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$[Ru(phen)_2dppz]^{2+}$ luminescence reveals nanoscale variation of polarity in the cyclodextrin cavity†

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Phosphorylation of β-cyclodextrin enhances binding with Ru(II)polypyridyl complexes, and promotes selectivity based on chirality and ligand hydrophobicity. For $[Ru(phen)_2dppz]^{2+}$, inclusion of dppz results in a dramatic increase in luminescence with multiple lifetimes. The sensitive response of photophysics to the environment reveals nanoscale variation of polarity.

Ruthenium(II) complexes with a dppz ligand (dppz = dipyrido[3,2-*a*:2′,3′-*c*]phenazine)‡ (Fig. 1) exhibit extraordinary environmental sensitivity of their luminescence lifetimes and quantum yields.[1] The complexes emit at 600–650 nm in organic solvents[2a,b] and in hydrophobic microenvironments,[2c–e] as well as intercalated with DNA.[3] However, in aqueous solution, the emission is dramatically quenched.[4a] This behaviour results from differential population of two close-lying dppz-localized ^{3}MLCT states,[4] with increased population of the low-lying non-emissive state favoured in polar and H-bonding solvents.[5] Hydrogen bonding of water to the dppz nitrogens in the excited state is proposed to be responsible for quenching, with H-bonds to both nitrogens required to completely extinguish luminescence.[5,6] To expand our knowledge of $[Ru(phen)_2dppz]^{2+}$ photophysics in restricted environments, we have studied its interaction and emission with neutral β-cyclodextrin and with its phosphorylated analogue.

β-Cyclodextrin (β-CD) is a cyclic oligosaccharide of seven D-glucopyranose units linked by α-1,4-glycosides to produce a conical cylinder with a hydrophobic inner cavity and hydrophilic rims (Fig. 1). Modifications on the rim alcohols with charged groups greatly enhance water solubility.[7] The more nucleophilic primary alcohols (6-position; narrow rim) are more readily modified than the secondary alcohols (2-,3-positions) by strong electrophiles such as phosphoryl chloride to produce phosphoric acid derivatives of β-CD.[8]

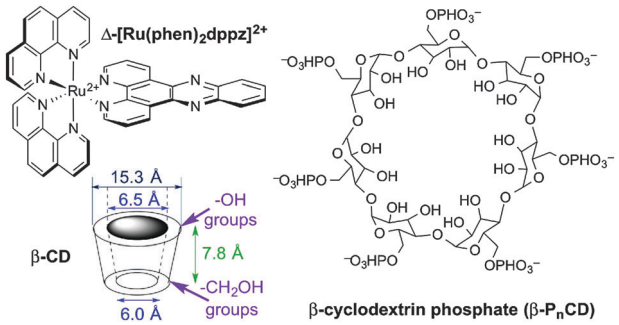

Fig. 1 Representative structures for this study.

Cyclodextrins form inclusion complexes with organic molecules that insert non-polar guest regions into the cavity, whilst polar or charged regions remain exposed to solvent.[9] Bicyclic and tricyclic heteroaromatic molecules and polyaromatic hydrocarbons form inclusion complexes with β-CD with their long axes oriented through the cavity. Of particular relevance for $[Ru(phen)_2dppz]^{2+}$ is the phenazine dye Neutral Red which forms 1 : 1 and 1 : 2 inclusion complexes with β-CD.[9b]

Neither $[Ru(bpy)_3]^{2+}$ (bpy = 2,2′-bipyridine) nor $[Ru(phen)_3]^{2+}$ bind significantly to β-CD,[10a,b] since the width of the phen and bpy ligands precludes full insertion into the cavity. However, they form tighter complexes with anionic cyclodextrins.[10] For example, carboxymethylthio-β-CD shows selectivity for *Δ*-$[Ru(phen)_3]^{2+}$, with NMR showing that at least one phenanthroline ligand is included from the anion-decorated primary alcohol rim, which is widened compared to β-CD by electrostatic repulsion of the carboxy groups.[10b] Large salt effects indicate significant Coulombic attraction but a failure strongly to bind $[Ru(bpy)_3]^{2+}$ suggests that hydrophobic inclusion is also important.[10b] In this report we compare the interactions with phosphorylated β-CD of $[Ru(L)_3]^{2+}$ and $[Ru(L)_2dppz]^{2+}$ (L = phen or bpy) to clarify how the dppz ligand influences binding.

Addition of neutral β-CD to aqueous *Δ*-$[Ru(phen)_2dppz]^{2+}$ has only minor effects on absorption and emission (ESI†). Low level hypochromism and emission suggest that the dppz ligand

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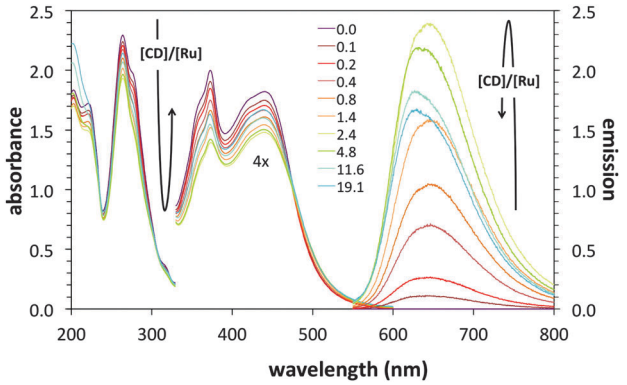

Fig. 2 Absorption and emission spectra of Δ-[Ru(phen)$_2$dppz]$^{2+}$ with added β-CD-phosphate. λ_{ex} = 410 nm; [Ru] = 20 μM; legend shows [β-P$_n$CD]/[Ru] ratios.

enters the β-CD cavity and becomes protected from water. However, the small magnitude of the changes indicates weak association, consistent with inclusion constants for tricyclic aromatic dyes of K ~10^2 M^{-1}.[9b]

In contrast, binding to anionic phosphorylated β-cyclodextrin (β-P$_n$CD) induces substantial changes in both the absorption and emission spectra of Δ-[Ru(phen)$_2$dppz]$^{2+}$ (Fig. 1), with two identifiable regimes. Addition of low concentrations of β-P$_n$CD to Δ-[Ru(phen)$_2$dppz]$^{2+}$ produces hypochromism across the absorption spectrum (Fig. 2) until [β-P$_n$CD]/[Ru] reaches ~2 (Regime I). At higher host concentrations, the behaviour is reversed and absorption increases (Regime II). In Regime I, strong hypochromism in the dppz-polarized (370 nm) band and band-shape changes are consistent with insertion of this ligand. In Regime II, hypochromism is partly reversed but the lack of a single isosbestic point in the visible band, together with irreversible band-shape changes, imply that this does not represent a reversion to free dye but instead results from population of a second binding mode. This is supported by the observation of two independent regimes in the emission spectra. In regime I, addition of β-P$_n$CD results in growth of a broad emission band centred at ~645 nm which gains intensity until [β-P$_n$CD]/[Ru]~2. In regime II, the emission intensity drops as [β-P$_n$CD] increases further, and a substantial blue shift is observed.

Emission lifetime data provide extra insight into the interaction. In single photon counting experiments, for all [β-P$_n$CD]/[Ru], at least three lifetimes are identified for Δ-[Ru(phen)$_2$dppz]$^{2+}$ of about 5, 35, and 180 ns (Table 1), suggesting that the bound complex occupies variety of environments. The integrated intensity (A_n) from the longest lifetime drops at higher binding ratios, mainly in favour of the 32 ns component, consistent with the intensity reduction. Although up to 22% (α_n) of the decay arises from the shortest lifetime, this contributes no more than 1% of the steady-state emission.

Changing the ancillary ligand from phen to bpy has little effect, with two regimes intersecting at [β-P$_n$CD]/[Ru] ~ 2 and binding constants of ~5 × 10^4 M^{-1} for both complexes (Table 2).

Spectrally, Λ-[Ru(phen)$_2$dppz]$^{2+}$ (ESI†) behaves like Λ-[Ru(phen)$_2$dppz]$^{2+}$, with an inversion [β-P$_n$CD]/[Ru] ~ 2, and Table 1 shows that their emission lifetimes and decay amplitudes are comparable. However, binding analysis (Table 2) produces an order of magnitude lower association constant for the Λ-enantiomer (4 × 10^3 M^{-1}) than for Δ-(6 × 10^4 M^{-1}), demonstrating moderate enantioselectivity.

With β-P$_n$CD, both Δ-[Ru(phen)$_3$]$^{2+}$ and Δ-[Ru(bpy)$_3$]$^{2+}$ show increases in emission lifetime (Table 3), together with changes in absorption and emission spectra (Fig. 3 and ESI†).§ For both complexes, absorption drops in the MLCT band and emission is enhanced as [β-P$_n$CD]/[Ru] increases, and small spectral shifts are observed. The change in environment on binding to

Table 2 Binding constants from Benesi–Hildebrand analysis[a]

Complex	$\log_{10} K$
Λ-[Ru(phen)$_2$dppz]$^{2+}$	3.6
Δ-[Ru(phen)$_2$dppz]$^{2+}$	4.8
Δ-[Ru(bpy)$_2$dppz]$^{2+}$	4.5
Δ-[Ru(phen)$_3$]$^{2+}$	5.5
Δ-[Ru(bpy)$_3$]$^{2+}$	5.6

[a] [Ru] = 20 μM, 20 °C. 1 : 1 binding. Uncertainty ±10%.

Table 3 Emission lifetimes (τ) from SPC decays of homoleptic complexes with β-P$_n$CD[a]

	Δ-[Ru(phen)$_3$]$^{2+}$ (N$_2$)		Δ-[Ru(phen)$_3$]$^{2+}$ (air)		Δ-[Ru(bpy)$_3$]$^{2+}$ (air)	
CD:Ru	τ/ns	τ/τ_0	τ/ns	τ/τ_0	τ/ns	τ/τ_0
0	919	1.00	438	1.00	360	1.00
1	1036	1.15	545	1.19	411	1.15
20	—	—	668	1.46	517	1.45

[a] [Ru] = 20 μM; λ_{ex} = 405 nm/λ_{em} = 625 nm.

Table 1 Emission lifetimes (τ), relative initial amplitudes (α), and relative integrated intensity contributions (A) from SPC decays of heteroleptic complexes with β-P$_n$CD. These complexes have no measurable emission in the absence of host[a]

Complex	CD:Ru	τ_1/ns	α_1 (A_1/%)	τ_2 /ns	α_2 (A_2/%)	τ_3 /ns	α_3 (A_3/%)
Δ-[Ru(phen)$_2$dppz]$^{2+}$	0.4	3	0.15 (0.3)	33	0.35 (9.0)	234	0.50 (90.7)
	1	5	0.22 (1.0)	39	0.37 (13.1)	230	0.41 (85.9)
	7.5	6	0.16 (1.2)	35	0.49 (21.2)	179	0.35 (77.6)
	20	5	0.20 (1.4)	35	0.48 (23.5)	168	0.32 (75.1)
Λ-[Ru(phen)$_2$dppz]$^{2+}$	1	3	0.07 (0.2)	28	0.42 (10.9)	200	0.48 (88.9)
	20	5	0.14 (0.8)	34	0.53 (22.4)	200	0.32 (76.8)

[a] [Ru] = 23 μM, 20 °C. Lifetime and amplitude uncertainties ±10%. λ_{ex} = 405 nm/λ_{em} = 625 nm.

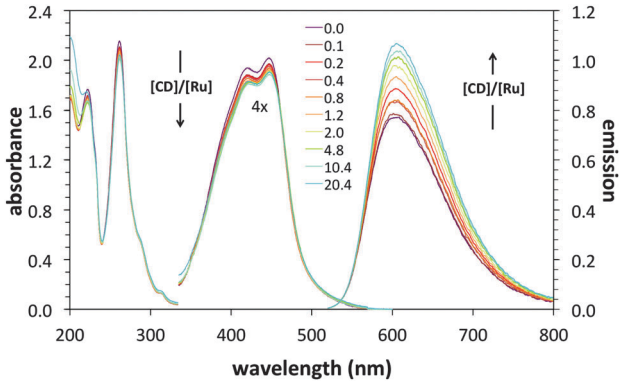

Fig. 3 Absorption and emission spectra of Δ-$[Ru(phen)_3]^{2+}$ with added β-CD-phosphate. λ_{ex} = 470 nm; [Ru] = 20 μM; legend shows [β-P_nCD]/[Ru] ratios.

the cyclodextrin results in increased lifetime and quantum yields, and reduced HOMO–LUMO gap energies. Binding constants were $\sim 3 \times 10^5$ M^{-1} for both complexes (Table 2); an order of magnitude higher than for the dppz complexes.

For homoleptic complexes, all emission decays fit excellently to single exponential functions. Stern–Volmer analysis (ESI†) shows that the O_2 quenching rate constant for $[Ru(phen)_3]^{2+}$ drops slightly from 4.0×10^9 M^{-1} s^{-1} to 2.9×10^9 M^{-1} s^{-1} in the presence of β-P_nCD. This may be attributed to a slightly smaller diffusion coefficient for β-P_nCD–bound $[Ru(phen)_3]^{2+}$ as well as reduced oxygen accessibility to the bound metal complex due to weak protection by the cyclodextrin.

Collectively, these results provide insights into how these ruthenium complexes interact with phosphorylated cyclodextrin in water. The emission and absorption spectra of $[Ru(L)_3]^{2+}$ and $[Ru(L)_2dppz]^{2+}$ (L = bpy or phen) show substantial changes on addition of β-P_nCD, whilst interactions with neutral β-CD and γ-CD are insignificant. The observation of strong $[Ru(L)_2dppz]^{2+}$ emission with β-P_nCD is consistent with insertion of the dppz ligand. Unexpectedly, although full ligand insertion cannot occur for $[Ru(L)_3]^{2+}$, these small complexes bind an order of magnitude more strongly than their $[Ru(L)_2dppz]^{2+}$ analogues. This suggests that although binding is strongly driven by attractive electrostatics between the dicationic ruthenium complex and the multiple negative charges of phosphorylated cyclodextrin, an unfavourable energetic contribution is associated with dppz insertion.

We anticipated that $[RuL_2dppz]^{2+}$ would form a 1 : 1 complex with β-P_nCD through insertion of the dppz ligand, and expected a single lifetime for the ensemble. However, the observed spectral variations paint a more elaborate picture. Different behaviours below and above [β-P_nCD]/[Ru] ≈ 2 indicate more than one binding mode. This is corroborated by the observation of at least three emission lifetimes for bound $[Ru(phen)_2dppz]^{2+}$, whilst single lifetimes are observed for $[Ru(phen)_3]^{2+}$. Correlating the steady-state and time-resolved emission data suggests that the ~180 ns lifetime is associated with the 645 nm emission that predominates at low [β-P_nCD]/[Ru], and the ~32 ns lifetime is associated with the 620 nm emission that becomes increasingly important as [β-P_nCD] is raised. The broad range of measured lifetimes can be interpreted to reflect population sampling of different environments, as previously concluded for binding to poly(dA).‡[11] We can elucidate the nature of different environments by comparing the photophysical characteristics of the complex bound to β-P_nCD with those in organic solvents.

The longest lifetime and emission maximum (~180 ns/645 nm) are similar to those in non-protic polar solvents such as acetonitrile (174 ns/634 nm; Table S1, ESI†). We assign this component to $[Ru(phen)_2dppz]^{2+}$ bound with its dppz ligand inserted deeply into the cavity, thus protecting the non-coordinating phenazine nitrogens from water. The intermediate lifetime and emission maximum (~32 ns/620 nm) is similar to that in methanol (27 ns/627 nm; Table S1, ESI†), suggesting that dppz in this environment is more accessible to H-bonding solvent. The shortest lifetime (~5 ns) is similar to that in ethylene glycol (6.5 ns; Table S1, ESI†),[6b] and complexes with this lifetime must have their phenazine nitrogens in a very polar H-bonding environment. Hence, the variable emission of $[Ru(phen)_2dppz]^{2+}$ in the restricted cyclodextrin cavity can be interpreted in terms of different local environments, the nature of which can be inferred by comparison with lifetimes in bulk solvents. Previous studies have reported the polarity of the β-CD cavity as similar to that of ethanol[12] or an alcohol/water mixture.[9b] Whilst this may reflect the average value, the emission of $[Ru(phen)_2dppz]^{2+}$ reveals considerable nanoscale variation of cavity polarity, as a consequence of its exquisite environmental sensitivity compared to other fluorescence probes.

It is not trivial to determine the exact geometry of bound $[Ru(phen)_2dppz]^{2+}$, but the variation of emission with [β-P_nCD]/[Ru] allows us to make plausible suggestions. The emission enhancement and absorption hypochromism observed up to [β-P_nCD]/[Ru] ~ 2 indicates deep insertion of the dppz ligand. We surmise that additional β-P_nCD molecules bind to the ancillary ligands, since titrations with $[Ru(phen)_3]^{2+}$ and $[Ru(bpy)_3]^{2+}$ show that the host interacts strongly with these ligands. Such binding would likely push the dppz-encapsulating β-P_nCD away from the ruthenium centre due to electrostatic repulsion between hosts. This would increase exposure of the phenazine nitrogens to water, resulting in reduced emission intensity. Such ancillary ligand binding by a second host molecule has been observed in crystal structures of $[Ru(phen)_2dppz]^{2+}$ with oligonucleotides.[13] Indeed, binding of $[Ru(phen)_2dppz]^{2+}$ to β-P_nCD shares some features with DNA intercalation from the minor groove,[3d] where dppz inserts into a hydrophobic cavity from a location of high negative potential due to the backbone phosphates. However, the lifetimes with cyclodextrin are shorter than those generally observed for intercalation,[3,14] and under suitable conditions, a single lifetime can be observed for a complex bound to an isolated intercalation site.[14b] We envisage the cationic ruthenium centre preferentially localizing at the anionic phosphorylated rim when [β-P_nCD]/[Ru] < 2, but we cannot exclude the possibility that dppz inserts from both rims, and that the equilibrium between the two orientations alters as [β-P_nCD] changes, producing the observed photophysical variations. Whatever the structure of the bound complex,

it is clear that the multiple lifetimes indicate population sampling of different environments, with the binding ratio causing subtle alterations in the distribution.

In conclusion, we report that phosphorylation of β-CD dramatically enhances interactions with ruthenium(II) polypyridyl complexes, including $[Ru(L)_2dppz]^{2+}$ which inserts the dppz ligand to turn on luminescence. However, contrary to our initial expectations, the dppz ligand decreased the affinity of the host for the metal complex. This suggests that as well as favourable electrostatic attraction, there is an unfavourable energetic contribution associated with dppz insertion. Although the ancillary ligand (phen *vs*. bpy) has little influence on binding avidity, chiral selectivity occurs, with Δ-$[Ru(phen)_2dppz]^{2+}$ binding an order of magnitude more strongly than Λ. As a result of cyclodextrin shuttling along dppz when additional β-P_nCD molecule bind to ancillary ligands, $[Ru(L)_2dppz]^{2+}$ exhibits tri-exponential emission lifetimes which we associate with population sampling of local cavity polarities in the range of ethylene glycol to acetonitrile. Future studies will be aimed at developing greater understanding of the influence of hydrophobic encapsulation on the photophysics of these complexes.

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Notes and references

‡ Abbreviations: dppz = dipyrido[3,2-*a*:2′,3′-*c*]phenazine; phen = 1,10-phenanthroline; bpy = 2,2′-bipyridine; poly(dA) = polydeoxyadenylic acid.

§ Under these conditions, no significant changes were observed with β-CD.

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