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Abstract

We describe the emission spectral properties of two rhenium metal–ligand complexes with one and two-photon excitation, $Re(bpy)_2(CO)_3Cl$ and $[Re(bpy)(CO)_3CH_3CN]^+$, where bpy is 2,2′-bipyridyl and CH_3CN is acetonitrile. Similar emission spectra and intensity decay times characteristic of the metal-to-ligand charge transfer state were observed for one- and two-photon excitation. The lifetime and quantum yield of the acetonitrile complex are approximately 14-fold higher than that of the chloride complex. Both complexes display high anisotropies near 0.33 in frozen solution with one-photon excitation. Two-photon excitation results in anisotropies about 40% larger, consistent with the increased photoselection expected for two-photon absorption. These complexes display single rotational correlation times in glycerol, but the correlation time of the charged acetonitrile complex is 3 to 4-fold larger. These results show that rhenium complexes can be used as hydrodynamic probes with one- or two-photon excitation. © 1999 Elsevier Science S.A. All rights reserved.

Keywords: Two-photon excitation; Metal–ligand complexes; Acetonitrile

1. Introduction

During the past few years we have seen the introduction of a new class of luminescent probes, the transition metal–ligand complexes (MLCs). The molecules typically consist of a transition metal and one or more diimine ligands. The best known molecule of this class is ruthenium *tris*(2,2′-bipyridine), $[Ru(bpy)_3]^{2+}$. A wide variety of such molecules have been synthesized and characterized [1–3], primarily with the goal of solar energy conversion. The basic idea is to harness the charge separation which occurs in the excited state into electrical or chemical storage.

The metal–ligand complexes are now known to display unique properties making them suitable as biophysical probes. The intensity decay times range from 100 ns to 10 μs [2,3]. Additionally, these molecules display polarized emission [4,5]. Hence, the powerful concepts of time-resolved intensity and anisotropy decays are now generally available on the microsecond timescale.

Another trend in recent years has been the introduction of two, three and multi-photon excitation (1PE, 2PE and MPE) [6]. In multi-photon excitation the fluorophore simultaneously absorbs two or more photons. MPE is typically accomplished with longer wavelengths so that the summed energy of the photons is comparable to that of the lowest energy single photon absorption. Because of the quadratic or higher dependence of light intensity, multi-photon excitation provides localized excitation at the focal point of the incident light. Importantly, the long wavelengths used for MPE are usually not absorbed by the sample, so photodamage is minimized. Multi-photon excitation is now being widely used in optical microscopy [7,8], and for studies of fluorophores in blood or scattering media [9,10].

In the present paper we describe the studies of two-photon excitation of rhenium metal–ligand complexes. Rhenium complexes have recently been shown to display unusually long decay times and high quantum yields in aqueous solution and when conjugated to proteins and membranes [11–13]. We now show that rhenium complexes can display two-photon excitation with wavelengths from 620 to 840 nm, which are available from dye lasers and Ti:Sapphire lasers. These studies extend our previous report [14] on two-photon excitation of $[Ru(bpy)_3]^{2+}$. Additionally, we present the first anisotropy measurements of a metal–ligand complex with two-photon excitation.

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Abbreviations: bpy, 2,2′-bipyridyl; FD, frequency-domain; 1PE, one-photon excitation; 2PE, two-photon excitation; MPE, muti-photon excitation; CH_3CN, acetonitrile; MLCT, metal-to-ligand charge transfer; MLC, metal–ligand complex
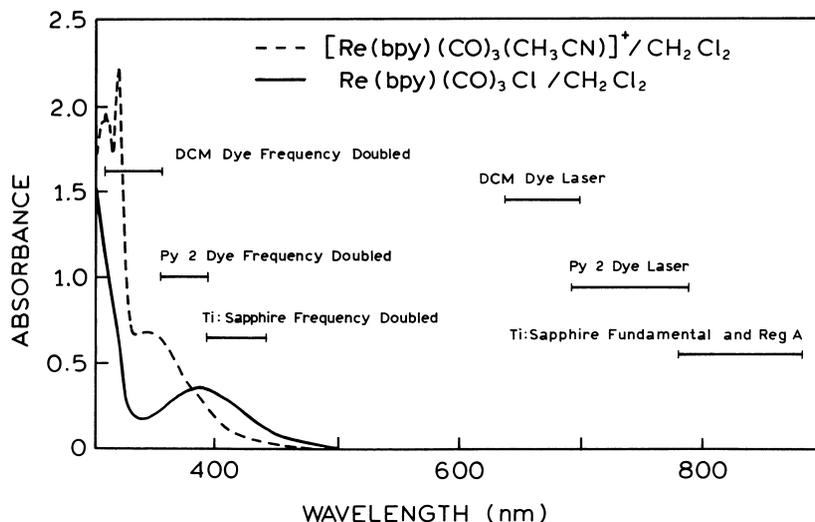
Fig. 1. Absorption spectra of the Re complexes. The horizontal bars indicate the excitation wavelengths and laser sources used for these experiments.

2. Materials and methods

The compounds fac-Re(bpy)(CO)$_3$Cl and fac-[Re(bpy)(-CO)$_3$(CH$_3$CN)](PF$_6$) were prepared as described in [15]. Reagent grade solvents and chemicals were obtained from commercial sources and used as received. All solutions were in glycerol–methanol (95:5, v:v). These samples were prepared by first dissolving a portion of the compound in methanol followed by mixing with anhydrous glycerol (EM Science). These 95% glycerol samples were used for all spectroscopic measurements. All samples reported were measured under ambient air saturated conditions unless otherwise stated. The chloride complex was stable at all temperatures. The acetonitrile (CH$_3$CN) complex was stable at room temperatures and lower. However, the decay times changed if the sample was heated to 50°C or higher, which we believed was due to the thermal dissociation of the acetonitrile ligand. UV–Vis measurements were obtained on a HP-8453 diode array spectrophotometer. Uncorrected emission spectra were obtained on an SLM AB-2 spectrofluorimeter. Low temperature (−60°C) steady-state luminescence anisotropy spectra were measured on an SLM 8000 spectrofluorimeter.

Intensity and anisotropy decays were measured using the frequency-domain method [16,17]. Intensity decay data were analyzed in terms of the multi-exponential model

$$I(t) = \sum_i \alpha_i \exp(-t/\tau_i), \qquad (1)$$

where α_i are the time-zero amplitudes for each decay time τ_i. Anisotropy decays were analyzed in terms of the multi-correlation time model

$$r(t) = \sum_j r_{0j} \exp(-t/\theta_j), \qquad (2)$$

where r_{0j} is the anisotropy amplitude which decays with a correlation time θ_j. The intensity (α_i, τ_i) and anisotropy

(r_{0j}, θ_j) decay parameters were determined by non-linear least squares. Intensity and anisotropy decays were measured using both one-photon and two-photon excitation, where the appropriate data were analyzed globally [18] with the decay times or correlation times constrained to be the same (i.e., global) for one and two-photon excitation.

The excitation sources for these experiments were the ps pulses of a DCM or pyridine 2 (Py 2) dye lasers, or the fs pulses from a Ti:sapphire laser (Fig. 1). The laser pulses were frequency-doubled to yield wavelengths for one-photon excitation. The fundamental outputs were used for two-photon excitation. One laser system used a mode-locked Ar$^+$ (Coherent) input into a synchronously-pumped cavity-dumped dye laser (DCM or pyridine 2, Coherent) which was used as a two-photon excitation source (630–700 or 690–790 nm, respectively) or was frequency doubled (315–350 or 345–395 nm, respectively) to provide conventional one-photon excitation. The pulse width of the dye laser output was typically on the order of 5 ps. We also utilized a mode-locked Ti:Sapphire laser (Tsunami, Spectra Physics) which was pumped by a 10 W CW–Ar$^+$ laser (Spectra Physics) as a two-photon (fundamental output) and one-photon (frequency doubled output) excitation source. The pulse width of the Ti:Sapphire laser was between 80 and 120 fs over the tuning range in which the measurements were performed (780–850 nm). In addition, some measurements were made using a regenerative amplifier (Coherent RegA 9000) which is seeded with a Ti:Sapphire laser (Coherent MIRA) and simultaneously pumped with 14 W of CW output from an Ar$^+$ laser (Coherent, 22 W Sabre). The RegA operates at 100 kHz with 0.2 W average power at 800 nm. The pulse energy is roughly 2 μJ/pulse with a fwhm of 120 fs. This red output was used as a two-photon excitation source in time-resolved measurements. In all cases, measurements were taken on freshly prepared samples and great care was taken to insure that decomposition during the measurements did not take place.
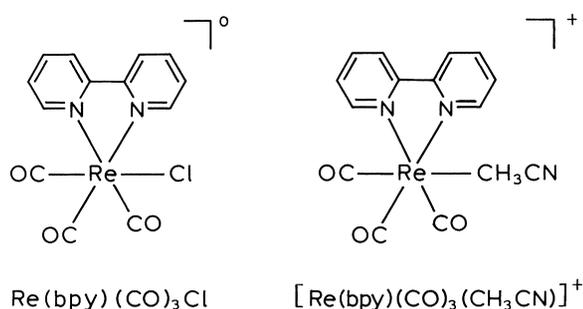
3. Results

Absorption spectra of the two rhenium complexes (Scheme 1) are shown in Fig. 1. The absorption below 330 nm is typically assigned to absorption of the ligand bpy. The structureless absorption above 330 nm is assigned to the metal-to-ligand charge transfer transition. In this state an electron is thought to be transferred from the metal to the bpy ligand.

Fig. 1 also shows the lasers used for excitation at various wavelengths. We found that the two rhenium complexes displayed one-photon excitation using the frequency-doubled pulses, and two-photon excitation for the laser fundamental wavelengths (see Fig. 3, below).

Emission spectra of the rhenium complexes in glycerol are shown in Fig. 2. These emission spectra display a large Stokes' shift with emission maxima from 550 to 600 nm. A large Stokes' shift is a favorable property for a fluorescent probe because the shift allows easy separation of the emission from scattered light, resulting in minimal resonance energy transfer. The chloride complex displays a longer wavelength emission maximum. Polarity-sensitive emission has been reported for other chloride complexes [19,20]. The emission intensity of the chloride complex is approximately 14-fold weaker than that of the acetonitrile complex. This relationship of the relative intensities is probably due to the energy gap law, which states that the non-radiative decay rate increases as the energy of an excited state decreases [21].

We questioned whether the 14-fold decrease in intensity and lifetime (below) was quantitatively in agreement with the energy gap law [21]. Quantum yields and decay times are known for a range of $Re(bpy)(CO_3)L$ complexes, where L is the ligand [15]. From these data one can predict an approximate 15-fold decrease in yield and lifetime for the chloride relative to the acetonitrile complex based on the spectral shift seen in Fig. 2. Hence the observed 14-fold decrease is quantitatively in agreement with the energy gap law data for Re complexes [15].

Emission spectra were measured using both one-photon and two-photon excitation (Fig. 2). The nature of the long wavelength excitation process is shown in Fig. 3. At 705 nm, and for longer wavelengths, the emission intensity depends on the square of the laser power. This result
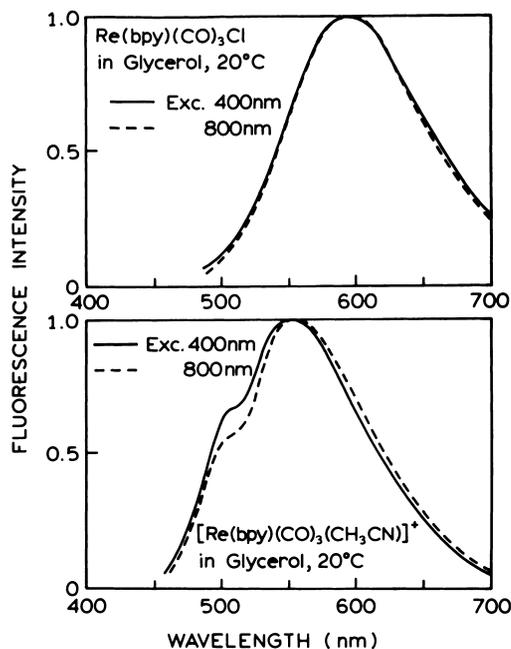

Fig. 2. Emission spectra of the two rhenium complexes for excitation at 400 and 800 nm.
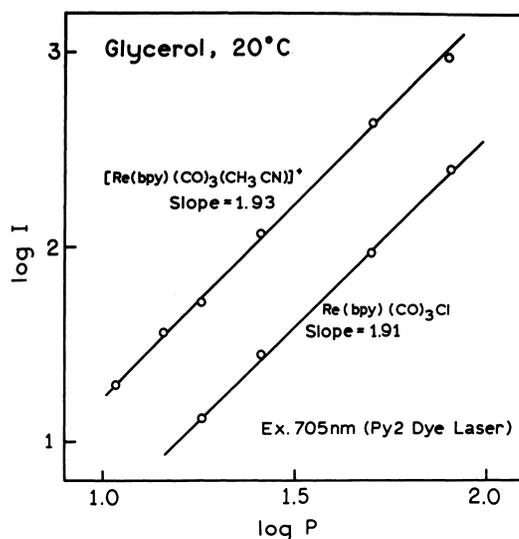

Fig. 3. Emission intensity vs. excitation intensity at 705 nm for the rhenium complexes.

indicates a two-photon process. The emission spectra were found to be essentially identical (Fig. 2) for excitation at 400 and 800 nm. This is not an obvious result because rhenium complexes are known to display emission from both the metal-to-ligand charge transfer (MLCT) state, and from the organic ligands [22,23]. The latter is referred to as ligand-centered (LC) emission, which is typically structured and resembles the phosphorescence emission spectrum of the ligand. In fact, the small feature on the short wavelength side of the emission from $[Re(bpy)(CO)_3(CH_3CN)]^{2+}$ may be due to such LC emission. However, the present data are

Scheme 1. Structures of the Re metal–ligand complexes.
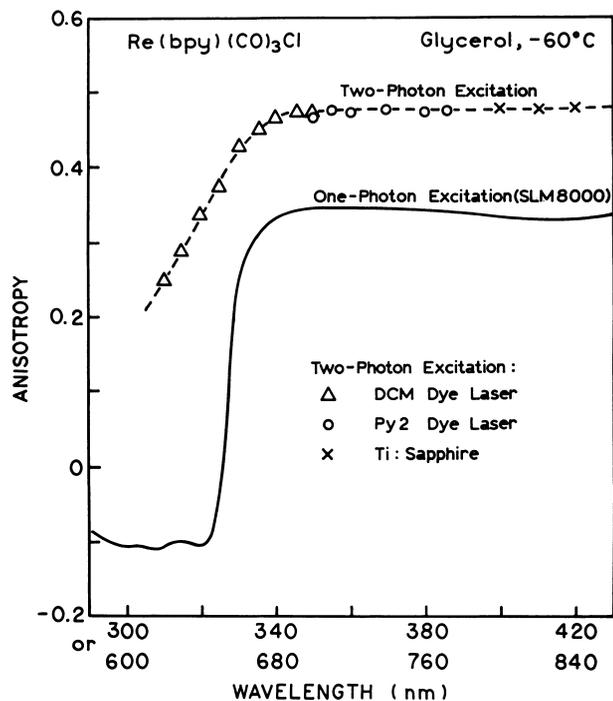
Fig. 4. Excitation anisotropy spectra of Re(bpy)(CO)$_3$Cl for one- and two-photon excitation.
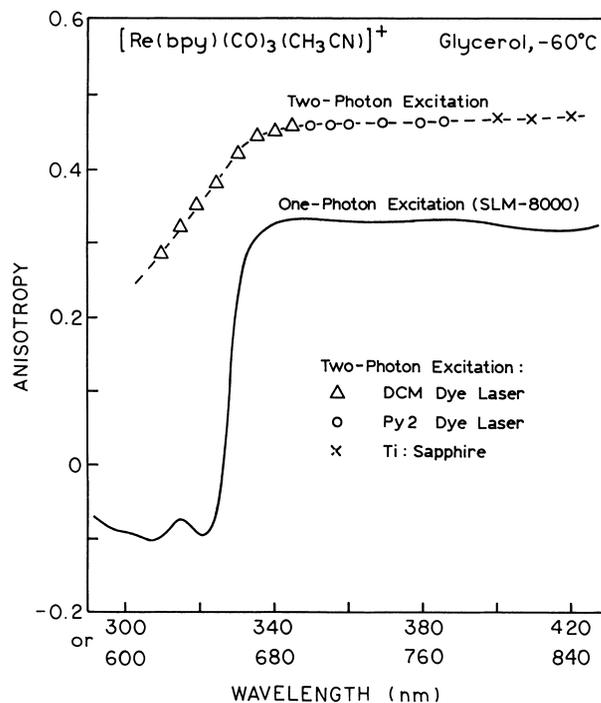
Fig. 5. Excitation anisotropy spectra of [Re(bpy)(CO)$_3$(CH$_2$CN)]$^+$ with one- and two-photon excitation.

inconclusive, and suggest that the emission spectra of [Re(bpy)(CO)$_3$(CH$_3$CN)]$^{2+}$ are the same for both one and two-photon excitation.

Excitation anisotropy spectra of the two rhenium complexes are shown in Figs. 4 and 5. Polarized emission from a rhenium complex has been reported previously [24]. The present spectra are measured in glycerol at −60°C to eliminate rotational diffusion during the excited state lifetime. For both complexes the anisotropy with one-photon excitation is high (≈ 0.33) and constant for excitation from 340 to 420 nm. The constant anisotropy suggests the same electronic transition over this range of wavelengths. The high anisotropy value that indicates the absorption and emission transition moments are nearly collinear. It is easy to imagine that the electronic transition points from the rhenium metal to the bpy ligand, in the same direction as the electron transfer process.

For one-photon excitation with wavelengths below 320 nm, the anisotropy is negative (Figs. 4 and 5). Such negative anisotropy values are due to absorption and emission transition moments which are nearly perpendicular [25]. The decrease in anisotropy as the excitation wavelength decrease below 330 nm is most probably due to a shift from MLCT to ligand centered (LC) absorption. Given the negative value of r_0, the LC transition is probably directed along the long axis of the bpy molecule, and thus perpendicular to the rhenium-bpy axis.

Anisotropy spectra of the rhenium complex were also measured with two-photon excitation (Figs. 4 and 5). The

anisotropies are high and constant for excitation from 680 to 840 nm. The values with two-photon excitation are about 1.4-fold larger than those found for one-photon excitation. This is the factor expected for two-photon vs. one-photon excitation, assuming the same direction for the electronic transitions [26]. Hence, the rhenium complexes appear to be excited to the same electronic state independent of the mode of excitation.

For two-photon excitation using wavelengths below 640 nm the anisotropy decreases (Figs. 4 and 5). However, the rate of decrease is less than expected for the rapid shift from MLCT to LC absorption seen with one-photon excitation. This suggests that the ligand centered absorption displays a lower relative cross-section for two-photon absorption as compared to one-photon absorption.

Intensity decays of the rhenium complex were measured using one-photon (Fig. 6) and two-photon (Fig. 7) excitation. For both complexes, independent of the mode of excitation, the frequency-domain data were consistent with a single decay time (Table 1). Additionally, the intensity decay data for one- and two-photon excitation of each complex could be analyzed globally to yield the same decay time and goodness-of-fit (not shown). The decay time of the chloride complex is about 14-fold shorter than that of the acetonitrile complex, consistent with the lower quantum yield of the chloride complex.

We also examined the anisotropy decay of the rhenium complexes, with one- and two-photon excitation (Figs. 8
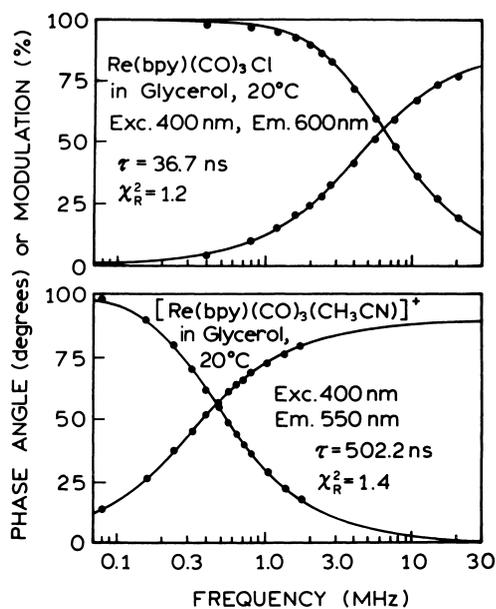
Fig. 6. Intensity decays of the rhenium complexes with one-photon excitation at 400 nm.
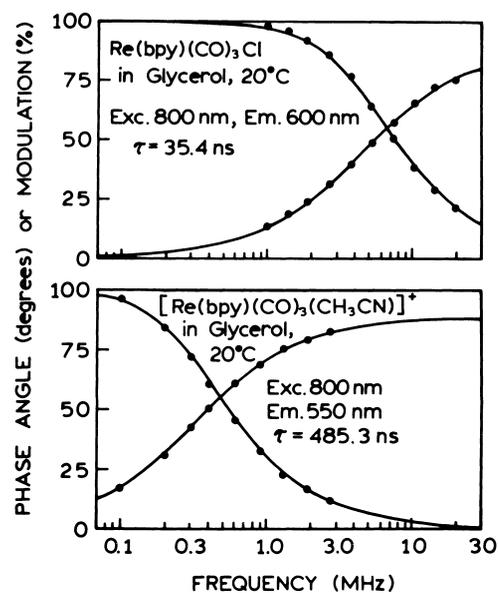

Fig. 7. Intensity decays of the rhenium complexes with two-photon excitation at 800 nm.
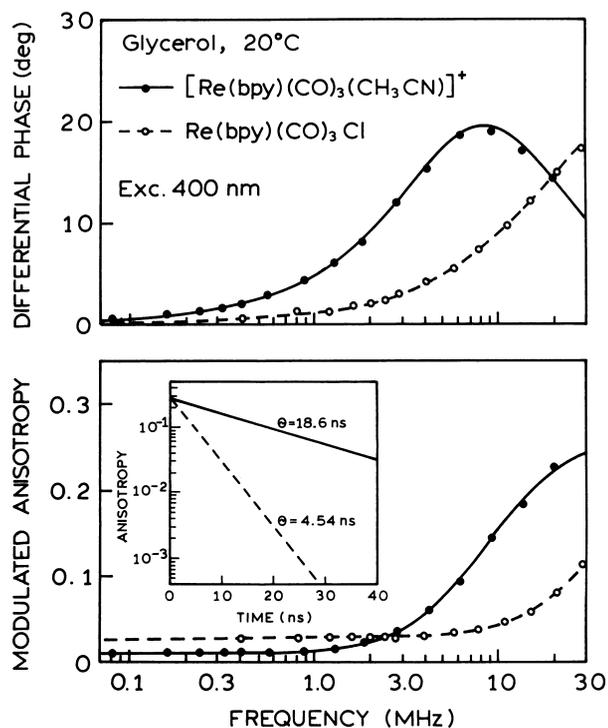

Fig. 8. Anisotropy decays of the rhenium complexes with one-photon excitation at 400 nm.
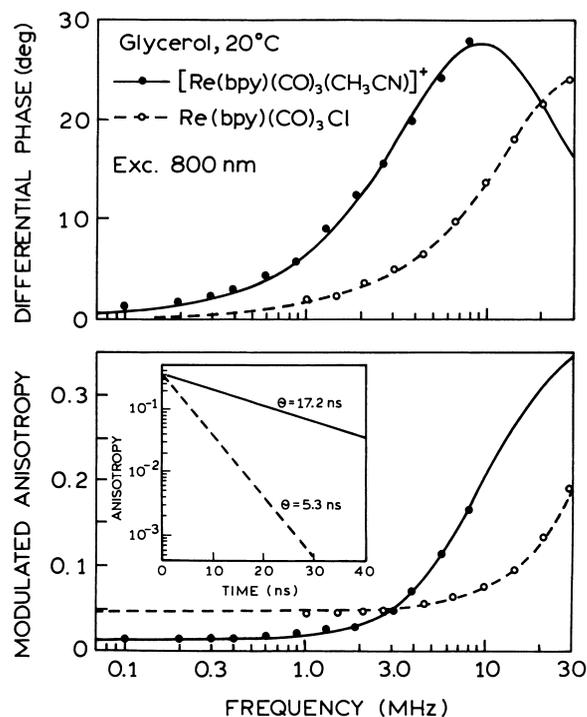

Fig. 9. Anisotropy decays of the rhenium complexes with two-photon excitation at 800 nm.

and 9). The anisotropy decayed with a single correlation time for both complexes. As expected from the fundamental anisotropies (Figs. 4 and 5), the time-zero anisotropies were 40% higher with two-photon excitation. A surprising aspect of the data is that the correlation time of the acetonitrile complex is 3 to 4-fold longer than that of the chloride complex. One explanation of the longer correlation time

is that the acetonitrile complex is positively charged, and has a counter ion PF_6^-, whereas the chloride complex is neutral. Another factor is that the effective radius of the acetonitrile complex may be larger due to the larger CH_3CN ligand and the PF_6^- counterion. In fact, an increase in radius of 50%, from 4 to 6 Å, is expected to increase the volume and correlation time by a factor of 3.4. Since the same overall

Table 1
Intensity and anisotropy decay analysis for the rhenium complexes with one-photon and two-photon excitation in 95% glycerol at 20°C

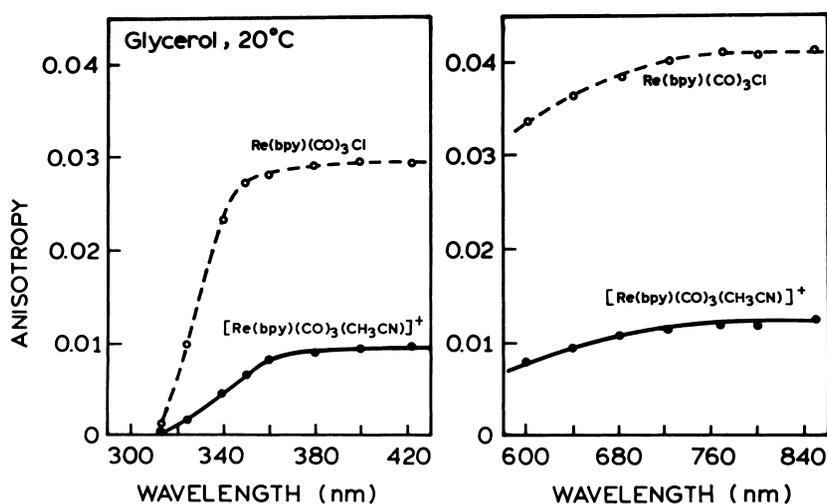| Compound	Excitation	Intensity decay			Anisotropy decay		
		α	τ (ns)	χ_R^2	$r(0)$	θ (ns)	χ_R^2
Re(bpy)(CO)$_3$Cl	400	1.0	36.7	1.2	0.264	4.54	0.6
	800	1.0	35.4	5.4	0.375	5.29	3.2
[Re(bpy)(CO)$_3$(CH$_3$CN)]$^+$	400	1.0	502.2	1.4	0.263	18.6	2.0
	800	1.0	485.3	4.9	0.379	17.2	2.6

Fig. 10. Steady-state anisotropies of the two rhenium complexes with one- and two-photon excitation, left and right panels, respectively.

correlation times were observed with one- and two-photon excitation, we feel the results are reliable and that the acetonitrile complex does display a decreased rate of rotational diffusion. The difference in correlation times is confirmed by the steady-state anisotropies (Fig. 10). As expected for its shorter lifetime the chloride complex displays a higher anisotropy. At this time we cannot explain the large difference in correlation times for these two similar molecules.

4. Discussion

The results described above demonstrate the usefulness of rhenium metal–ligand complexes as biophysical probes. These complexes, and a number of other rhenium complexes [27], were all found to display large fundamental anisotropies above 0.3. We believe the large anisotropies are a consequence of the single chromophoric ligand present in these complexes, as compared to the three ligands usually present in ruthenium complexes. Another favorable feature of these molecules is the constant anisotropy over a wide range of excitation wavelengths, allowing their use as anisotropy probes with a variety of laser sources. And finally, the rhenium complexes can be excited at long wavelengths by a multi-photon process, making them potentially useful as probes in multi-photon microscopy or in tissues.

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