV together with PS:PEG:COOH to synthesize nanoparticles with better biocompatibility and surface functionalization.[16] NIR775, a small molecular dye with a near-infrared emission at 780 nm (Figure 1a), was encapsulated in the nanoparticles to enable near-infrared emission. The size

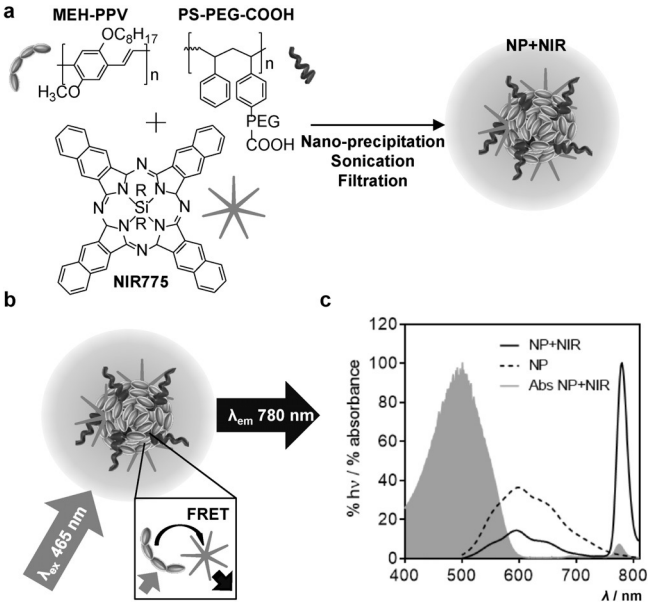

Figure 1. Synthesis and fluorescence properties of the persistent luminescent polymer nanoparticles. a) Nano-precipitation of polymer nanoparticles doped with NIR775 followed by sonication and filtration. b) Schematic of the Förster resonance energy transfer (FRET) from the MEH-PPV polymer to the NIR775 dye. c) Fluorescence spectra of the nanoparticle (NP) with and without NIR775 dye, and absorbance spectrum of the NP with NIR775.

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of the nanoparticles measured by dynamic light scattering (DLS) was in the range of 20–50 nm, which is similar to that of previously reported polymer nanoparticles.[12e] The absorbance spectrum of the NIR775-doped nanoparticles (Figure 1c) shows the peak absorption in the violet to orange range: 400–600 nm. There is a small absorption peak at 770 nm due to the doped NIR775 dye. The fluorescence emission spectra (Figure 1c) of the nanoparticles with and without encapsulated NIR775 show a broad emission from 500–700 nm attributed to the MEH-PPV polymer. There is a sharp, strong peak at 775 nm arising from the NIR775 doped nanoparticles. Emission from the MEH-PPV at 500–700 nm is clearly lower in the NIR775-doped nanoparticles compared with the undoped nanoparticles at the same concentration of nanoparticles, suggesting a significant transfer of energy from the MEH-PPV polymer to the NIR775 dye by the FRET mechanism (Figure 1b).

The NIR775-doped nanoparticles emit near-infrared persistent luminescence upon excitation with white light. A maximum near-infrared persistent luminescence radiance of $1\times10^{7}\,\mathrm{p\,(s\,cm^{2}\,sr)^{-1}\,mg^{-1}}$ (λ_{em} at 780 ± 10 nm) was attained following excitation (Figure 2a,b and Supporting Information (SI) Figure S1). The persistent luminescence spectrum closely resembles its fluorescence emission spectrum (Figure 2a), which also shows a broad emission from 500–700 nm originating from the MEH-PPV and a sharp peak at 780 nm corresponding to the NIR775 dye. The nearly identical spectra suggest that the persistent luminescence originates from the MEH-PPV polymer, and that energy transfer from MEH-PPV to the encapsulated NIR775 dye also occurs in the persistent luminescence. The persistent luminescence emission decay was quantified at three temperatures: 4 °C, 28 °C and 35 °C (Figure 2b). The emission curve could be fitted to a bi-exponential decay, with two rate constants shown in Table 1. The maximum radiance of persistence luminescence emission decreased by 40 % when the temperature decreased from 35 °C to 28 °C, and 94 % from 35 °C to 4 °C (Table 1). The decrease of the temperature significantly lowered the slow half-life. Interestingly, no persistent luminescence was detected from nanoparticles made of a different polymer PFO-DPT, though it is unclear what in the MEH-PPV polymer is essential for the persistent luminescence (Figure 2c,d). Multiple re-excitations over the time course of a week were possible while there was a 29 % decline in the intensity by the end of 4 days after 7 re-excitation cycles (Figure 2e). The presence of NIR dye is not absolutely required for the persistent luminescence emission since the non-doped MEH-PPV nanoparticles also possess this property (SI Figure S2). Interestingly, at the same concentration, the persistent emission from the MEH-PPV polymer core (600 nm) is higher when nanoparticles are doped with the NIR dye, suggesting the encapsulated NIR dye can help store more excitation energy in the nanoparticles.

Table 1: Persistent luminescence rate constants.

	4 °C	28 °C	35 °C
Half-life fast [$\mathrm{min^{-1}}$]	1.2	3.1	2.3
Half-life slow [$\mathrm{min^{-1}}$]	104.0	16.0	14.0
Max average radiance [$\mathrm{p\,(s\,cm^{2}\,sr)^{-1}}$]	7947	76677	126737
R^2	0.91	0.90	0.99

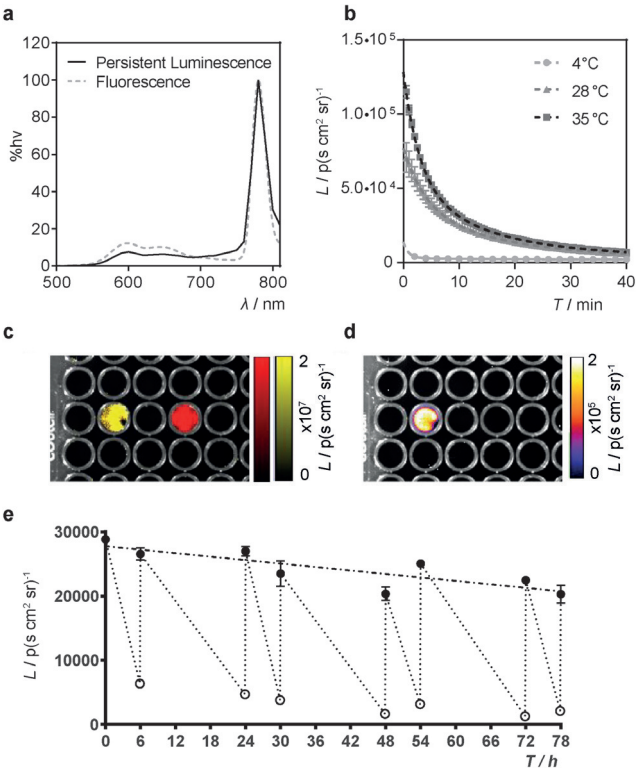

Figure 2. a) Fluorescence (FL) and persistent luminescence (PL) spectra of MEH-PPV nanoparticles doped with NIR775. b) Time-dependent persistent luminescence intensity at 4, 28 and 35 °C. c) Fluorescence emission from MEH-PPV (yellow) and PFO-DPT (red) nanoparticles. d) Persistent luminescence from two nanoparticles in (c). e) Persistent luminescence from MEH-PPV nanoparticles (average over 5 min) measured immediately after excitation (full circles) and just before re-excitation (open circles). Nanoparticles were stored in dark at 4 °C between excitations.

The potential of the persistent luminescence nanoparticles for in vivo imaging was evaluated by subcutaneous injection of NIR775-doped MEH-PPV polymer nanoparticles on the back of anaesthetized mice. Prior to injection, nanoparticles (16 μg in 40 μL) were excited with white light, and animal imaging was performed in the absence of excitation light (Figure 3). A maximum radiance of $5\times10^{5}\,\mathrm{p\,(s\,cm^{2}\,sr)^{-1}}$ was observed immediately after injection, which is at the same order of magnitude as that reported by Masne de Chermon et al.[5] after subcutaneous injection of pre-excited $Ca_{0.2}Zn_{0.9}Mg_{0.9}Si_2O_6$ nanoparticles (1 mg). The injected nanoparticles could be re-excited by placing the animal directly under a white light source for repetitive imaging. At 2 h post injection, there was a small decline in the luminescence intensity after re-excitation, and a larger decline in intensity after 24 h possibly due to the clearance of the nanoparticles through the lymphatic system as shown previously.[12e]

To further evaluate the in vivo distribution and clearance of the nanoparticles, we intravenously injected nanoparticles (16 μg/40 μL of nanoparticles in 160 μL saline) that had been

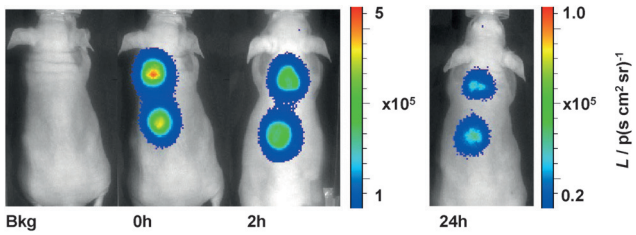

Figure 3. In vivo optical persistent luminescence imaging of a mouse before (Bkg) and after (0 h) subcutaneous injection of nanoparticles (≈16 μg) that had been excited prior to injection. At 2 h and 24 h later, the subcutaneous injection site was exposed to white light for 3 min first before the collection of persistent luminescence (in the absence of any excitation light).

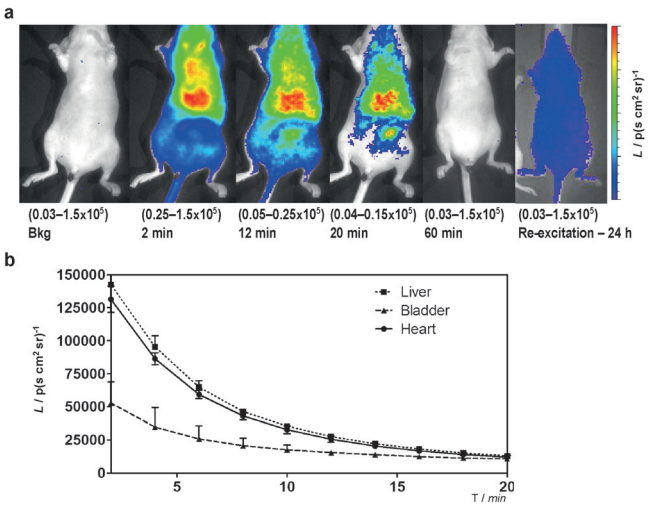

Figure 4. In vivo systemic persistent luminescence imaging. a) Images of a mouse before (Bkg) and after intravenous injection of nanoparticles (≈16 μg) which had been excited prior to injection and imaged at 2, 12, 20, and 60 min, also at 24 h following 3 min exposure to excitation light; the number in brackets indicates the intensity scale of the individual images. b) Time-course luminescence emission intensity at three regions of interest.

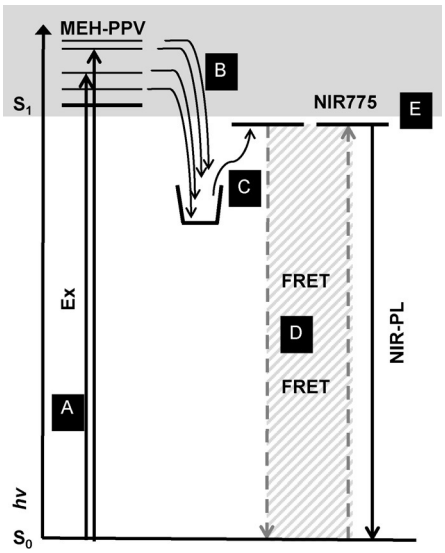

Figure 5. Proposed mechanism of persistent luminescence in polymer nanoparticles: The nanoparticles are first excited (A) and the excitation energy is trapped within in the semiconducting layer of the polymer (B) before released from the trap after activation energy (heat) is applied (C). Part of the relaxation energy from the energy trap may be transferred to the near-infrared dye NIR775 (D), and emit persistent luminescence from the nanoparticle and the near-infrared dye (E).

excited prior to injection, and continuously imaged the persistent luminescence emission in the absence of any further excitation light for 60 min (Figure 4a). There was an initial high uptake of nanoparticles in the heart and liver, with a local average radiance exceeding 1×10^5 p(s cm^2 sr)$^{-1}$ (Figure 4b). The initial overall intensity was comparable to that of other metal-based persistent luminescence nanoparticles.[6,17] As expected, the persistent luminescence imaging offers much improved signal-to-noise ratio in comparison to fluorescence imaging due to its extremely low background emission (SI Figure S4).

In comparison to other luminescent nanoparticles such as those enabled by bioluminescence proteins[18] and Cerenkov emission,[19] the persistent luminescent emission does not require the injection of substrates or the use of radioactive isotopes, and works in a different mechanism. Based on previous reports about the mechanism of persistent luminescence,[1] we propose a similar mechanism for the energy transformation in the polymer nanoparticles (Figure 5): Upon light excitation, the excitation energy is captured and stored in the semiconducting layer of the MEH-PPV polymer double bonds. Conjugating polymers are known for their ability in storing light energy due to their long π-conjugation conducting band.[20] The trapped excitation energy may re-emit as light through stimulated release by application of activation energy (heat).[21] In the presence of a near-infrared dye, this released energy can also be transferred through a mechanism similar to FRET, resulting in near-infrared persistent luminescence. In consistence with this mechanism, we observed temperature-dependent persistent luminescence emission (Figure 2b). Furthermore, the emission ratio between the NIR dye (780 nm) and the polymer core (600 nm) appears to be dependent on the temperature too (SI Figure S3), which may be explained in light of different excitation energy required for the NIR dye and polymer core in step (C). Further mechanistic studies should help understand the trapping mechanism, explain the bi-exponential decay mode and elucidate the structure requirement for persistence emission.

Many temporal scales of cellular events are already within the lifetime of our luminescence nanoparticles, so they may be continuously imaged with just one excitation so autofluorescence background and photobleaching will be greatly reduced. The organic structure also enables straightforward synthetic modification to introduce functional groups, for example, cyclic RGD for tumor targeting imaging,[22] in addition to low in vivo toxicity and better biocompatibility.[8,9]

In summary, we report a semiconducting conjugated polymer nanoparticle with both near-infrared persistent luminescent and fluorescent emissions. The persistent lumi-

nescent emission has been applied for in vivo optical imaging in mice, which provides support for future applications in cell tracking and molecular imaging. To the best of our knowledge, this is one of the first examples of conjugated polymer nanoparticles that emit persistent luminescence. More investigations will help understand the luminescent emission mechanism in order to exploit its full potential as novel biomaterials for optical imaging applications.

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