



# Facile preparation of water dispersible red fluorescent organic nanoparticles and their cell imaging applications



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## ABSTRACT

Red fluorescent organic nanoparticles (FONs) based on a cyano-substituted diarylethylene and tetraphenylethene derivative conjugated molecule (**R-TPE**) were facily prepared via surfactant modification with lecithin for the first time. The obtained **R-TPE-LEC** FONs were characterized by a series of techniques including fluorescence and UV spectroscopy, Fourier transform infrared spectroscopy, and transmission electron microscopy. Biocompatibility evaluation and cell uptake behavior of **R-TPE-LEC** FONs were further investigated to explore their potential biomedical application. We demonstrated that such red FONs exhibit anti-aggregation-caused quenching property, broad excitation wavelength, high water dispersibility, uniform morphology (40–60 nm), and excellent biocompatibility, making them promising for cell imaging application.

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## 1. Introduction

Optical bioimaging is one of the rapid growing areas in biological and biomedical research.<sup>1</sup> Fluorescence cellular probes with red/near-infrared (R/NIR, >600 nm) emission are highly desirable for biological applications due to their low optical absorption and autofluorescence of biological media.<sup>2–5</sup> Up to date, various fluorescent materials including organic dyes,<sup>6</sup> fluorescent proteins,<sup>7</sup> fluorescent inorganic nanoparticles (NPs)<sup>8–13</sup> have been used as R/NIR probes. However, many of these fluorescent materials often suffer from obvious disadvantages, such as water insolubility, photobleaching, and toxicity, which severely limited their practical bioimaging applications. For example, most of organic dyes are intrinsic hydrophobic and instable in biological media.<sup>14</sup> Usage of fluorescent proteins is often limited due to the high cost, low molar absorptivity, and low photobleaching thresholds.<sup>15</sup> While inorganic NPs or quantum dots are non-biodegradable and often toxic to living organisms.<sup>16–19</sup> Compared with the current used fluorescent bioprobes, fluorescent organic nanoparticles (FONs) have recently received more and more attentions due to flexible synthetic approaches of these small organic molecules and their biodegradation potential.<sup>20–25</sup> Because of these virtues, various FONs

including fluorescent conjugated polymers, polydopamine nanoparticles, and aggregation-induced-emission (AIE) or aggregation induced emission enhancement (AIEE) materials based FONs have been reported in recent years.<sup>26–37</sup> Among them, the AIE or AIEE based FONs have attracted great research interest because they could overcome the notorious aggregation-caused quenching (ACQ) effect of most organic dyes. Different AIE or AIEE units, such as siloles,<sup>38</sup> diphenylacrylonitrile,<sup>39–43</sup> tetraphenylethene,<sup>44–48</sup> triphenylethene,<sup>49–54</sup> distyrylanthracene derivatives<sup>55–59</sup> conjugated molecules have been examined for chemosensors and bioimaging applications.

However, direct using of AIE or AIEE based FONs for bioimaging has been proven problematic due to the strong hydrophobicity of most of AIE or AIEE units. The introduction of charges into AIE (or AIEE) materials could improve their solubility in aqueous media, but the electric charges of the highly concentrated ionized dyes may affect intracellular physiology and sometimes even kill the cells.<sup>52</sup> Therefore, facile preparation of novel FONs, which exhibited enhanced water dispersibility, excellent biocompatibility, and photoluminescent properties simultaneously is of great significance.

In our recent report, we have demonstrated that hydrophobic AIE unit (**An18**) could be facily transferred into hydrophilic FONs via mixing of **An18** and a commercial surfactant pluronic F127.<sup>30</sup> The **An18** based FONs showed good water solubility and excellent biocompatibility are promising for cell imaging applications. However, one of the disadvantages of these AIE-based FONs for

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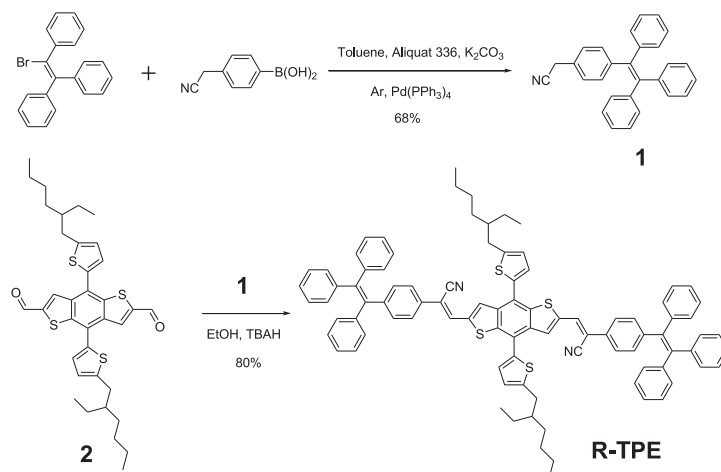
bioimaging applications is that their maximum emission wavelength is not long enough (filled into 530–550 nm), which will inevitably be interfered by the body optical absorption, light scattering, and autofluorescence of biological media.

Herein, we report a facile method for the preparation of red FONS based on a cyano-substituted diarylethylene and tetraphenylethylene derivative (**R-TPE**) (derivatized from 4,8-bis[5-(2-ethylhexyl)-2-thienyl]benzodithiophene core structure with bis[2-(tetraphenylethene)-acrylonitrile] side groups, its synthetic route was shown in Scheme 1) by surfactant modification of lecithin (Scheme 2). **R-TPE** was firstly dissolved in THF, mixed with the aqueous solution of surfactant lecithin by sonication. With the removal of THF, the red FONS **R-TPE-LEC** formed. The hydrophobic surface of **R-TPE** was changed to hydrophilic by surrounding with lecithin, thus the **R-TPE-LEC** FONS exhibit good water dispersibility and anti-ACQ property. To exploit the potential biomedical applications of such red FONS, their biocompatibility as well as cell imaging applications were further investigated.

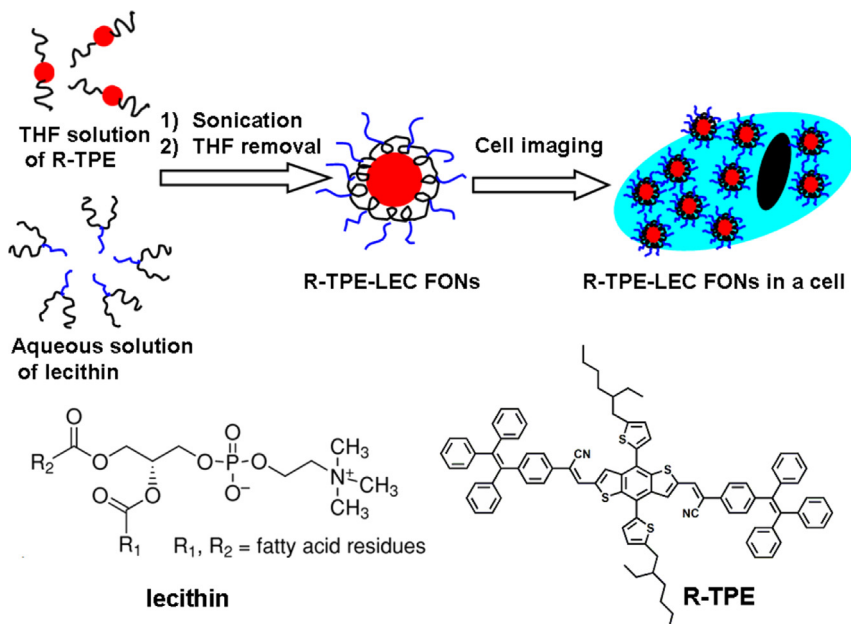
## 2. Results and discussion

### 2.1. Characterization of **R-TPE-LEC** FONS

**2.1.1. Fluorescence spectra.** The fluorogen **R-TPE** was prepared following the synthetic route shown in Scheme 2 (see Experimental procedure). Its structure was characterized and confirmed by standard spectroscopic methods. The fluorescent dye **R-TPE** is hydrophobic and emits strong crimson fluorescence in solid state. When **R-TPE** is dissolved in DMSO, it has strong orange fluorescence in dispersed state. After modified by surfactant lecithin, **R-TPE** could be dispersed well in water by the hydrophobic interaction of lecithin, meanwhile, the fluorogen is still in aggregated state in the **R-TPE-LEC** nanoparticles, and the **R-TPE-LEC** composite emitted strong red fluorescence (612 nm for the maximum emission wavelength) in water with almost the same fluorescent intensity like that in a pure DMSO solution (Fig. 1(A)), which show obvious anti-ACQ property. Meanwhile, fluorescence quantum



Scheme 1. Synthetic route of **R-TPE**.



Scheme 2. Schematic showing transformation of **R-TPE** from hydrophobic to hydrophilic red **R-TPE-LEC** FONS with lecithin and their use as cell imaging probes.

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