Red-to-NIR Emissive Radical Cations Derived from Simple Pyrroles for Bio-Imaging

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Abstract

Red-to-near-infrared (NIR) fluorophores are highly desirable for bio-imaging with advantages of excellent tissue penetration ability and less interference from auto-fluorescence, but their synthesis usually require tedious procedures and it's thus highly desirable for red-to-NIR fluorophores directly generated from easily available substrates. Compared with the conventional closed-shell fluorophores, radical cations are featured with a large red-shift absorption, but most of them are not fluorescent due to the fast internal conversion between excited and ground state with a small energy gap. Moreover, radical cations suffer from instability because they can easily undergo radical coupling or nucleophilic addition reactions. Herein, we found that 2,5-

dimethylpyrroles can rapidly generate red-to-NIR emissive radical cations, which can be stabilized by adsorption on thin layer chromatography (TLC) plate or being encapsulated in cucurbit[7]uril (CB[7]). The NIR-emissive radical cations derived from pyrroles were verified by electron paramagnetic resonance (EPR) spectroscopy and theoretical calculations. Importantly, the pyrrole-derived radical cations encapsulated in CB[7] can be used for mitochondrial imaging in living cells and tumor imaging in vivo with a high signal-to-noise ratio. The easily available and NIR-emissive radical cations derived from simple pyrroles are promising for applications in biomedical study.

1. Introduction

The red-to-near-infrared (NIR) fluorophores are highly desirable for bio-imaging with advantages of excellent tissue penetration ability and less interference from auto-fluorescence.[1] However, their structures are usually composed with electron-donating/-withdrawing groups connected by lengthy conjugation units, which require tedious procedures for their preparation.[2] Therefore, it's highly desirable for facile preparation of red-to-NIR emissive fluorophores from easily available substrates.[3] Compared with the conventional closed-shell fluorophores, radical cations are featured with signficantly red-shift absorption, but very few of them are fluorescent due to the fast internal conversion between excited and ground state with a small energy gap.[4] Moreover, the stability of radical cations are usually very poor in solution because they can easily undergo further reactions, such as radical coupling, bond cleavage or nucleophilic addition reactions.[5]

The 2,5-dihydropyrroles are featured with a strong electron donating ability and can be easily oxidized to afford oligo- or poly-pyrroles for optoelectronic applications via unstable radical cation intermediates.[6] Herein, we unexpectedly found that the 2,5-dimethylpyrroles can easily generate stable and red-to-NIR emissive radical cations by adsorption on thin layer chromatography (TLC) plate or being encapsulated in cucurbit[7]uril (CB[7]). Moreover, the pyrrole-derived radical cation encapsulated in CB[7] can be used for mitochondrial imaging in living cells and tumor imaging in vivo with an excellent signal-to-noise ratio and long-term stability (Scheme 1).
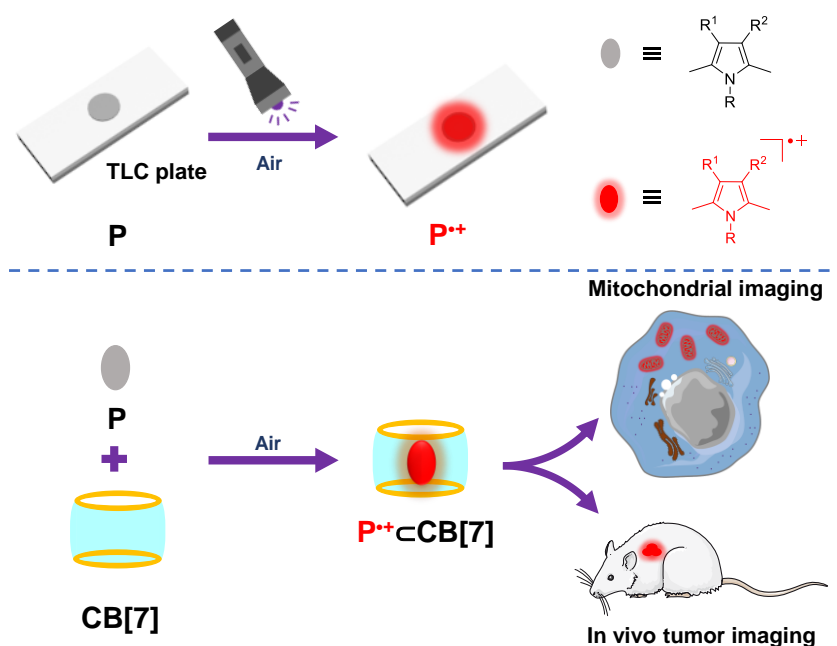
Sheme 1. The 2,5-dimethylpyrroles derived NIR-emissive radical cations stabilized by adsorption on TLC plate or being encapsulated in CB[7] and their applications for mitochondrial and tumor imaging.

2. Results and Discussion

The pyrrole compunds **P1-P10** in Figure 1 are commercially available or can be easily prepared by Paal–Knorr reaction from 1,4-butanediones and primary amines (Figure S1-S6).[7] We first investigated the photophysical properties of pyrrole compound **P1** decorated with *N*-phenyl and 2,5-dimethyl substituents, which in THF solution exibited a maximum absorption and emission at 245 and 305 nm, respectively (Figure 1A). To our surprise, when it was dropped on silica gel-coated thin layer chromatography (TLC) plate under air, a red fluroescence emission was observed with a maximum excitation and emission wavelength at 600 and 630 nm, respectively (Figure 1B). Compared with the control group under dark, an accelerated generation of red fluorescence was observed under UV irradiation (365 nm) and the fluorescence intensity at 630 nm quickly increased to a plateau within 30 min (Figure 1C-D, Figure S7). It's noteworthy that the maximum emission at 630 nm is independent of the excitation wavelength (Figure S8). Importantly, the generation of red fluorescence was efficiently inhibited

under argon, which indicates oxygen in air is neccesary for generation of fluorophores with red fluoresence (Figure S9).[8] We suppose that the electron-rich pyrrole **P1** can undergo single electron oxidation promoted by oxygen to generate radical cation **P1$^{\bullet+}$**, which can be stabilized by the porous silica gel and emit red fluorescence.[9] The in situ generated **P1$^{\bullet+}$** on TLC plate showed a quantum yield of 8.54% and a lifetime of 1.63 ns (Figure S10, Table S1), which suggests it's promising for bio-imaging applications.

We then investigated the substituent effect of pyrroles for the generation of radical cations (Figure 1E-L, Figure S11-S12). Compounds **P2** and **P3** with 2,5-dihydrogen or 2,5-diphenyl subsitutents on TLC plate showed a very weak fluorescence signal (Figure S11A-D), while compound **P4** with 2-phenyl-5-methyl substituent showed a slowly increased red fluorescence under UV irradiation for 120 min (Figure S11E-F). Compound **P5** decorated with an electron-withdrawing 3-aldehyde group on the pyrrole ring showed a very weak fluorescence signal even after a long-term UV irradiation (Figure S11G-H). In contrary, compound **P6** with 3-hydroxylmethyl group on the pyrrole ring showed a high efficiency for generation of **P6$^{\bullet+}$** within 5 min (Figure 1E-F, λ_{em} = 670 nm, Φ_F = 11.32%, τ = 2.22 ns). These results suggest that the electron-rich pyrroles decorated with alkyl groups are beneficial for the generation of radical cations.

We further investigated the effect of *N*-substituents for the generation of radical cations. Interestingly, compared with compound **P7** decorated with electron-donating *N*-(4-methoxyphenyl) substituent for generation of **P7$^{\bullet+}$** with UV irradiation for 120 min (Figure 1G-H, Φ_F = 7.22%, τ = 1.87 ns), compound **P8** decorated with *N*-(4-carboxylic acid phenyl) substituent showed a much higher efficiency for generation of radical cation **P8$^{\bullet+}$** within 3 min (Figure 1I-J, Φ_F = 9.59%, τ = 1.64 ns). Moreover, compound **P9** with *N*-methyl group showed a high efficiency for generation of **P9$^{\bullet+}$** within 15 min (Figure 1K-L, Φ_F = 4.86%, τ = 1.93 ns), but compound **P10** with *N*-H group showed a much lower efficiency for generation of the corresponding radical cation **P10$^{\bullet+}$** (Figure S11I-L).
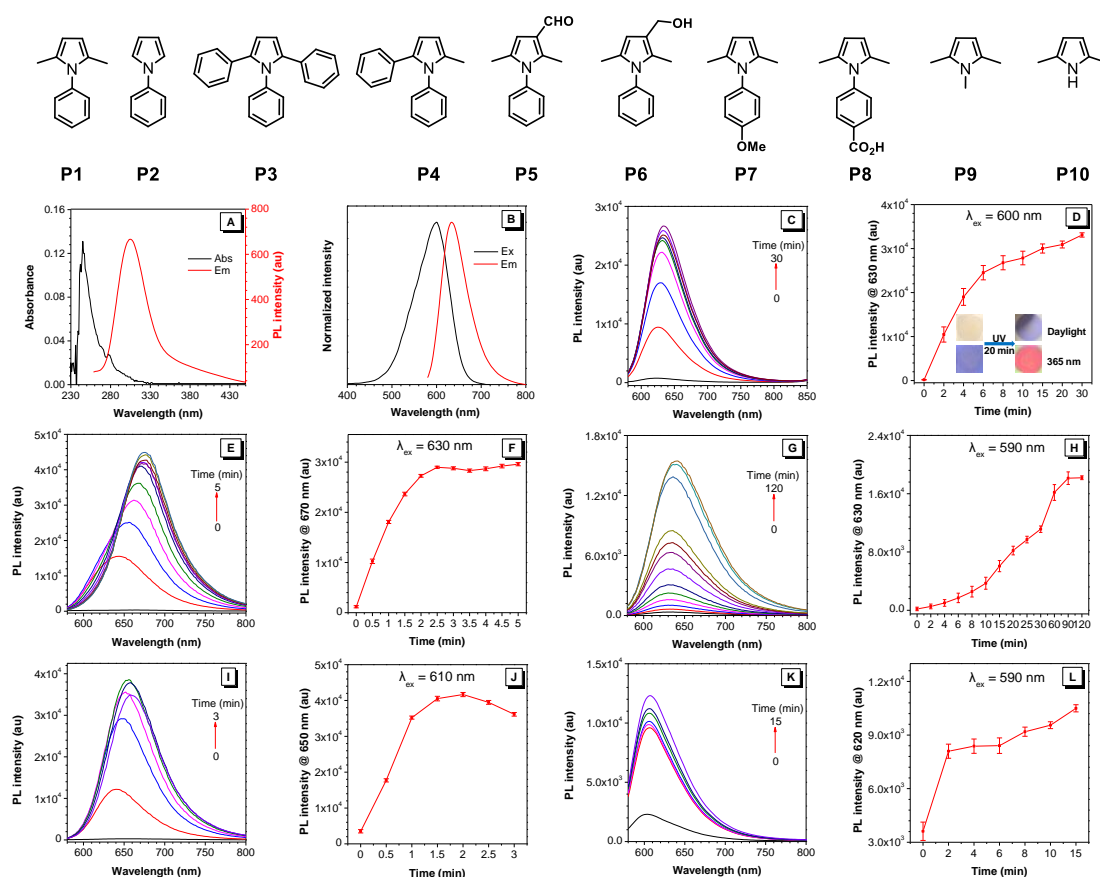
Figure 1. (A) UV-Vis absorption and fluorescence spectra of **P1** in THF (10 μM); (B) Exciation and emission spectra of **P1**$^{•+}$ on TLC plate; (C) PL spectra of in situ generated **P1**$^{•+}$ on TLC plate under air and 365 nm light irradiation for different time; (D) Plots of relative PL intensity of **P1**$^{•+}$ at 630 nm versus irradiation time; Insets are photos of **P1** and in situ generated **P1**$^{•+}$ on TLC plate under daylight and UV light; (E, G, I, K) PL spectra of in situ generated **P6**$^{•+}$, **P7**$^{•+}$, **P8**$^{•+}$, **P9**$^{•+}$ on TLC plate under 365 nm light irradiation for different time; λ_{ex} = 550 nm; (F, H, J, L) Plots of relative PL intensity of **P6**$^{•+}$, **P7**$^{•+}$, **P8**$^{•+}$, **P9**$^{•+}$ at 670, 630, 650 and 620 nm versus irradiation time, respectively.

To verify the generation of radical cations, we then measured their electron paramagnetic resonance (EPR) spectra. Because compound **P6**$^{•+}$ showed the highest emission efficiency, it was chosen as the model compound. As shown in Figure 2A, a single line signal was observed for the in situ generated **P6**$^{•+}$ adsorbed on silica gel powder with g = 2.003, which suggests the existence of unpaired electron and is in good

agreement with the calculated EPR spectrum.[10] For other pyrrole compounds, they also showed a similar single line signal with $g \sim 2.003$ (Figure S13). The redox properties of **P6** were then examined by cyclic voltammetry in CH_2Cl_2 at room temperature, which exhibited an irreversible oxidation wave with a low oxidation potential ($E_{ox} = + 0.62$ V vs Fc/Fc^+, Figure 2B). Moreover, pyrroles **P1** and **P7-P9** with high efficiencies for generation of red-to-NIR fluorescence signals also showed irreversible low oxidation potentials (Figure S14, Table S2, $E_{ox} = + 0.53–0.73$ V vs Fc/Fc^+). These results suggest that electron-rich pyrroles can easily undergo oxidation reaction to generate radical cations.

We then prepared **P6**$^{•+}$ in CH_2Cl_2 solution with silver hexafluoroantimonate ($AgSbF_6$) as the one-electron oxidant.[11] A maximum absorption and emission intensity at 633 and 676 nm was respectively observed for the in situ generated **P6**$^{•+}$ within 60 min (Figure 2C-F). Further reaction led to a new absorption peak at 460 nm and disappearance of the absorption and emission peaks at 633 and 676 nm, respectively. These results indicates that **P6**$^{•+}$ in CH_2Cl_2 solution may undergo further coupling reactions. In contrary, the in situ generated **P6**$^{•+}$ on TLC plate showed a much higher stability for at least 240 min (Figure S15). These results verified that the pyrrole-derived radical cations can be stabilized by adsorption on porous silica gel.
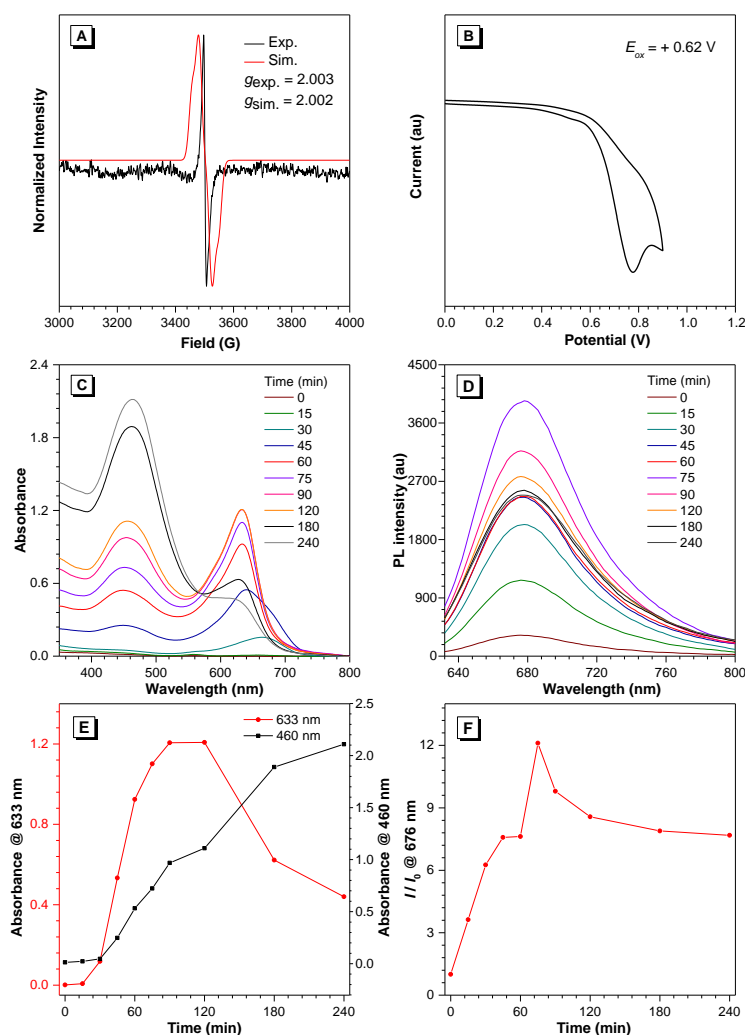
Figure 2. (A) Experimental and calculated EPR spectra of **P6**$^{\bullet+}$ absorbed on silica gel; (B) Cyclic voltammogram (oxidation) of **P6**$^{\bullet+}$ in CH$_2$Cl$_2$ containing 0.1 M *n*-Bu$_4$NPF$_6$; (C, D) Time-dependent UV-Vis absorption and PL spectra of **P6**$^{\bullet+}$ in CH$_2$Cl$_2$ with AgSbF$_6$ as the oxidant; (E, F) Plots of absorbance intensity of **P6**$^{\bullet+}$ in CH$_2$Cl$_2$ at 633 nm and 460 nm and PL intensity I/I_0 at 676 nm (λ_{ex} = 600 nm) versus reaction time.

To further get insight into the photophysical properties of pyrrole radical cations, energy levels of the frontier molecular orbitals of **P6**$^{\bullet+}$ were calculated based on density functional theory (DFT). Based on UωB97XD/def2-TZVP level, the optical energy gap for **P6**$^{\bullet+}$ was calculated to be 1.96 eV (631 nm), which agrees well with experimental value (633 nm) and can be ascribed to the D$_0$ → D$_1$ transition consisting of β-NH-3 to

β-SUMO (37.6%), β-NH-2 to β-SUMO (30.6%) and β-SOMO to β-SUMO (29.2%) (Figure 3A). The Mulliken spin population distribution of **P6**$^{•+}$ radical indicates that the single electron mainly locates at the pyrrole ring, especially the C-2 and C-5 position (Figure 3B). Therefore, the exceptional stability of the 2,5-dimethyl substituted pyrroles can be ascribed to the steric shielding of the reactive positions of C-2 and C-5 by the methyl substituents, and the electron-donating alkyl substituent can further stabilize the radical cations through δ–π hyperconjugation effect.[12]
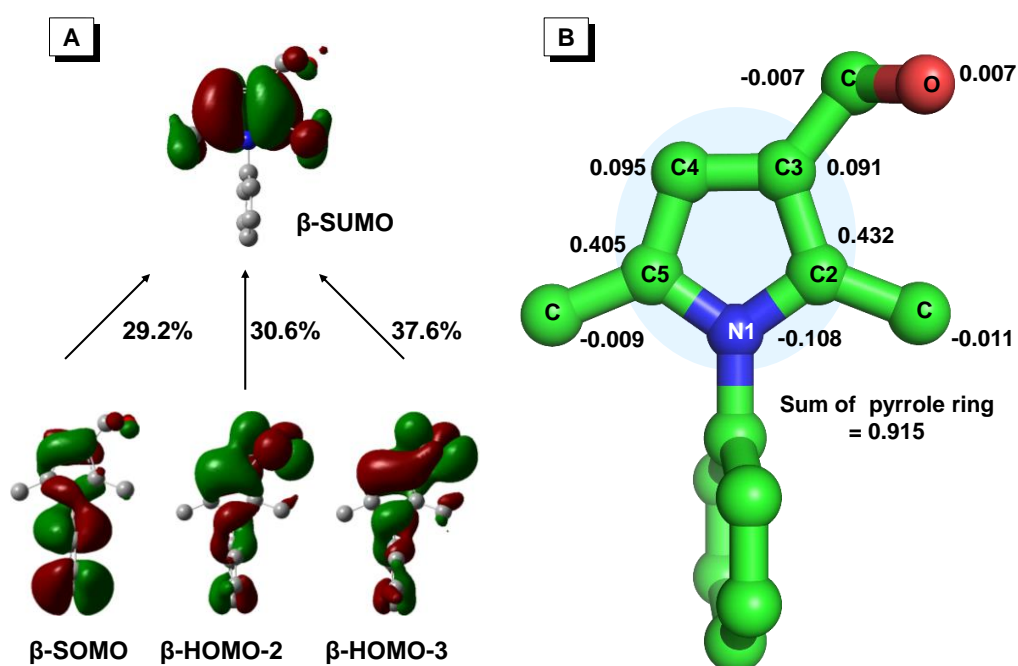

Figure 3. (A) Molecular orbitals and (B) Mulliken spin distribution of **P6**$^{•+}$. The spin density was drawn at the isovalue of 4×10^{-4} e/bohr^3. Positive and negative values respectively refer to α and β electron spin. Hydrogen atoms are omitted for clarity.

We then used the supramolecular host cucurbit[7]uril (CB[7]) to stabilize the pyrrole-derived radical cation.[13] After screening experiment (Figure S16), we found that the mixture of **P6** and CB[7] under air quickly became clear under sonication and showed a light blue color, which suggests that **P6** can quickly transform into **P6**$^{•+}$ and further being encapsulated by CB[7] to generate **P6**$^{•+}$⊂CB[7] complex with NIR emission (Figure 4A-B). Moreover, the formation of **P6**$^{•+}$ in the presence of CB[7] was

verified by EPR spectra (Figure S13). The **P6$^{\bullet+}$⊂CB[7]** complex in aqueous solution showed a stable absorption and emission spectra at room temperature under air for at least 240 min without any apparent decrease (Figure S17). Its markedly enhanced stability can be attributed to the interaction between the pyrrole cation radical and the hydrophobic cavity with a slightly negative electrostatic potential (Figure S18).[14]

We then investigated the application of the pyrrole radical cations for cell imaging. The CLSM images of HeLa and A375 cells stained by **P6$^{\bullet+}$⊂CB[7]** complex showed a red fluorescence signal, which mainly distributed in mitochondria and was verified by co-staining with MitoTracker Green (Figure 4C). The Lambda mode scanning further verified the red fluorescence emission generated from radical cation of **P6$^{\bullet+}$** with a maximum emission wavelength at ~670 nm (Figure S19). Importantly, the **P6$^{\bullet+}$⊂CB[7]** complex can be used for in vivo tumor imaging with an excellent fluorescence signal stability and tumor retention ability for at least 24 h (Figure 4D). These results suggest that **P6$^{\bullet+}$⊂CB[7]** complex is suitable for bio-imaging study with a low cytotoxicity (Figure S20).
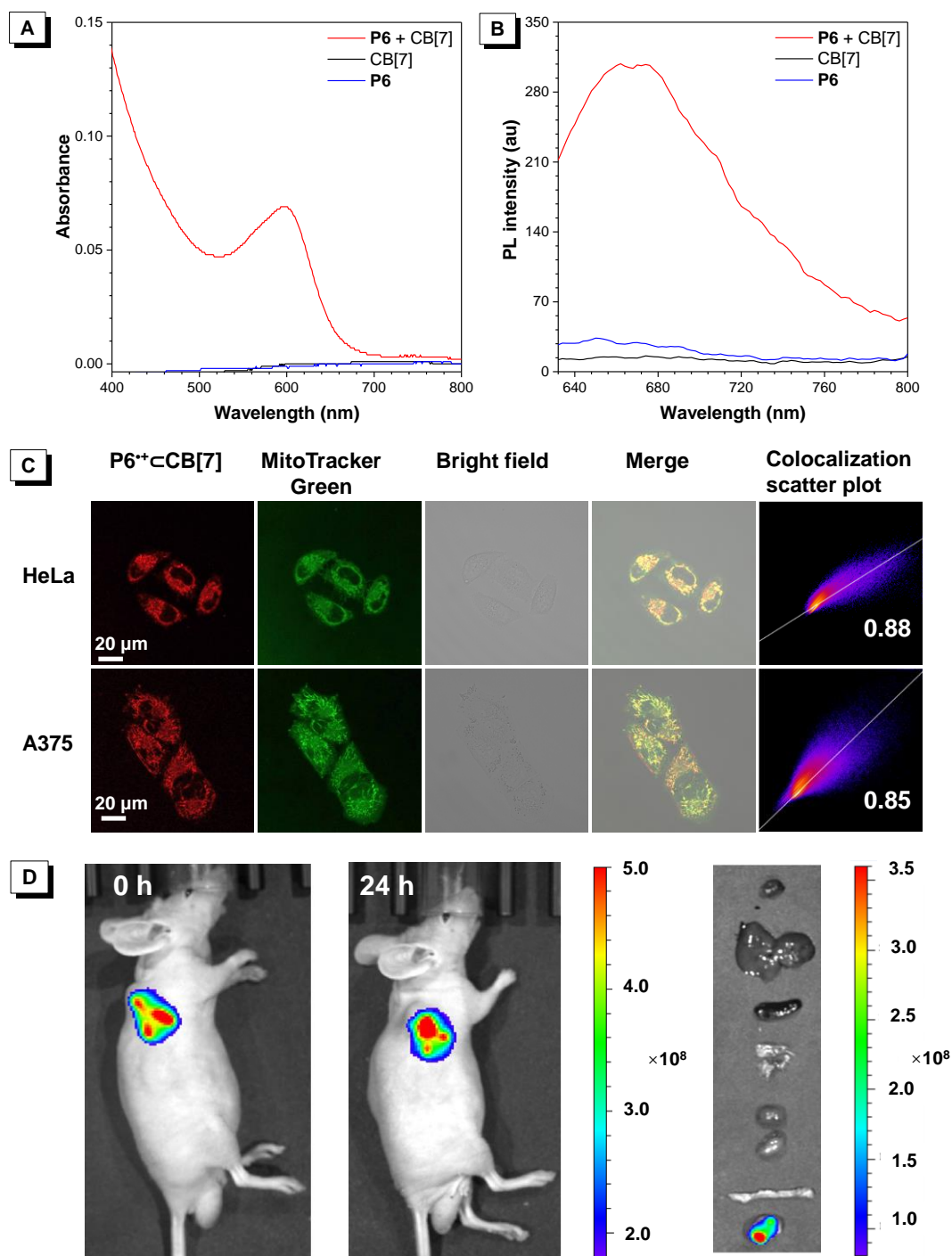
Figure 4. (A, B) UV-Vis absorption and PL spectra of **P6,** CB[7] and "**P6** + CB[7]";

[P6] = 300 μM, CB[7] = 100 μM; (C) Colocalization images of **P6•+⊂CB[7]** and

MitoTracker Green in living HeLa and A375 cells; For **P6•+⊂CB[7]**, λ_{ex} = 633 nm; λ_{em}

= 640-750 nm; for MitoTracker Green, λ_{ex} = 488 nm; λ_{em} = 500-550 nm; (D) Left: In

vivo tumor images at 0 and 24 h after injection of **P6•+⊂CB[7]**; Right: fluorescence

images of anatomic organs after 24 h (from top to bottom: heart, liver, spleen, lung, kidney, small intestine and tumor), λ_{ex} = 608 nm, λ_{ex} = 680 nm.

3. Conclusions

In conclusion, we unexpectedly found that stable and red-to-NIR emissive radical cations can be easily obtained from 2,5-dimethylpyrroles. Their excellent stability can be ascribed to the restriction of radical coupling reactions by blocking of the reactive positions of C-2 and C-5 with methyl groups and further adsorption on porous silica gel or being encapsulated in CB[7]. The pyrrole-derived radical cations were verified by EPR spectra and theoretical calculations. Moreover, the **P6$^{\bullet+}$⊂CB[7]** complex can be used for mitochondrial imaging in living cells with a high signal-to-noise ratio and long-term in vivo tumor imaging. The further exploration of pyrrole-derived radical cations in biomedical study is under way in our laboratory.

measurements.

Conflict of interest

The authors declare no conflict of interest.

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