Anion-Specific Aggregation Induced Phosphorescence Emission (AIE) in an Ionic Iridium Complex in Aqueous Media†

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Efficient Aggregation Induced Phosphorescence Emission (AIE) of an ionic Ir(III) complex occurs when the counterion (PF6 −) is exchanged specifically by ClO4 − in aqueous media. As a result, a rapid, highly selective “turn-on” phosphorescent response to ClO4 − is observed in aqueous media. These studies pave the way for a new efficient phosphorescence-based detection strategy for anions.

Ionic transition metal complexes (iTMCs), notably those of Ir(III) and Pt(III), are of considerable interest due to their high photoluminescence quantum yields (PLQYs) at room temperature, physicochemical stability and long (several microseconds) PL lifetimes (τ), leading to their applications as light-emitting electrochemical cells (LECs), biological labels, chemosensors for inorganic ions, data recording and security protection devices. The photophysical properties of iTMCs are attributed primarily to strong spin–orbit coupling within the cations. However, the counter anions can also have a profound effect on the photophysical properties and LECs performance. Therefore, it is important to investigate further the role of counterions in the applications of iTMCs.

The selective detection of certain anions is of vital significance. For example, perchlorate is a major contaminant resulting from the industrial and commercial applications of perchlorate salts which are highly soluble and stable in water. Due to their similar ionic radii, perchlorate can inhibit iodide intake in the thyroid gland, thereby impacting human health. Therefore, the determination of trace levels of ClO4 − in environmental and biological samples is very important. The detection of anions by fluorescence techniques has limitations due to: 1) fluorescence “turn-off” rather than “turn-on” is usually observed and therefore the sensitivity is limited due to competing fluorescence quenching by heavy metal ions; 2) many systems work in organic solvents, but when water is added, the response becomes difficult to detect.

Aggregation-induced emission (AIE) fluorophores, which show weak emission when dispersed, but emit efficiently when aggregated or in the solid state are attracting great attention, and have been applied for the fluorescent turn-on detection of various ions. However, the highly selective turn-on detection of anions in aqueous media by AIE is rare. For anion detection in vivo, the output signal of AIE-active fluorophores always suffers from the interference of short-lifetime background fluorescence and scattered light. We considered that aggregation-induced phosphorescent emission (AIE) using iTMCs may be an alternative, unexplored strategy for the turn-on detection of anions due to the long lifetime of phosphorescent emission. Moreover, compared with neutral complexes, iTMCs could show highly selective recognition of anions through the interactions between the cation and anion moieties.

Herein, we demonstrate that the new ionic dinuclear Ir(III) Schiff base complex 1•2PF6 (Fig. 1a) shows a highly selective response for the turn-on phosphorescent detection of ClO4 − in aqueous media and HeLa cells. To the best of our knowledge, this is the first report of an iTMC used in phosphorescent turn-on detection of an anion by AIE.

Fig. 1 (a) Chemical structure of complexes 1•2PF6 and 1•2ClO4 −; (b) Phosphorescence spectra of 1•2PF6 (10 μM) in the presence of different anions (15 equiv.) in 10 mM HEPES buffer (CH3CN:HEPES, 1:4, v/v); (c) The corresponding phosphorescence variations of 1•2PF6. Insert: photographs of 1•2PF6 with different anions (15 equiv.) under UV lamp irradiation (top) and sunlight (bottom).
Complex 1·2PF$_6$ is AIPE-active$^{14}$ (Fig. S1 and Table S1). The faint emission in pure acetonitrile solution can be ascribed to structural distortions in the complex’s T$_1$ geometry compared to the S$_0$ geometry (Table S2). These distortions induce excited-state relaxations which suppress radiative decay.$^{15}$ However, in the solid state, intermolecular π-π or C—H⋯π interactions between the phenyl rings can restrict intramolecular relaxation favouring a more planar geometry which activates the AIPE (Fig. S2).

Anion recognition was studied by monitoring the phosphorescence of 1·2PF$_6$ (10 μM) in HEPES buffer (10 mM in CH$_3$CN/H$_2$O, 1/4, v/v, pH 7.4) upon addition of 15 equiv. of ClO$_4^-$, F, Br, Cl, HCO$_3^-$, Ac$, NO_2^-$, NO$_3^-$, SO$_3^{2-}$, CO$_3^{2-}$, SiO$_2^{4-}$, ClO$^-$ or HPO$_4^{2-}$ anions and shaking the sample by hand for a few seconds (Fig. 1b and 1c). A 1:4 ratio of CH$_3$CN:H$_2$O was optimum for solubilizing the complex and observing AIPE. For ClO$_4^-$, phosphorescent turn-on was observed with a long decay time (0.24 μs), longer than that of the blank (0.29 × 10$^{-4}$ μs) (Fig. S3). The blue shift may be due to a counterion effect, as observed previously in anion exchange experiments.$^{3b}$ Remarkably, the addition of all other anions caused no significant change in the emission. The variation of photophysical data for ClO$_4^-$ is accompanied by aggregation which enables the detection of anions readily by the naked eye in daylight (Fig. 1c).

![Fig. 2 TEM images of nanoparticles obtained from anion titration experiments of 1·2PF$_6$ (10 μM) with (a) blank; (b) 10 equiv. HPO$_4^{2-}$; (c) 10 equiv. F$^-$; (d) 3 equiv. ClO$_4^-$; (e) 5 equiv. ClO$_4^-$; (f) 10 equiv. ClO$_4^-$ in 10 mM HEPES buffer (CH$_3$CN:H$_2$O, 1/4, v/v).](image)

The formation of nano-aggregates was established by transmission electron microscopy (TEM) and dynamic light scattering (DLS) experiments. TEM reveals the nano-aggregates in the titration of 1·2PF$_6$ (10 μM) with ClO$_4^-$; their average sizes increased with the incremental addition of ClO$_4^-$ (Fig. 2a, 2d, 2e and 2f). However, the average sizes are unperturbed in the presence of HPO$_4^{2-}$ and F$^-$ (Fig. 2b and 2c). DLS measurements revealed an enhanced light-scattering intensity on addition of 15 equiv. of NaClO$_4$ in HEPES buffer to 1·2PF$_6$ (10 μM), with an average particle size of 405 nm (Fig. S13). However, there was no significant change when 15 equiv. of NO$_3^-$ or Br$^-$ ions were added to 1·2PF$_6$ and the average particle size decreased to 120 nm. These results indicate that aggregation was specifically controlled by perchlorate salt. We propose that nano-aggregate formation is a critical factor in enhancing the emission.

Titration experiments with the addition of ClO$_4^-$ to 1·2PF$_6$ in HEPES buffer showed no further increase in phosphorescence intensity beyond 15 equiv. of ClO$_4^-$ (Fig. S4a). There is a good linear relationship between the emission intensity of 1·2PF$_6$ and ClO$_4^-$ concentration ($R^2 = 0.96$, 2-120 μM), suitable for quantitative detection of ClO$_4^-$.$^{12}$ The Hill coefficient $n$ is 0.67 indicating that the binding between 1·2PF$_6$ and ClO$_4^-$ is 1:2 stoichiometry (Fig. S5) which is consistent with the theoretical ion ratio in the complexes. The UV titration experiments showed that the absorption bands of 1·2PF$_6$ at ca. 330 nm decreased upon addition of ClO$_4^-$ and the tail above 500 nm increased gradually (Fig. S4b). This is ascribed to the light scattering effect of the formed nano-aggregates which is consistent with the TEM and DLS results.$^{16}$

Competition experiments for the system composed of 1·2PF$_6$ and 15 equiv. of ClO$_4^-$ showed that the emission intensity is unperturbed in the presence of 15 equiv. of a range of other anions (Fig. S6). Moreover, the titration of 1·2PF$_6$ with ClO$_4^-$ in the presence of potential competing cations, such as Na$^+$, Li$^+$, Mn$^{2+}$ and Hg$^{2+}$, showed only a slight effect on the emission (Fig. S8). These results demonstrate that 1·2PF$_6$ has a strong affinity and selectivity for ClO$_4^-$.$^{17}$ The phosphorescence intensity of 1·2PF$_6$ containing 15 equiv. of ClO$_4^-$ is also unaffected over the pH range 1-14 (Fig. S9). The lowest detection limit of ClO$_4^-$, as determined from a plot of normalized phosphorescence intensity as a function of the concentration of added ClO$_4^-$, is as low as 0.05 ppm (Fig. S10). Therefore, this complex shows highly selective and sensitive phosphorescent turn-on detection of ClO$_4^-$ in the presence of different cations, anions and pH values which is sufficient to detect ClO$_4^-$ pollution in Eco-water.

To understand the origin of the selectivity of 1·2PF$_6$ towards ClO$_4^-$, $^{19}$F NMR experiments were performed. 1·2PF$_6$ was added to an internal standard of 1-iodo-3-(trifluoromethyl)benzene in the molar ratio (standard: 1·2PF$_6$ = 16:1). Figure S14a shows that the integral area ratio between the standard and 1·2PF$_6$ is 4:1. However, for a solution of the light yellow precipitate from a ClO$_4^-$ titration experiment, the integral area ratio (standard: complex) is 20:1 (Fig. S14b) showing that partial anion exchange has occurred from PF$_6^-$ to ClO$_4^-$.

MALDI-TOF negative-ion mass spectrometry further verified which anions exchange the PF$_6^-$ of 1·2PF$_6$. After adding 15 equiv. of ClO$_4^-$, Br$^-$ or NO$_3^-$ to 1·2PF$_6$ (in CH$_3$CN/H$_2$O, 1/4, v/v), the mass spectra of the filtrates were recorded (Fig. S14c, S14d and S14e). The peaks at m/z 98.7 and 144.7 correspond to ClO$_4^-$ and PF$_6^-$, respectively. In contrast, only the peak at m/z 144.7 appeared with added Br$^-$ and NO$_3^-$.$^{17}$ These results are in agreement with the $^{19}$F NMR studies and indicate that only ClO$_4^-$ readily exchanges PF$_6^-$.

The specificity for ClO$_4^-$ is supported by DFT calculations. The binding energy for complex 1 with ClO$_4^-$, ClO$_4^-$, Br$^-$, Cl$^-$, HCO$_3^-$, Ac$^-$, NO$_2^-$, NO$_3^-$, SO$_3^{2-}$, CO$_3^{2-}$, SiO$_2^{4-}$ or HPO$_4^{2-}$ anions in the presence of counter cations, is listed in Table S3. The formation of the perchlorate adduct is exothermic by ~10.1 kcal mol$^{-1}$ in the gas phase and ~8.6 kcal mol$^{-1}$ in acetonitrile solution (Fig. 3 and entry 1 in Table S3) whereas all the other reactions are endothermic (entries 2-10 in Table S3). Therefore, the highly
selective detection of ClO$_4^-$ is consistent with thermodynamics data.

To investigate the mechanism of the phosphorescent turn-on response to perchlorate, trans-cis isomerization for 1•2PF$_6$ and 1•2ClO$_4$ was evaluated by DFT calculations (Fig. 3).

The trans-conformation is more stable than the cis in both cases; however, the energy difference between the two conformations decreases upon exchanging PF$_6^-$ for ClO$_4^-$ in both solution and the gas phase. This implies that isomerization of 1•2ClO$_4$ can occur more readily and the ratio of cis-conformation will be larger for 1•2ClO$_4$ than for 1•2PF$_6$. This is verified by the following experimental observations. Firstly, single crystal X-ray structures of 1•2ClO$_4$ reveal two conformations, namely trans-1•2ClO$_4$ and cis-1•2ClO$_4$. However, for 1•2PF$_6$ only the trans-conformation was obtained, because the large energy difference between cis- and trans-conformations favors the more stable isomer. Secondly, the $^1$H NMR data for 1•2PF$_6$ and 1•2ClO$_4$ (Fig. S16, S17 and S18) show that both complexes have two conformations in the solid state. The ratio of cis:trans is larger in 1•2ClO$_4$ than in 1•2PF$_6$ and the two conformations of 1•2ClO$_4$ have been readily isolated (Fig. S19 and S20) due to the high solubility of trans-1•2ClO$_4$ in hot dichloromethane compared to cis-1•2ClO$_4$. This is a rare example where the cis- and trans-conformations of a dinuclear metal complex have been separated.

$^1$H NMR titration experiments of 1•2PF$_6$ with ClO$_4^-$ were carried out in CH$_3$CN–H$_2$O (1:4 v/v, pH 7.4) (Fig. S21). Compared with 1•2PF$_6$ (Fig. S16), the ratio of cis-conformation increases upon the addition of ClO$_4^-$. Thirdly, the X-ray crystal structures of 1•2PF$_6$ and 1•2ClO$_4$ (Fig. 4) show that the bridging ligand of cis-1•2ClO$_4$ has a smaller distortion (46°, 55°) and a more planar geometry than both the trans-1•2ClO$_4$ and trans-1•2PF$_6$ conformations. We conclude that this change of geometry affects the solubility of the complexes, and aggregation (as observed in the TEM studies, Fig. 2) is induced by the increased ratio of the cis-conformation during the titration process.

![Fig. 3](image-url) Optimized structures and relative energies (in kcal mol$^{-1}$) of cis and trans isomers of 1•2PF$_6$ and 1•2ClO$_4$. In parentheses, CPCM(acetonitrile)/M06-2X/6-31G*/LANL2DZ(Ir) data are shown.

![Fig. 4](image-url) The dication in the X-ray molecular structures of (a) trans-1•2PF$_6$, (b) cis-1•2ClO$_4$, and (c) trans-1•2ClO$_4$ in the crystal. The anions and solvent molecules are omitted for clarity.

To further understand the large enhancement of the emission when nano-aggregates are formed by addition of ClO$_4^-$, PLQYs and lifetimes of the complexes in the solid state were determined (Table S1). Cis-1•2ClO$_4$ has a significantly higher PLQY (31%) than both 1•2PF$_6$ (17%) and trans-1•2ClO$_4$ (13%). Moreover, cis-1•2ClO$_4$ has a higher radiative rate than both 1•2PF$_6$ and trans-1•2ClO$_4$. A distorted geometry (trans-conformation) would favour non-radiative processes, whereas the more planar geometry (cis-1•2ClO$_4$) would increase radiative processes, as observed previously. Furthermore, the photoluminescence (PL) spectra of cis-1•2ClO$_4$ and trans-1•2ClO$_4$ in CH$_3$CN–H$_2$O mixtures with various water contents were investigated (Fig. S11). Trans-1•2ClO$_4$ in pure CH$_3$CN exhibits faint emission which is only slightly enhanced when the water content increased to 90% v/v. However, the PL intensity of cis-1•2ClO$_4$ is significantly enhanced when the water content exceeds 80% v/v, and is about 20-fold greater than for the trans-1•2ClO$_4$ system. The result confirms that cis-1•2ClO$_4$ has a significant AIE effect. Therefore, the large ratio of the cis-conformation with enhanced AIE performance is responsible for turning on the phosphorescence in ClO$_4^-$ titration experiments.

All the data obtained point to the anion-exchange induced AIE being responsible for the large phosphorescence enhancement in the detection of ClO$_4^-$, consistent with the crystal structure analysis, $^1$H NMR titration experiments and DFT calculations. We explain this anion recognition mechanism as: 1) PF$_6^-$ is exchanged specifically by ClO$_4^-$, and 1•2PF$_6$ has high selectivity for ClO$_4^-$, 2) ClO$_4^-$ induces trans to cis conformational isomerisation, 3) turn-on phosphorescence anion recognition is realized by the larger ratio of cis-1•2ClO$_4$ inducing AIE.

We have further studied the potential anion recognition properties of 1•2PF$_6$ in vivo. Traditional iTMCs are often very sensitive to oxygen in solutions and exhibit significantly shortened lifetimes which limit their practical applications. However, AIE-active iTMCs can avoid interference from oxygen due to the complexes being encapsulated within the nanoparticles formed in suspension, which can protect them from...
oxygen. Therefore, AlPE-active iTMCs may be an ideal choice as an in vivo probe. Preliminary results on 1•2PF6 as a probe for ClO4− in living cells have been obtained. HeLa cells (from liver cancer cells) were incubated with ClO4− (30, 90, 200 μM, respectively) for 15 min at 37 °C and then supplemented with 1•2PF6 (10 μM) at 37 °C for another 30 min, and washed with phosphate buffer saline (PBS) to remove the remaining complex. Confocal laser scanning microscopy revealed a clearly enhanced red phosphorescence in the cell membrane with an increased amount of ClO4− compared to the data obtained without added ClO4− (Fig. 5). Complex 1•2PF6 is, therefore, a potential probe for phosphorescence imaging of ClO4− in living cells. These are initial proof-of-concept results.

Fig. 5 Phosphorescence images of the HeLa cells cultured in the presence of complex 1•2PF6 (10 μM) at 37 °C for 30 min (a); Phosphorescence images of the HeLa cells cultured in the presence of NaClO4•H2O (30, 90, 200 μM, respectively) (b), (c), (d) for 15 min, followed by addition of complex 1•2PF6 (10 μM) at 37 °C for another 30 min.

In summary, a new dinuclear ionic iridium complex 1•2PF6 shows a rapid, highly selective phosphorescent turn-on response to perchlorate in aqueous media by aggregation induced emission. The structural versatility of iTMCs will enable the scope of this new “anion-exchange induced AlPE” process to be explored in the selective phosphorescence-based detection of anions, for which it may be a general strategy. Dinuclear complexes may be beneficial as the bridging ligand confers additional conformational flexibility compared to mononuclear analogues.

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