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PEG-Linked luminescent platinum(II) complex as aqueous polymeric molecular light switch for protein binding reactions†

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Attachment of poly(ethylene glycol) (PEG) to [Pt(4-HOPh-C^N^N)Cl] *via* covalent etheric bonds yields the luminescent polymer PEG-[Pt] (2), the photoluminescence of which is enhanced in hydrophobic regions of protein molecules (binding constant up to 10^4 M^{-1}) in aqueous solution; complex 2 can function as a luminescent probe for protein denaturation due to urea unfolding reaction.

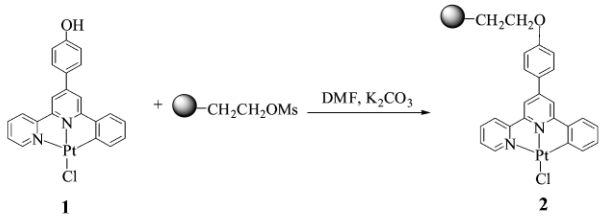
Despite the extensive works on using organic dyes such as 8-anilino-1-naphthalene sulfonate to probe conformational changes in proteins, related studies on employment of luminescent metal complexes for probing the structure and dynamic properties of proteins remain sparse.[1] In this context, square planar d^8 platinum(II) derivatives such as $[(C^N^N)PtL]^+$ (HC^N^N = 6-aryl-2,2′-bipyridine; L = neutral nitrogen or phosphine donor) are good systems for developing into chemical sensing applications.[2] They exhibit high stability at room temperature and are easily prepared with diverse structural modification. Importantly, their photoluminescent properties are sensitive to the micro-environment.[3] With judicious choice of auxiliary ligands, these Pt(II) complexes can emit in the visible region where there is no interference from fluorescence of proteins. However, most luminescent Pt(II) complexes are not soluble and/or non-emissive in aqueous solution and their emissions are easily quenched by oxygen, so their application has been limited in the biosensor field. Therefore, appropriate structural modification of luminescent Pt(II) complexes that can function as chemosensors has a great potential in biology.

Our recent studies showed that platinum(II) complexes such as $[Pt(C^N^N)L]^+$ can act as probes for pH and SDS micelles.[3] Because of the physical and intriguing biological properties of poly(ethylene glycol) (PEG), such as broad solubility profile, high stability, and excellent biocompatibility,[4,5] we envision water-soluble polymer supported luminescent cyclometalated platinum(II) complexes, such as **2** depicted in Scheme 1, would circumvent the previously encountered problems. We anticipated that photoluminescence enhancement would be observed if [(C^N^N)PtCl] moieties of **2** protrude into hydrophobic region of proteins upon interaction with ethylene glycol 'arms'. Herein, the synthesis of **2** and its photoluminescent properties in aqueous solution in the presence of proteins such as albumins are described. We demonstrated that luminescent platinum(II) complexes supported by the water-soluble polymer PEG can be employed as a molecular light switch for protein binding reactions.

Reaction of [(4-HOPh-C^N^N)PtCl] [(4-HOPh-C^N^N) = C-deprotonated form of 4-(4-hydroxyphenyl)-6-phenyl-2,2′-bipyridine, **1**] with methoxypoly(ethylene glycol) mesylate (PEG-OMs, Mw = 5000) in DMF in the presence of anhydrous potassium carbonate gave **2** in high yield (92%). ^{1}H-NMR spectra showed the absence of the Me signals of $MeSO_2$-PEG at 3.07 ppm and the appearance of a new peak at 4.06 ppm attributed to (PEG-CH_2CH_2O-[Pt]). The loading (0.17 mmol g^{-1}) was determined by ^{1}H-NMR spectroscopy using the PEGCH_2OMe signal at *ca.* δ = 3.30 to be the internal reference. The UV/vis absorption spectrum of **2** exhibits several intense absorption bands at λ < 350 nm that are similar to related $[Pt(C^N^N)L]^+$ complexes;[3] the broad and structureless absorption band at 420 nm (ε = 685 ± 30 dm^3 mol^{-1} cm^{-1}) in water is assigned as ^{1}MLCT [(5d)Pt → π*(C^N^N)] in nature.

The emission spectra of **2** measured in methanol, ethanol, acetonitrile, chloroform and water are depicted in Fig. 1. Significant enhancement of emission intensity and small red-shift in emission maximum (λ_{max} = 543–560 nm) are observed with decreasing solvent polarity.

In water, only a weak and broad emission is recorded, the emission maxima of which is concentration-dependent. At 5 × 10^{-6} M, a weak emission centred at 543 nm is observed. At concentration ≥ 10^{-4} M, a new emission centred at 710 nm develops. Previous studies ascribed the 710 nm emission to metal–metal and/or ligand–ligand interactions.[3] Because the PEG polymer behaves like a typical non-ionic surfactant in water, micellar assemblies can form with the hydrophobic [Pt(C^N^N)] moieties embedded inside, leading to aggregation of the [Pt(C^N^N)] moieties. Fig. 2 shows the ratio of the intensity of the MLCT emission at 543 nm to that of the MMLCT emission at 710 nm plotted against the concentration of **2**. The ratio of emission intensity at 710 nm to that at 543 nm increases with the concentration of **2** up to 1.1 mM and reaches a maximum at 1.2 mM. The emission is virtually unaffected by the ionic strength of the solution.



Scheme 1 Synthesis of water soluble polymer supported cyclometalated platinum(II) complex.

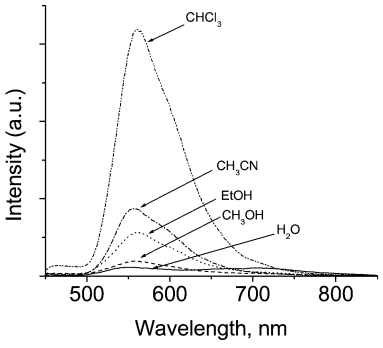


Fig. 1 Emission spectra of **2** measured in different solvents (20 μM, λ_{ex} = 390nm) at 298 K.

† Electronic supplementary information (ESI) available: general experimental procedure, synthesis and characterization of **1** and **2**, titration experiments and urea unfolding of BSA. See http://www.rsc.org/suppdata/cc/b2/b207395a/

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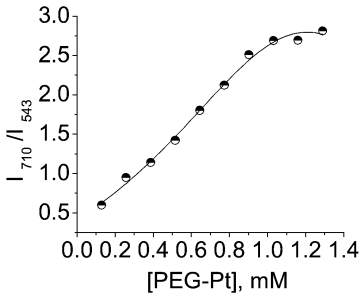

Fig. 2 Variation of ratios of luminescence intensity at 710 nm to that at 543 nm *vs.* [**2**] (measured at 543 nm, λ_{ex} = 390 nm, 298 K).

We examined the photoluminescent properties of **2** in the presence of bovine serum albumin (BSA), which functions biologically as a carrier for fatty acid anions and other simple amphiphiles in the bloodstream.[6] The emission intensity is significantly enhanced upon addition of BSA (0.15 μM), as shown in Fig. 3. The binding constant, obtained by analyzing the Scatchard plot (Fig. 3, insert curve.), is 2.7×10^4 mol^{-1} dm^3.[6] Based on the variation of emission intensity with the [BSA]/[**2**] ratio, the number of molecules (*n*) of **2** bound to BSA was estimated to be one. At saturation binding conditions, where luminescent intensity remains constant, only one site of BSA is occupied by **2**. We have investigated the salt effect on the luminescence properties of **2**. The emission spectral profile and intensity are unaffected by the addition of NaCl, revealing that the binding of **2** to a specific site of BSA occurs through non-ionic interaction.

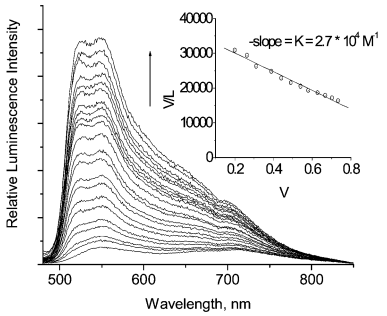

Fig. 3 Emission spectra of **2** (20 μM) in BSA aqueous solution (pH = 7.40, 100 mM Na_2HPO_4–NaH_2PO_4 with 0.9% NaCl.). From bottom to top, [BSA] (μM): 0.00 to 3.15, step: 0.15. (Insert: the Scatchard plot for obtaining the binding constant.)

To elucidate the relationship of emission enhancement to the binding of **2** to BSA, urea was added to unfold the BSA structure and subsequent effects on the emission were studied. Previous studies showed that BSA is a large single polypeptide chain organized into homologous domains (**I**, **II** and **III**) through helical extensions, which leads to hydrophobic cavities.[6] During urea denaturation of BSA, the domains are gradually unfolded and separated from each other leading to disintegration of the hydrophobic void cavities. Results from the binding of **2** to BSA at different urea concentrations are shown in Fig. 4. When [urea] was increased from 0.5 to 4.5 M, the emission intensified and reached a maximum at 2.0 M; then remained virtually constant up to 4.5 M. This finding is consistent with rearrangement in domain **II** at urea concentration less than 4.5 M, resulting in tighter binding of **2** to BSA due to helical twisting.[8] The emission intensity then decreased sharply as the [urea] was increased from 4.0 to 6.0 M. At [urea] of 6.0 M, the emission intensity was similar to that obtained before the addition of urea. At [urea] > 6.0 M, a marked decrease of the emission intensity was noticed. The emission of **2** became very weak at [urea] = 8.0 M, indicating that the protein has unfolded completely, and hence the emission

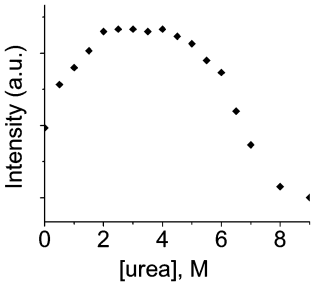

Fig. 4 The effect of urea concentration on the luminescence intensity (measured at 543 nm, λ_{ex} = 390 nm, 298 K, [**2**] = 20 μM).

enhancement resulting from the binding of **2** to the hydrophobic region of BSA has disappeared. It should be noted that, in the absence of BSA, the emission of **2** was quenched by addition of urea from 0.0 to 8.0 M (ESI†) in Fig. 4 and no enhancement of intensity was observed when the concentration of urea was increased from 0.0 to 4.0 M.

To demonstrate the versatility of **2** as a molecular light switch for protein-binding reactions, we have studied the binding of **2** towards human blood γ-globulin, human albumin and chicken egg albumin; and high binding constants (1.3×10^4, 4.2×10^4 and 2.7×10^4 M^{-1}, respectively) have been obtained. Similarly, the urea denaturation of human blood γ-globulin demonstrated that the emission enhancement is also related to the binding of **2** to the protein in aqueous solution.

To our knowledge, **2** is the first luminescent Pt(II) complex supported by a water-soluble polymer that can sensitively detect proteins under aqueous conditions without degassing procedures. This is also an example showing the interaction between protein and PEG. We believe that rational modification of water-soluble polymeric Pt(II) complexes will facilitate the development of research into luminescent biosensors for protein binding reactions.

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