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Labeling phospholipid membranes with lipid mimetic luminescent metal complexes

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ABSTRACT

Lipid-mimetic metallosurfactant based luminophores are promising candidates for labeling phospholipid membranes without altering their biophysical characteristics. The metallosurfactants studied exhibit high structural and physicochemical similarity to phospholipid molecules, designed to incorporate into the membrane structure without the need for covalent attachment to a lipid molecule. In this work, two lipid-mimetic phosphorescent metal complexes are described: $[Ru(bpy)_2(dn\text{-}bpy)]^{2+}$ and $[Ir(ppy)_2(dn\text{-}bpy)]^{+}$ where bpy is 2,2′-bipyridine, dn-bpy is 4,4′-dinonyl-2,2′-bipyridine and ppy is 2-phenylpyridine. Apart from being lipid-mimetic in size, shape and physical properties, both complexes exhibit intense photoluminescence and enhanced photostability compared with conventional organic fluorophores, allowing for prolonged observation. Moreover, the large Stokes shift and long luminescence lifetime associated with these complexes make them more suitable for spectroscopic studies. The complexes are easily incorporated into dimyristoil-phosphatidyl-choline (DMPC) liposomes by mixing in the organic solvent phase. DLS reveals the labeled membranes form liposomes of similar size to that of neat DMPC membrane. Synchrotron Small-Angle X-ray Scattering (SAXS) measurements confirmed that up to 5% of either complex could be incorporated into DMPC membranes without producing any structural changes in the membrane. Fluorescence microscopy reveals that 0.5% label content is sufficient for imaging. Atomic Force Microscopic imaging confirms that liposomes of the labeled bilayers on a mica surface can fuse into a flat lamellar membrane that is morphologically identical to neat lipid membranes. These results demonstrate the potential of such lipid-mimetic luminescent metal complexes as a new class of labels for imaging lipid membranes.

1. Introduction

Imaging and spectroscopic characterization methods based on fluorescent labeling of biomolecules, such as confocal imaging, Förster resonance energy transfer (FRET) and fluorescence recovery after photobleaching (FRAP) are performed routinely in biophysical membrane studies [1]. The majority of fluorescent probes used in these studies is based on fused aromatic or heterocyclic rings [2] such as anthracene [3,4], fluorene [5] and pyrene [6,7]. These probes are typically characterized by high quantum yields, small Stokes shifts, short luminescence lifetimes, and often high susceptibility to photobleaching [8]. While a short luminescent lifetime and photochemical instability might occasionally be advantageous, many applications such as super resolution microscopy demand longer luminescent lifetimes, increased photostability and a larger Stokes shift [8–11].

Beyond the shortcomings in their photophysical properties, in the context of membrane labeling, planar heterocyclic luminophores also suffer from unfavorable molecular geometries. Lipid membranes are dynamic self-assembled systems, where any small change in the constituents can alter the balance of the second order interactions that stabilize the bilayer, affecting its physical properties [12,13]. For example, the effect of cholesterol on biophysical properties such as lipid mobility and membrane thickness is well documented [14]. Cholesterol is a weakly amphiphilic planar fused heterocyclic molecule. Consistently, the incorporation of other large fused heterocycles such as organic fluorophores has a similar effect [15–20]: it can change the membrane phase transition temperature [16], decrease the acyl chain order and inhibit lateral diffusion [17], increase inter-bilayer lipid transfer [19] and can even lead to loss of membrane integrity [20]. Some labels are less intrusive, however even subtle changes in lamellar thickness, surface tension and bending rigidity affect the stable morphologies of the membrane, such as its ability to form liposomes or to fuse into

Abbreviations: AFM, atomic force microscopy; DLS, dynamic light scattering; DMPC, 1,2-ditetradecanoyl-sn-glycero-3-phosphocholine

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supported bilayers on surfaces [13]. Accordingly, novel approaches to labeling membranes in a biomimetic manner are required.

Transition metal complexes such as those based on Ru(II) and Ir(III) fulfill photophysical requirements and can be easily tailored synthetically to possess desired structural attributes. These complexes display molecular photoluminescence from a metal-to-ligand and/or intraligand charge transfer excited state and relaxation occurs via a partially forbidden transition, so excited state lifetimes are often relatively long (10 ns–10 μs) [21–23]. The complexes also exhibit large Stokes shifts (up to few hundred nanometers) [8]; enhanced photostability as well as comparable and sometimes higher quantum yield than organic fluorophores [24,25]. While there has been a recent increase in reports of using metal coordination complex labels in cell biology [9,26–29], relatively few such probes have been proposed for phospholipid membrane studies. Ruthenium complexes that anchor to bilayers by a thioether-cholestanol hybrid ligand [30], dipyridophenazine–ruthenium complexes that "float" in the bilayer [31], as well as lipophilic Ir(III) polypyridine complexes with pendant alkyl chains of 2, 10 and 18 carbons [32] have been proposed as membrane probes. However, these probes do not imitate the geometry and fundamental physical properties of the phospholipids closely enough. As a solution, head group modification of lipids was proposed, such as coupling a luminescent Ru(II) complex to the amino group of phosphatidyl ethanolamine which was used to study membrane motion on a nanosecond timescale [33,34]. An alternative and easier strategy is to use metallosurfactants that have been developed to create neat micellar systems and/or to create micellar mixtures with simple surfactants [35,36]. There is no information, however, on the physical properties of phospholipid membranes labeled with metallosurfactant luminophores.

Here, the use of two lipid-mimetic metallosurfactants, based on well-known structural motifs of luminescent iridium and ruthenium complexes, is proposed for this purpose: $[Ru(bpy)_2(dn\text{-}bpy)]^{2+}$ and $[Ir(ppy)_2(dn\text{-}bpy)]^{+}$ where bpy is 2,2′-bipyridine, dn-bpy is 4,4′-dinonyl-2,2′-bipyridine and ppy is 2-phenylpyridine. Our results suggest that the use of these metallosurfactants as lipid-mimetic labels has only negligible effect on the membrane properties, while offering significant photophysical advantages over traditional fluorophores.

2. Materials and methods

2.1. Materials

1,2-Ditetradecanoyl-sn-glycero-3-phosphocholine (dimyristoil-phosphatidyl-choline, DMPC) 1,2-ditetradecanoyl-sn-glycero-3-phosphoglycerol (dimyristoil-phosphatidyl-glycerol, DMPG) and cholesterol were purchased from Avanti Polar Lipids (Alabaster, AL, USA). Chloroform (ACS Reagent, ≥99.8%) was purchased from Sigma–Aldrich (Castle Hill, NSW, Australia). Lipid mimetic metal complexes; the precursors used for synthesis of the complexes 4,4′-dinonyl-2,2′-dipyridyl and bis(2,2′-bipyridine)dichlororuthenium(II) hydrate were purchased from Sigma Aldrich (>99%), and used without further purification. Sodium chloride (Ultra, ≥99.5% (AT)), potassium phosphate monobasic (ACS reagent, ≥99%), and potassium phosphate dibasic (ACS reagent, ≥99%) were purchased from Sigma–Aldrich (Castle Hill, NSW, Australia). Ultrapure water with a resistivity of 18.2 MΩ-cm was used (Milli-Q). Dry CH_3CN for analytical purposes was distilled from CaH_2 under nitrogen.

2.2. Synthesis

The molecular structure of the complexes is shown in Fig. 1. It should be noted that the actual three dimensional equidistant alignment of the ligands around the central metal leads to a spherical "head group" with two parallel alkane chains.

$[Ru(bpy)_2(dn\text{-}bpy)][PF_6]_2$: The ruthenium complex was synthesized using a small variation on a well-established method [37,38]. Briefly, 80 mg bis(2,2′-bipyridine)dichlororuthenium(II) hydrate (0.165 mmol) and 77.6 mg 4,4′-dinonyl-2,2′-dipyridyl (0.189 mmol, 15% excess) were dissolved in 75 mL 90:10 methanol:water under N_2 atmosphere. The mixture was heated and refluxed for 5 h, a color change from dark purple to dark red was observed. The solvent was removed via rotary evaporation, and the residue was dissolved in acetone. Saturated potassium hexafluorophosphate solution was added in excess, causing precipitation of the crude hexafluorophosphate complex salt. The crude complex was recrystallized from acetone/water and dried for a final yield of 82%.

$[Ir(ppy)_2(dn\text{-}bpy)][PF_6]$:$[Ir(ppy)_2(dn\text{-}bpy)]^{+}$ was synthesized by a well-established method [39] from the iridium dichloro-bridged dimer, tetrakis(2-phenylpyridine-C [2],N′)(μ-dichloro) diiridium–$[Ir(ppy)_2Cl]_2$, which was synthesized using the method of Sprouse et al. [40] 100 mg iridium dichloro-bridged dimer (0.093 mmol) and 80 mg 4,4′-dinonyl-2,2′-dipyridyl (0.196 mmol, 5% excess) were dissolved in 40 mL N_2 purged dichloromethane. The solution was refluxed under N_2 atmosphere for 24 h. The solvent was removed via rotary evaporation, and the residue was dissolved in the minimum amount of acetone. Saturated KPF_6 was added in excess, causing a bright yellow solid to precipitate. This solid was washed sparingly with cold ether, and finally purified on a sephadex LH-20 column using acetonitrile mobile phase. The bright yellow luminescent (UV, 365 nm) band was collected and the solvent was removed; the collected solid was dried for a final yield of 85%.

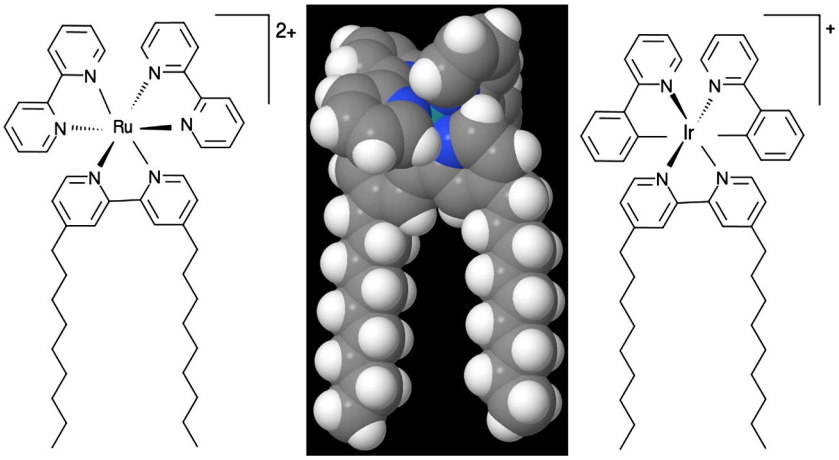

Fig. 1. Schematic molecular structure (left) and space filling model (middle) of $[Ru(bpy)_2(dn\text{-}bpy)]^{2+}$; molecular structure of $[Ir(ppy)_2(dn\text{-}bpy)]^{+}$ (right).