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Lanthanide luminescence from self-assembled supramolecular hydrogels consisting of bio-conjugated picolinic acid based guanosine quadruplexes

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SUMMARY

The formation of bio-inspired healable lanthanide luminescent hydrogels is detailed. These materials are composed of K(I)-stabilized guanosine quadruplex (G4) that were bio-conjugated to mannose derived picolinic acid ligand 1 using boric acid chemistry (G4-1). The supramolecular self-assembly between G4-1 and europium (Eu(III)) was confirmed, including the use of time-gated luminescence spectroscopy. The most stable hydrogel was formed when the ratio between 1 and Eu(III) was 1:1 and this gel was found to assemble into a helical column. The obtained gel samples were further characterized using techniques such as scanning electron microscopy (SEM), circular dichroism (CD) and luminescence spectroscopy. We demonstrate that a Eu(III)-centered circularly polarized luminescence (CPL) signal from guanosine hydrogels can be observed even via long range interaction, while the rheology of these gels exhibits their healable properties.

Lanthanide luminescence• self-assembly • circularly polarized luminescence • supramolecular hydrogels • guanosine quadruplex • picolinic acid ligands

INTRODUCTION

The design and development of functional self-assembly materials is at the heart of supramolecular and nanochemistry research.[1,2,3] Self-assembly is driven by complimentary and synergetic effects from a great number of non-covalent intermolecular interactions, reversible covalent bond formations, and interactions with media/solvents.[4,5,6] Therefore, it is of no surprise that the design of supramolecular materials remains inspired by supramolecular interactions, 'building blocks' and substrates commonly employed by nature, such as peptides, oligonucleotides and steroids, to name just a few.[7,8,9] Guanosine quadruplexes (**G4**) are important bio-motifs that are formed through hydrogen bonding interactions between four guanine units, which arranged into a helical planar quartet and stabilized by group I cations (M(I)).[10] These structures have been found within telomeric DNA strands and are believed to participate in the regulation of transcription and genome stability, as well as having a potential role in cancer therapy.[11] Hence, it is no surprise that **G4** quadruplex structures have been used as building blocks in supramolecular self-assembly chemistry, such as in the development of sensors, catalysts, (hydro)gels,[12] tissues, and in the formation of controlled-release drug delivery agents.[13,14] For a long time the main limitation in the use of guanosine hydrogels was their relatively low stability which is due, in part, to crystallization leading to the subsequent collapse of the gel. Since then, the stability of the gels has been tailored,[15,16,17] and recently it was shown that stable hydrogels can be obtained

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through the formation of anionic borate diesters with guanosine, following self-assembly and stabilization of the **G4** quadruplex with M(I).[18,19] Such hydrogels were recently developed as photoactivatable drug delivery systems that can deliver platinum(IV) anticancer complexes selectively to cancer cells.[20] Other recent examples include self-healing hydrogels with on-demand release of acyclovir[21] or doxorubicin[22] and stimuli-responsive hydrogels with antibacterial activity.[23] Moreover, when obtained within deep eutectic solvents these systems were shown to reveal excellent electrochromic activity.[24]

In the work presented herein, we set out to develop multi-component bio-inspired self-assembled **G4** gels and investigate if their luminescent, morphological, and rheological properties could be modulated using lanthanide ions. It has been demonstrated that lanthanides can be used as cross-linking agents in both polymeric and supramolecular gels, where some of the physical/functional properties of the lanthanide are transferred to the bulk samples.[25] We foresaw that this could be achieved by introducing the mannose unit as a bridge between guanosine and **1** using borate ester chemistry (Scheme 1). With this in mind, we decided to employ the luminescent Eu(III) ion as the 'cross-linking ion'. We also sought to explore if the chiral nature of **G4** quadruplexes would lead to Eu(III)-centered circularly polarized luminescence (CPL) from the resulting supramolecular metallo-hydrogels, which has not been demonstrated before. The current and growing interest in the application of lanthanide CPL originates from, but is not limited to, their use as optical bio-probes and in the generation of luminescent materials for display technologies, where the evolution of Ln(III)-centered CPL may be induced by including chiral centers within various ligand designs.[26] Here we were interested in determining if CPL would be observed from such self-assembled gels despite the fact that the distance between the emitting center and the chiral **G4** quadruplexes is quite large. We were also interested in whether such a transfer of chirality would affect the overall properties of the bulk sample. The use of Ln(III) ions in this work allows for direct probing of their role in the formation of supramolecular hydrogels.[27] It should be noted that the ligand **1** design chosen in this work is based on the known fact that Eu(III) complexes with 2,5-dipicolinic acid (DPA) form racemic systems containing Δ- and Λ- $[Eu(DPA)_3]^{3-}$ complexes and that the equilibrium between these two enantiomers may be disturbed upon addition of chiral systems to the solution,[26] including histidine-containing peptides,[28] and protein fibrils, through non-covalent interactions.[29]

Herein, we demonstrate that the combination of various supramolecular interactions indeed results in the formation of functional, multi-component, supramolecular Eu(III) gels. The coordination arrangement of **1** around Eu(III) centers is perturbed by guanosine quadruplexes during formation of the hydrogel, which concomitantly is displayed in the luminescent, rheological and morphological properties of the resulting hydrogel.

RESULTS and DISCUSSION

Synthesis and characterisation

The synthesis of the ligand **1** was carried out in six steps (Scheme S1) starting from peracetylated mannose. Glycosylation, followed by azidation, gave intermediate **3** which was further reacted with freshly prepared dimethyl 4-(trimethylsilyl)ethynyl-2,6-pyridinedicarboxylate (**5**) in a copper catalyzed azide alkyne cycloaddition reaction to furnish the corresponding triazole (**6**). Ester hydrolysis with deacetylation was achieved through treatment of **6** with 5% NaOH solution to yield target ligand **1** in 80% yield (see Supplemental Information for reaction conditions). The composition of each compound was confirmed using ^{1}H and ^{13}C NMR spectroscopy, mass-spectrometry, and IR spectroscopy (Figures S1-S10). For the final ligand **1**, elemental analysis was performed to complete its characterization.

Self-assembly and photophysical studies of Eu(III) assemblies with 1 in aqueous medium

Initially we investigated the self-assembly interaction between **1** and Eu(III) in buffered solution with the following evaluation of binding model and binding constant values. The pH behavior of the **Eu1$_3$** system was also investigated in aqueous medium. The data obtained from these studies was then used to prepare several different **G4-1**:Eu(III) gel ratios, and their luminescent, rheological, and morphological properties, as well as their overall stabilities

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were studied. The chirality of the gels was also assessed using circular dichroism (CD) and circularly polarized luminescence (CPL) spectroscopy.

The self-assembly between water soluble ligand **1** (ε_{287} = 6555.4±145.4 $M^{-1} \times cm^{-1}$; Figure S11) and Eu(III) was investigated in DPBS buffered solution (pH = 7.1) by monitoring the changes in the absorbance, fluorescence and Eu(III)-centered emission of **1** (c = 8.84 × 10^{-5} M) upon addition of Eu(III) ions to the solution (0→2 equivalents) (Figure 1A; Figures S12–S14). In all cases, the changes occurred gradually up until the addition of 1 equivalent of Eu(III), upon which the plateau was observed. The changes were analyzed using the non-linear regression analysis program SPECFIT and the binding constants calculated for the chosen binding model where the formation of 1:1, 1:2 and 1:3 (M:L) species was proposed (Table S1). The observed binding constants were lower than those found for Eu(III) and Tb(III) assemblies with DPA,[30] most likely due to the presence of the mannose substituent in the 4-position of the pyridine group in **1** affecting the overall stability of the assemblies, as well as the difference in the buffer used in these measurements.

The design of **1** was motivated by the fact that 4th position of the ring can give high stability and high quantum yield values for Eu(III) tris-complexes in aqueous media.[31] In order to evaluate such properties for **Eu1$_3$** we studied its self-assembly in DPBS buffered solution by reacting 3 equivalents of the ligand **1** with 1 equivalent of $Eu(CF_3SO_3)_3$ (Figure S15). The kinetics of the self-assembly formation was monitored over a period of ~12 hours by recording the changes in the Eu(III)-centered emission and it was found that a plateau was achieved after 5 hours, where we considered that 100% formation of Eu**1**$_3$ had occurred in solution. This was further confirmed by the Eu(III)-centered emission decay of the assembly being mono-exponential in both H_2O and D_2O media after this time, where τ_{H2O} = 1.29±0.01 ms and τ_{D2O} = 2.49±0.01 ms. These values confirm the number of water molecules directly bound to the lanthanide ion (*q* value) to be *q* = 0 (Table S2).[32] The luminescence quantum yield of **Eu1$_3$** was found to be 18.6±0.3%, which is slightly lower than the original $Cs_3[Eu(DPA)_3]\cdot 9H_2O$, and is most likely due to vibrational quenching and lower sensitization efficiency (Table S3) caused by the presence of the triazole moiety. Nevertheless, this luminescence quantum yield is significantly higher than values we have recorded for other chiral based DPA derived systems in our laboratory.[33]

Having formed a self-assembled system in solution we then investigated its dissociation upon dilution in DPBS buffer medium from 1.00 × 10^{-4} M to 1.00 × 10^{-6} M. In this experiment we recorded absorbance, fluorescence, Eu(III)-centered emission spectra and decays of the metal-centered emission (Figure S16, S17A), the spectra of Eu**1**$_3$ self-assembly at the concentration of 2.25 × 10^{-5} M are shown in Figure S17B. It was found that the self-assembly stays as the Eu**1**$_3$ species until dilution to 7.60 × 10^{-6} M. Below this concentration bi-exponential decay was observed, suggesting the presence of two different species, with lifetimes of τ_1 = 1.29±0.01 ms and τ_2 = 0.29±0.01 ms. This is likely attributable to dissociation of the Eu**1**$_3$ assemblies following the equation $Eu\mathbf{1}_3 \rightleftarrows Eu\mathbf{1} + 2\mathbf{1}$.[30,34]

As we intended to create functional and Ln(III) mediated cross-linked hydrogels, we also studied the pH response of both ligand **1** and Eu**1**$_3$. Starting with the ligand and changing the pH from 2.45 to 11.00, the carboxylic and pyridine groups of **1** became deprotonated and the main absorption band centered at 293 nm was gradually shifted to 256 nm (Figure S18) at pH 6.00. Similarly, the maximum ligand fluorescence was observed at pH 6.00, followed by a gradual decrease in the fluorescence intensity at pH 7.00, after which the fluorescence reached a plateau in its intensity (Figure S19). Both titrations were fully reversible where upon ligand protonation, the original spectra were restored (Figures S18, S19). We subsequently carried out an analogous titration on Eu**1**$_3$, changing the pH from 2.0 to 10.4. Again, the photophysical properties of the complex were shown to be pH dependent. The absorption band centered at 275 nm was gradually blue shifted to 272 nm upon deprotonation of the carboxylic acid groups (Figure S20) and a plateau was observed within the pH range of 5 to 9. Concomitantly, the fluorescence and the Eu(III)-centered emission of Eu**1**$_3$ were monitored and it was found that upon changing the pH from acidic to basic solutions, the ligand based fluorescence emission was quenched while the Eu(III)-centered emission increased (Figure S21) (the same trend was observed for Eu(III)-centered emission measured in the phosphorescence mode (Figure S22)). At a pH below 5.00, the carboxylic groups of the ligand

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and the pyridine nitrogen moiety get protonated, leading to the partial dissociation of the complex in solution. The measured luminescence decays are best fitted to bi-exponential decays, resulting in lifetimes of τ_1 = 0.30±0.02 ms and τ_2 = 1.25±0.01 ms at pH = 5.0, and τ_1 = 0.29±0.01 ms and τ_2 = 0.95±0.03 ms at pH = 2.0 corresponding to dissociation following the equilibria: $Eu\mathbf{1}_3 \rightleftarrows Eu\mathbf{1}_2 + \mathbf{1}$ and $Eu\mathbf{1}_2 \rightleftarrows Eu\mathbf{1} + \mathbf{1}$.[30,34] However, above pH = 5.0 the intensity of Eu(III)-centered emission stays constant, with lifetime values being predominantly mono-exponential, with τ = 1.26±0.01 ms suggesting the presence of Eu**1**$_3$ in solution.[30,34]

We also monitored the response in the CD and CPL spectra of **1** upon addition of Eu(III) ions (Figure 1B, S23, S24). Unlike the titrations performed above only selected additions of Eu(III) to **1** (c = 8.84 × 10^{-5} M, PBS, pH = 7.4) were recorded. In the case of CD only a very weak signal centered at 290 nm was observed (Figure S23). The CPL response was detected upon addition of 0.33 equivalents of Eu(III) to the solution of **1**, whereby a negative signal was observed at the emission wavelength centered at 594 nm corresponding to the $^5D_0 \rightarrow {}^7F_1$ transition of the Eu(III) ion, while a positive signal was observed for the $^5D_0 \rightarrow {}^7F_2$ transition (Table 1 and S4, Figure S24). This titration continued until the addition of 1.0 equivalent of Eu(III) ion, with no CPL response observed when adding 2.0 or 3.0 equivalents of metal ion into the solution. Among this titration series, the maximum dissymmetry factor values (g_{lum}) were observed for the addition of 0.33 equivalents of Eu(III) ion to the solution of **1** which corresponds to the highest yield of Eu**1**$_3$ along with minor presence of Eu**1**$_2$ and Eu**1** species (see Figures S12C, S13D and S14C). This result suggests that the presence of a D-mannose substituent in the 4-position of DPA affects the equilibrium within the racemic system containing Δ- and Λ-$[Eu(DPA)_3]^{3-}$ complexes[26] resulting in the presence of the Eu(III)-centered CPL response (Figure 1B, S24).

The interaction between the mannose unit of **1** and Eu(III) is possible at very high concentrations.[35] However, during our self-assembly experiments and pH response studies it was not observed. This is because the DPA binding unit possesses higher binding affinity and sensitization efficiency to Eu(III) ions in comparison to the carbohydrate. Also, no photoluminescence could be detected reflecting that interaction. We further confirmed this finding by investigating the possibility of this interaction using ^{1}H NMR experiments in the following section. Hence, here it can be concluded that the Eu**1**$_3$ complex stays intact within a pH range of 5.00 to 11.00 and gives a CPL response. With this detailed solution study at hand, we moved towards preparing the **G4** hydrogels and investigated their supramolecular properties.

Synthesis and characterization of G4 hydrogels modified with 1 and Eu(III)

We set out to form four different hydrogels, and in Figure 2 the schematic representation of anticipated guanosine functionalized quadruplexes is given following the previously published work.[18] Their synthetic procedure is described in the Supplemental Information along with the cooling rate used (see Figure S25). A hydrogel using **G4** alone was formed for comparison purposes, but such a hydrogel has previously been reported in the literature,[19] and was found to be long-lived. Using the same synthetic approach (see Supplemental Information, Schemes S2–6)[20] we incorporated ligand **1** onto **G4** through borate ester linkages resulting in the formation of the **G4-1** hydrogel. In these only small number of quadruplexes is functionalized with **1** (the ratio between guanosine and **1** being 10:1 equivalents). The Eu(III)-containing hydrogels were then obtained by adding various equivalents of the Eu(III) ion and monitoring the formation of a possible cross-linked Eu-gel. The best results in terms of gel stability and luminescent properties were obtained when the ratio between Eu(III) and **1** was 1:1 resulting in the formation of the hydrogel **G4-2**. Upon adding 2 more equivalents of ligand **1** unbound to guanosine we obtained hydrogel **G4-3**. Similarly to the gel **G4-2**, when the ratio between the ligand and Eu(III) was 3:1 the hydrogel **G4-4** was obtained. As can be seen from Figure 2, all the Eu-crosslinked gels were found to be luminescent, the red Eu(III) centered emission being clearly visible to the naked-eye.

Out of the five gels obtained, only **G4**, **G4-1** and **G4-2** resulted in long-lived stability over a period of more than four years and their overall pH was found to be at ~7.0–7.5. Both **G4-3** and **G4-4** resulted in the formation of gels immediately after the synthesis. Unfortunately, **G4-3** was only shown to be stable for 24 hours while **G4-4** was stable for a period of 20 days upon standing at room temperature.

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For the long-lived hydrogels, thermogravimetric analysis was performed under a nitrogen atmosphere and in each case the loss of water molecules can be clearly observed (Figure S26). The presence of solid component was found at 1.7–2.2 weight % corresponding to the amounts taken to prepare the hydrogels. Solid state ^{11}B NMR spectra were also recorded for these gels (Figure S27), where the signals are observed for the guanosine unit containing the borate ester linkages. The coordination of **1** through the DPA moiety to Eu(III) did not result in any significant changes in the ^{11}B NMR spectrum, probably because the metal centered coordination occurs far enough from the borate ester linkages. For all the gels (**G4**, **G4-1** and **G4-2**) the signals at δ 7.6-8.0 and 11.3-11.4 ppm (Figure S27B) were observed and can be assigned to the presence of borate mono and diesters.[18] The signals at δ 18.8-20.0 ppm suggest the presence of the borate[36] as the counter anion to the potassium stabilizing the structure of guanosine quadruplexes. It should be noted that due to the statistical distribution of the molecules within the gels, various types of packing may be observed where not only one guanosine molecule is connected to **1** within the quadruplex, but also two, three or four (see Figures S28A–D). These quadruplexes may then be assembled into the helical columns as depicted on Figure S28E–H where the repeating unit is indicated. However, due to the complexity of the system, it would be challenging to identify them experimentally in the frame of the current manuscript.

The addition of Eu(III) to the hydrogel containing guanosine and ligand **1** may potentially result in the competitive binding of the lanthanide to the DPA unit of the ligand, and also to the donor oxygen atoms of the guanosine quadruplex. However, we believe that in this instance Eu(III) will preferentially bind to the picolinic acid moiety of the ligand **1** as: 1) guanosine quadruplexes are already preformed before the addition of the lanthanide ion and they are positive in charge due to the stabilization with K(I) ions, thus repelling positively charged Eu(III) ions; 2) the binding constants calculated for Eu(III) assemblies with **1** are high (Table S1) while the interaction with guanosine was previously found to be very weak or non-occurring;[37] 3) the ratio between guanosine and Eu(III) in this work is significantly higher (10:1; 25:1 equivalents) compared to the work postulating the binding of Tb(III) ions into the quadruplex-forming oligonucleotide.[38] Nevertheless, in order to understand the binding processes occurring within Eu(III) containing hydrogels, we monitored the changes by ^{1}H NMR spectroscopy as well as in the absorbance and emission spectra of guanosine upon addition of Eu(III) in water (Figure S29, S30). From ^{1}H NMR spectra, we monitored the possibility of competitive binding of the Eu(III) ion to guanosine or mannose substituent rather than DPA part of ligand **1**. When **1** ($c = 1.26 \times 10^{-3}$ M) was mixed with 1 equivalent of Eu(III) (for example, the ratio between **1** and Eu(III) within the gel **G4-2** is 1:1) in D_2O at 20 °C the signals corresponding to DPA moiety at 8.48 and 8.24 ppm completely disappeared while the signals of mannose containing substituent broadened suggesting the binding of paramagnetic Eu(III) ion into the DPA with the following rearrangement of mannose substituents (Figure S29). It should be noted that the concentration of the ligand used in this NMR experiment is only 4 times lower than within the actual gel. When monitoring the interaction of guanosine ($c = 2.06 \times 10^{-3}$ M) with 0.1 equivalent of Eu(III) ion (the ratio between guanosine and $Eu(OTf)_3$ within the gel **G4-2** is 1:0.1) in D_2O at 20 °C no changes in the proton signals of guanosine was observed. However, when the mixture was heated to 90 °C for three hours the disappearance of the signal corresponding to the purine proton at 8.00 ppm was observed as a result of proton exchange in D_2O (Figure S30A). The experiment was repeated with just guanosine in the NMR tube at the same concentration as the experiment above and 25 times lower then within **G4-2** gel. This solution was warmed up to 90 °C for three hours resulting in the disappearance of the same purine proton of guanosine nucleoside which confirms the proton exchange (Figure S30B). Further spectroscopic titration confirms the above result where no changes were observed in absorbance, fluorescence and Eu(III)-centered emission spectra of guanosine upon addition of 0→2.00 equivalents of Eu(III) ions (Figures S31A–C). However, the addition of 0.10 equivalents of **1** resulted in the immediate increase of the Eu(III)-centered emission intensity (Figures S31B,C) with excitation spectrum indicating that the observed emission occurs due to the energy transfer from **1** (Figure S31D, S17B) to the Eu(III) energy levels. The lifetime of the Eu(III)-centered emission decay was recorded and fitted to the

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mono-exponential decay with $\tau = 0.15 \pm 0.01$ ms suggesting the formation of Eu(III):**1** in the aqueous medium.[34,35]

These experiments clearly indicate the presence of preferable binding of the DPA moiety of **1** to the Eu(III) ion over mannose or guanosine within Eu(III)-based guanosine hydrogels, as anticipated within this work.

Morphological characterization of G4, G4-1, G4-2, and G4-4

Morphological analysis was performed using optical and scanning electron microscopy (SEM) techniques. In the first case 20 μL of each hydrogel was dispersed on a glass surface and the sample was imaged immediately, or after drying (xerogel). The latter was also the case for the SEM where the same amount of the gel was dispersed on a silica surface that was further dried in closed Petri dishes under ambient conditions (Figure 3, S32–S42).[39] The original guanosine-only hydrogel (**G4**) was imaged first, and its very fine fibrous structure was observed using polarized light optical microscopy (Figure S32). This structure is retained upon storing **G4** for a period of over four years. However, once this gel is dried, its fibrous structure is collapsed and is not visible anymore using optical microscopy (Figure S33). Similarly, SEM of the xerogel samples show that **G4** consists of very fine fibrous structure (Figure S34). The following modification of the gel with **1**, however, resulted in hydrogel **G4-1**; which lacked any defined structure using optical microscopy and SEM (Figure S35). However, the formation of crystalline centers could be observed upon storage which appear initially in distinct areas (within two weeks of storage) and over a period of four years it expands throughout the sample. The length of these inclusions was found to be of 0.13±0.03 mm, obtained from the original gels before drying using optical microscopy (Figures S36). The imaging of the xerogel using SEM revealed the length of the inclusions within 0.05±0.03 mm range (Figure S37). Overall, the morphology of **G4-1** (Figure S38) shows increased roughness of the surface compared to that seen for **G4** along with the formation of the observed crystalline inclusions. The addition of Eu(III) ions to **G4-1** results in further morphological modifications. When **G4-2** was formed (where the ratio between the ligand **1** present within the gel and Eu(III) ion is 1:1) the immediate formation of crystalline inclusions was observed. The length of these is within a 0.11±0.03 mm range from the optical microscopy imaging of the samples for initial gel and xerogel (Figure S39), which remains unchanged upon storage of the gels. The SEM imaging for the xerogel **G4-2** reveals 3–6 μm width of the inclusions and the length ranging from 0.04 mm to 0.08 mm (Figure 3A). We were able to check the unit cell parameters of the obtained crystals and they correspond to those known for guanosine dihydrate (see Experimental Procedures part). In this case it is possible that 3 equivalents of guanosine (out of the total 10 equivalents) remained unused for the quadruplex formation and hence these molecules crystalized within this hydrogel. This is due to the ratio of the components used for the preparation of the gels. Closer analysis of **G4-2** morphology shows the fibrous structure of the gel (Figures 3B,C) with an approximate width of the fibers being 28±7 nm. Adding 2 more equivalents of **1** to **G4-2** resulted in the gel **G4-3** which, as discussed above, remained in the gel state only for a period of 24 hours. The morphology of this gel revealed the presence of intertwined rods with width of 1.6±0.5 μm and length of 18±3 mm (Figure S40). The morphology of hydrogel **G4-4**, where the ratio of guanosine functionalized with **1** and Eu(III) was 3:1, did not show the presence of fibers. It is possible that the higher level of cross-linking caused by the interaction of **1** with Eu(III) ions, with the following formation of Eu:$\mathbf{1}_3$ assemblies (see luminescence characterization of the Eu(III) containing hydrogels), disrupted the hydrogen bonding arrangement within and between the neighboring **G4** quadruplexes (Figure S41, S42). This subsequently decreased the overall stability of the hydrogel. The results demonstrate that the supramolecular hydrogel networks collapsed on itself during the drying process to yield xerogel, but other structural changes could also have occurred during the drying process. It is possible that the drying process of the samples for microscopy analysis can affect their overall morphology, however, our results were fully reproducible, and consequently demonstrated consistency using our technique.

Rheological characterization of G4, G4-1, G4-2, and G4-4

The rheological properties were investigated only for the hydrogel samples that were stable over a long period of time (**G4**, **G4-1**, **G4-2**, and **G4-4**). For these samples strain and frequency

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sweeps were performed (Figure 4, Figure S43) and all the samples exhibited gel-like behaviour.[9,39] The storage modulus (G') of **G4-2** and **G4-1** (Figure 4A, Figure S43B) shows little frequency dependence in the linear viscoelastic regime with the response being solid-like (G' > G'') up to ~10Hz, beyond which the loss modulus (G'') starts to increase significantly. This is different to the moduli of the **G4** sample which show a monotonic increase over the measured frequency range with the storage modulus collapsing at ~10Hz (Figure S43A). Similarly, the **G4-4** hydrogel shows gradual increase of the loss modulus following both storage and loss moduli collapse at ~60 Hz (Figure S43C). **G4-2** shows a crossover between G' and G'', indicating a transition to the liquid-like regime at strains above ~20% (Figure 4B), at ~7% for **G4-4**, while for **G4** and **G4-1** the crossover strain amplitudes are at ~400% and ~25%, respectively (Figures S43D,E,F).

Each of **G4-1**, **G4-2** and **G4-4** were stiffer in comparison to **G4**, with the values for the G' plateau being ~5700 Pa, ~1200 Pa, ~180 Pa and 13 Pa (Figure 4B, S43D,E,F), respectively, indicating the critical role of **1** and the metal binding on the rheological properties of the hydrogels. The recovery test was performed after the sample was yielded during the strain sweep. As such, all samples had not returned fully to their rested G' values and all exhibited "post-yield" G' values ~20% of their rest values. All gels demonstrated partial self-healing properties with rapid recovery of G' to 70% of their post-yield values (Figures 4C, S43H,I) which is an important property for possible applications of such gels. Gel **G4** did not recover fully to its pre-yield state, with G'' > G' throughout the recovery test, although the gel was seen to reform to ~70% of its post-yield G' within 20 seconds of high strain cycles (Figure S43G). The **G4** hydrogel exhibits a storage modulus (G') plateau of ~13 Pa which is three orders of magnitude lower than the value observed previously.[19] This is possibly due to the slight difference in the water content used to prepare the gels *versus* the amount of solid components; in our work we prepared gels at 1.7 wt% (50.9 mM of guanosine and 25.5 mM of $KB(OH)_4$), while previously they were made at 2.0 wt% (72 mM of guanosine and 36 mM of $KB(OH)_4$).[19] Another possible explanation for the observed discrepancy in rheology could be variations in the cooling rate where previously authors[19] heated the solution to 90–100 °C until the components dissolved and then removed the reaction mixture from the heat and allowed it to cool down to the room temperature when the formation of the hydrogel occurred. In our work we heated up the mixture to 90 °C for 1 hour upon gradual addition of each component also ensuring that all components fully dissolved. Then the reaction mixture was cooled down to room temperature with the cooling rate given on Figure S25 when the formation of the hydrogel was observed. This discrepancy may lead to differences in the molecular organization within **G4** or variations in the borate diester ratio. The effect of the synthetic conditions on the rheological properties of the gels prepared from low molecular weight hydrogelators has recently been thoroughly discussed and it was observed for other hydrogel systems, indicating the importance of the detailed synthetic procedures to ensure reproducibility.[40] Nevertheless, this gel was stable over a period of four years and for this duration withstood the reverse test.

Luminescence, Circular Dichroism (CD) and Circularly Polarized Luminescence (CPL) Properties of the Hydrogels

The spectroscopic properties of the hydrogels were recorded by placing 10 μL of each sample between two quartz slides (see Experimental Procedures section). The absorbance of the gels shows generally two main bands centered at 250 nm and 285 nm. These two bands are well pronounced in the absorption spectrum of the **G4** hydrogel (Figure S44). Functionalization of **G4** with ligand **1** did not significantly change the absorbance of **G4-1** (Figure S45). However, the coordination to the Eu(III) in the **G4-2** gel resulted in the formation of an additional shoulder at ~350 nm as the transparency of the hydrogel decreased (Figure 5A, S46). The structure of the absorption spectrum of **G4-3** was even more perturbed (Figure S47A). As mentioned above, this sample stayed in the hydrogel form for only 24 hours, being irreversibly transformed into the sol state. The stability of **G4-4** was longer (evolution from gel to sol phase occurred within 20 days) and therefore for this sample we were able to record the absorbance in both gel and sol forms whereby the ratio between 255 nm, 283 nm and shoulder at 334 nm significantly changed (Figure S48A,B).

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Next the circular dichroism spectra were recorded, as it is well known that changes in the CD can be employed to probe the fluctuations in the arrangement of the molecules within supramolecular system, and often in a better way than that seen in the absorption spectrum.[41] When starting with the guanosine hydrogel, a weak positive signal at 298 nm, a strong negative signal at 265 nm, and a positive signal centered at 240 nm were observed (Figure S44). The position of the maxima in the CD spectrum corresponds to the previously reported data[18,42,43] and we can tentatively assign the presence of **G4** quartets being stacked together in both head-to-tail and head-to-head orientations as previously observed. The modification of **G4** with **1**, which gave rise to the formation of the **G4-1** hydrogel, resulted in a CD spectrum containing a negative band at 265 nm and a weak positive band centered at 223 nm with shoulder at 240 nm (Figure S45). This indicates that the structure of the **G4** quadruplexes is retained but the interaction between them has changed favoring head-to-head interactions.[43–45] **G4-2** again indicated the presence of a negative band centered at 265 nm with the shoulder at 285 nm, and relatively more positive in relation to its bands centered at 247 nm and 224 nm (Figure 5B). The baseline for this gel is shifted as its transparency is now decreased. We believe that here the guanosine quadruplexes structure is indeed retained again as the sigmoidal curve containing bands centered at ~260 and 240 nm was observed.

G4 did not show any emission (Figure 44B) and only a very weak luminescence signal centered at 430 nm was observed for **G4-1** upon excitation at 285 nm (Figure S45B). The coordination of the free 2,5-dipicolinic acid group of the ligand **1** to Eu(III) within **G4-2** resulted in the formation of red emissive gels upon excitation with UV light. This is ascribed to the typical long wavelength Eu(III) centered emission, which is the consequence of population of the Eu(III) excited state *via* sensitization from the DPA ligand (see Figure S46 for the excitation spectrum). Upon excitation of the ligand within the hydrogel at 285 nm, no ligand-centered fluorescence emission was observed. Indeed, highly intense characteristic Eu(III)-centered emission was observed, pointing towards efficient energy transfer from the ligand to metal centers, with sharp line-like bands centered at 578, 591, 613, 648 and 692 nm assigned to $^5D_0 \rightarrow {}^7F_J$ (J = 0–4) transitions (Figure 5A). For this gel, **G4-2**, we also recorded the lifetime of its Eu(III)-centered emission, where a bi-exponential decay profile was observed with τ_1 = 0.15±0.01 ms (15%) and τ_2 = 0.29±0.01 ms (85%). This suggests the formation of Eu(III):**1** and Eu(III):$\mathbf{1}_2$ species as they correspond to the previously obtained values of Eu(III):DPA assemblies in aqueous medium.[30,34] In order to confirm the type of species present within the gel experimentally (namely the ratio between Eu(III) and **1**) we prepared the same **G4-2** gel, but instead of H_2O we used D_2O. Again we recorded the lifetime of the Eu(III)-centered emission which followed bi-exponential decay with τ_1 = 0.75±0.02 ms (4%) and τ_2 = 1.60±0.01 ms (96%). This data allowed us to calculate the q-values for the Eu(III) ion,[32] with q_1 = 5.9±0.7 and q_2 = 3.1±0.1. From this we can confirm again the presence of Eu(III):**1** and Eu(III):$\mathbf{1}_2$ binding ratios within this gel as each ligand has three donor atoms (the coordination number of Eu(III) ion being 9). This coordination would be possible either within the same or between neighboring helical columns containing ligand **1** (Figure S28). The fluorescence quantum yield of the **G4-2** hydrogel was found at 4.7±1.0 % which is lower compared to the value obtained previously for the Eu$\mathbf{1}_3$ complex in solution (Table S3). However, within **G4-2**, the presence of lower emissive species Eu**1** and Eu$\mathbf{1}_2$ was confirmed, which was reflected in lower values of the fluorescence quantum yield along with concentration quenching.

The long stability of this hydrogel allowed us to investigate this system further and probe its possible Eu(III)-centered circularly polarized luminescence. It was shown recently that guanosine monophosphate-based quartet nanofibers stabilized with Sr(II) and K(I) ions may act as chiral templates for CPL emission in the case of AuAg nanoclusters,[45] ionogels[46] and various organic dyes where chirality transfer occurred with g_{lum} values ~10^{-3}–10^{-2}.[47] Here, by undertaking CPL spectroscopy of the **G4-2** hydrogel (Figure 5C), a relatively weak signal was observed, which reflected in the low dissymmetry factor values (g_{lum}; Table 2), when compared to these known for Eu(III) systems.[26] Interestingly, when comparing the g_{lum} values for the **G4-2** hydrogel and the self-assembled system in solution, a clear differences can be noticed (Table 2). Here we compared **G4-2** with the self-assembled system where the ratio between **1** and Eu(III) is 1:1 as within the hydrogel). Firstly, the g_{lum} for $^5D_0 \rightarrow {}^7F_2$ reverses from

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a positive to a negative value when going from the self-assembly to the hydrogel, indicating a change in the chiral environment of the Eu(III) ion. The observation of the CPL from the gel is particularly intriguing given the fact that normally the CPL signals arising from lanthanide ions are only significant provided that the ion sits within the chiral environment itself.[33] This can occur from the ligand (with induced ring inversion and subsequently locked arm rotation that governs the overall enantiopure nature of the complex) or within the active site in a protein, *etc*. as we[48] and others[26] have demonstrated. Indeed, it has been previously shown that the presence of the chiral centers in the 4-position of the pyridine ring of 2,5-dipicolinic acid resulted in Ln(III) helicate species exhibiting weak CPL signals.[49] We have also shown that in Ln(III) complexes with 2,6-bis(1,2,3-triazol-4-yl)pyridine (btp) ligands, the magnitude of g_{lum} values significantly decreased when the chiral centers were located further away[50] *vs* closer in distance to the metal ion centers.[51] One of the main factors contributing to the relatively low observed magnitude of g_{lum} values, however, is the presence of guanosine within the hydrogel and the turbidity of the gel itself, which scatters photons, causing them to depolarize and thereby significantly diminishing the magnitude of observed CPL.[52] Despite these strong scattering and depolarization effects, the g_{lum} values obtained here (g_{lum} ~10^{-3}) are similar to these found for BODIPYs, self-assembled chiral nanoparticles and quantum dots.[53]

Addition of two more equivalents of **1** to **G4-2** resulted in the formation of the significantly less stable gel **G4-3**. Despite the lower stability, we were able to record its emission spectrum which showed once again that no ligand-centered emission was observed, but the characteristic emission bands for Eu(III) that were centered at 578 nm, 592 nm, 614 nm, 648 nm and 692 nm, assigned as before to deactivation of the $^5D_0 \rightarrow {}^7F_J$ (J = 0–4) transitions were observed (Figure S47A). The Eu(III)-centered emission decay was also recorded and fitted to a mono-exponential function with τ = 0.63±0.03 ms suggesting the eventual formation of Eu**1**$_2$ assemblies within the gel by comparing to previously obtained data by Horrocks *et al.* and Bünzli *et al*.[30,34] The CD signal of the **G4-3** hydrogel was significantly different, resulting in a positive CD signal centered at 316 nm and a negative signal at 240 nm (Figure S47B). This suggests the disruption of the guanosine quadruplexes structure and possibly the prevalent formation of the ribbon-like assemblies within this system.[54,55]

Conversely, changing the ratio between guanosine quadruplex functionalized with **1** (see anticipated structure shown on Figure S28A) and Eu(III) to 3:1 resulted in the formation of the slightly more stable hydrogel **G4-4** that, as discussed above, retained its gel-like structure over a period of 20 days. This was also reflected in the shape of its CD spectrum, where initially well-resolved CD signals were observed (Figure S48A) which became significantly disrupted upon storage (Figure S48B). The luminescence spectrum of the freshly made **G4-4** hydrogel was recorded and again no ligand-centered emission was observed, with only intense characteristic Eu(III)-centered emission bands detected at 593 nm, 614 nm, 650 nm and 693 nm corresponding to $^5D_0 \rightarrow {}^7F_J$ (J = 1–4) transition bands (Figure S49). The lifetime of the Eu(III)-centered emission for the **G4-4** hydrogel was recorded and best fitted to a mono-exponential decay profile, with τ = 1.20±0.01 ms, which corresponds to the ratio between **1** and Eu(III) within **G4-4** gel being 3:1 (Table S2).[30,34] This 3:1 ratio in turn seems to disrupt the interactions between the guanosine quartets leading to the dissociation of the hydrogel or the formation of the ribbon-like structures, as the sample eventually becomes CD silent (Figure S48B).[54,55] Again **1** may be attached to one quadruplex or to neighboring ones (Figure S28). Nevertheless, the fluorescence quantum yield of the **G4-4** is 9.3 ± 2.1 % which is almost two times higher when compared to the value observed for **G4-2**, which contains less emissive Eu:**1** and Eu:**1**$_2$ species. Generally, the fluorescence quantum yields for the hydrogels are lower when compared to the values found for Eu**1**$_3$(CF_3SO_3)$_3$ (Table S3). This is due to either the presence of less emissive Eu:**1** and Eu:**1**$_2$ species, concentration quenching, and/or higher vibrational quenching by C–H, N–H and O–H vibrations of the neighboring molecules within the hydrogels.

One of the possible representations of the interaction between guanosine quadruplexes containing ligand **1** and Eu(III) ions within **G4-2**, **G4-3** and **G4-4** is schematically shown in Figure S50. It should be noted that such interactions may be more complex following the possibility of various types of quadruplex formations and their packing into the helical columns (see the Figure S28). However, we believe the type of species (in terms of Eu(III) to

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1 ratios) would remain the same, as determined following the spectroscopic evaluation of these hydrogels.

Finally, in order to further investigate the possible binding of guanosine donor atoms to the Eu(III) ions we formed **G4** and added 0.10 equivalents of Eu(III) (see the Supplemental Information for the synthetic details) and evaluated its absorption, fluorescence and CD properties (Figure S51). The absorption and CD spectra of this hydrogel did not change significantly in comparison to the CD spectrum of the original **G4** hydrogel (although slight change in transparency and the ellipticity of the signals was observed) suggesting the retention of the quadruplexes. Almost no emission signal could be observed in the fluorescence spectrum of this hydrogel, and it was found to be 250 times lower compared to the emission intensity of **G4-2**. The excitation spectrum of **G4** with 0.10 equivalents of Eu(III) suggests that very low emission can be excited by the presence of guanosine molecules, but no clear binding interaction between Eu(III) ions and guanosine was detected by us. This reaffirms that within all the obtained hydrogels, Eu(III) had preferential binding to the DPA of **1** resulting in their bright red luminescence.

Conclusions

We have demonstrated the incorporation of ligand **1** within a guanosine quadruplex structure through its mannose unit using boric acid and potassium hydroxide.[18,20] This in turn allowed us to build up self-assembled structures using Eu(III) ions which, upon coordination through the 2,5-dipicolinic acid unit of **1**, resulted in the formation of hydrogels of different robustness which were highly emissive in the red range of the visible spectrum. Their luminescent, morphological, and rheological properties were investigated, demonstrating that these Eu-based hydrogels consist of morphologies made up of dense networks containing crystalline inclusions of guanosine dihydrate. Two of the gels (**G4-1** and **G4-2**) were exceptionally stable and partially self-healable. Their physical properties and formation were further probed using various spectroscopic techniques including CD and CPL for **G4-2** which confirmed the presence of a Eu(III)-centered CPL response. The g_{lum} values of **G4-2** were found to change amplitude and be lower than those observed for the self-assembly in solution between Eu(III) and **1**. However, the magnitude of CPL emission was likely diminished by the presence of guanosine and multiple scattering and depolarization of photons as the photons traversed the gel substrate. The inversion of the CPL signal is likely to have occurred due to the presence of a chiral arrangement of guanosine quadruplexes. Overall magnitude of the CPL emission was comparable to the CPL emission of various organic molecules and quantum dots. It is important to emphasize that the system studied here is a complex self-assembly and, as such, further studies will help to understand the arrangement of its components within the overall structure. However, the study of such supramolecular systems becomes especially interesting within the view of the recent discovery of the biological activity of lanthanide ions, whereby such gels could be used as a model environment to monitor biological processes.[56]

EXPERIMENTAL PROCEDURES

Resource availability

Lead contact

Further information and requests for resources should be directed to and will be fulfilled by the lead contact, Thorfinnur Gunnlaugsson (gunnlaut@tcd.ie).

Materials availability

All materials generated in this study are available from the lead contact upon request.

Data and code availability

Data and code generated during this study are available from the lead contact upon request.

Materials and Methods

All solvents and chemicals were purchased from commercial sources and used without further purification. Where indicated compounds were purified using a TeledyneIsco CombiFlash Rf 200 automated purification system. Water was purified using a Millipore Milli-Q water purification system. Stock solutions of **1** and $Eu(CF_3SO_3)_3$ were prepared just before

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use in Millipore water. Exact concentration of $Eu(CF_3SO_3)_3$ solutions were determined by complexometric titrations using a standardized Na_2H_2EDTA solution in urotropine buffered medium and xylenol orange as the indicator. Deuterated solvents used for NMR analysis ($CDCl_3$, D_2O) were purchased from Apollo Scientific. Melting points were determined using an Electrothermal IA9000 digital melting point apparatus. Mid-infrared spectra were recorded using a Perkin-Elmer Spectrum One FT-IR spectrometer equipped with a universal attenuated total reflection (ATR) sampling accessory. Elemental analysis was conducted at the Microanalytical Laboratory, School of Chemistry and Chemical Biology, University College Dublin.

NMR studies

The ^{1}H and ^{13}C NMR spectra were recorded using an Agilent DD2/LH spectrometer at frequencies 400.1 MHz for ^{1}H NMR and 100.6 MHz for ^{13}C NMR. Chemical shifts are reported in ppm using deuterated solvents as internal standards. For solid state ^{11}B NMR the gels were packed into 3.2 mm O.D. ZrO_2 rotors, and NMR experiments were conducted using a Bruker AvanceIII HD NMR spectrometer ($\mathbf{B}_0$ = 9.40 T, $\nu_L(^{11}B)$ = 128.38 MHz). Spectra were acquired using TopSpin 3.6 software. A Bruker 3.2 mm HX double-resonance MAS probe tuned to ^{11}B on the X channel was used. A high power decoupling pulse sequence used and a 50kHz proton decoupling field was applied. The samples were spun at 10kHz and the temperature was 20 °C. The solid state NMR had no internal standard.

Mass Spectrometry

Mass-spectrometry was carried out using HPLC grade solvents. Electrospray mass spectra (ESI) were determined on a Micromass LCT spectrometer and high resolution mass spectra were determined relative to a standard of leucine enkephaline.

Thermal gravimetric analysis

Thermal gravimetric analysis was performed on Perkin Elmer Pyrus 1 TGA equipped with and ultra-micro balance with a sensitivity of 0.1 microgram. The temperature range is from 20-800 °C with a scan rate 10 °C/min.

Microscopy studies of the gels

To image the gel samples by scanning electron microscopy (SEM), they were deposited manually (volume: 20 μL) onto clean silicon samples with a thick silicon dioxide layer. Gilson pipettes were used for dosing and silicon pieces used as substrates were all cleaned thoroughly by sonication in HPLC grade acetone followed by HPLC grade propan-2-ol. All components were dried in two steps using a high pressure nitrogen gun and further dried under ambient conditions. The gels were manually drop cast on to the silicon at room temperature and dried during 5 days at ambient conditions inside closed Petri dishes. SEM was carried out using the Zeiss ULTRA Plus using either an SE2 or in-lens detector in the Advanced Microscopy Laboratory, CRANN, Trinity College Dublin. The samples prepared for the imaging using SEM did not have any additional conductive layer cover unless otherwise stated that sample was covered with Au/Pd layer.

Optical microscopy of the gels was performed by depositing 20 μL of the sample on the glass substrate in a similar manner described above. The gel was then imaged as soon as prepared and after it was dried using Olympus SZX16 optical microscope.

X-ray analysis

A sample of crystalline material from the hydrogel **G4-2**, approximate dimensions 0.17 mm x 0.02 mm x 0.01 mm, was used for the X-ray crystallographic cell determination. The X-ray intensity data were measured (λ = 1.54178 Å) on a Bruker Apex Kappa Duo with 30s exposures and 3 x 30 frames collected. Data were collected at 100K using an Oxford Cobra Cryosystem low temperature device and the sample was mounted on a MiTeGen micromount. The cell was determined using APEX3[57] indexing software based on a minimum I/σ = 4, yielding 120 reflections, a = 6.75 Å, b = 11.71 Å, c= 17.73 Å, β = 99.96°, crystal system = monoclinic, lattice = P. A comparison of this cell with the CCDC shows the closest match to the reagents involved to be guanosine dihydrate, CCDC 1170419.

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Rheological studies of the gels

The rheology of the hydrogels was measured with a MCR 301 rheometer from Anton Paar. We used a 50 mm parallel plate geometry with a sand-blasted surface to prevent slip. After placing the sample, the upper plate was slowly lowered until a gap of size 0.5 mm (0.2 mm for sample **G4-1** due to limited sample volume) was reached. Excess gel was trimmed just before the final gap height was reached. A solvent trap was placed to avoid evaporation. To ensure reproducible measurements we allowed the sample to rest after loading for 16 hours. At that point, we performed first the frequency sweep, then the strain sweep and finally the recovery test. The Peltier plate kept the samples at a constant temperature of 20 °C. The oscillatory strain sweep measurements were performed at a frequency of 1 Hz while the frequency sweeps were performed at a constant strain amplitude of 1%.

Photophysical studies

Unless otherwise stated, all the solution measurements were performed at 298 K in Dulbecco's DPBS buffered solutions (without calcium and magnesium chloride, Sigma). UV-visible absorption spectra were measured in 1 cm quartz cuvettes or by spreading 10 μL of each hydrogel between two quartz slides (9.5 mm × 34 mm) on a Varian Cary 50 spectrophotometer, FluoroMax-4 or Fluorolog-3 (Horiba Jobin Yvon). Baseline correction was applied for all spectra. Emission fluorescence, phosphorescence (delay time – 0.10 ms; total decay time: 0.020 s) and excitation spectra and lifetimes were recorded on a Varian Cary Eclipse Fluorimeter or FluoroMax-4. Quartz cells with a 1 cm path length from Hellma were used for these measurements or again 10 μL of each hydrogel sample was spread between two quartz slides (9.5 mm × 34 mm). The temperature was kept constant throughout the measurements at 298 K by using a thermostated unit block. Phosphorescence lifetimes of the Eu($^5D_0$) excited state were measured in time-resolved mode at 298 K. They are averages of three independent measurements, these were made by monitoring the emission decay at 616 nm, which correspond to the maxima of the Eu(III) $^5D_0 \rightarrow {}^7F_2$ transition, enforcing a 0.1 ms delay, and were analysed using Origin 7.5®. The details for determination of the *q* values and relative quantum yield measurements are given in the Supplemental Information. A F-3018 integrating sphere accessory mounted on a Fluorolog-3 spectrophotometer was used to measure photoluminescence quantum yields of the hydrogels **G4-2** and **G4-4** (λ_{ex} = 340 nm). The reported values are average of three repeat measurements, while the accuracy of the method is 10%. Emission and excitation spectra were corrected for the wavelength response of the system and the intensity of the lamp profile over the excitation range, respectively, using correction factors supplied by the manufacturer.

Circular dichroism (CD) spectra

Circular dichroism (CD) spectra were recorded for solutions and hydrogels where 10 μL of the hydrogel was dispersed between two quartz slides and the measurement was then performed on a Jasco J-810-150S spectropolarimeter. Spectra were obtained using a scanning speed of 50 nm/min, response time of 4 s, bandwidth of 1 nm and data pitch of 0.5 nm at 20 °C. Each spectrum is an average of three scans recorded within 400 (or 300) to 200 nm range.

Circularly polarised luminescence (CPL) spectra

For CPL measurements, a solution of **1** (c = 8.84 × 10^{-5} M) was prepared in PBS buffer (pH = 7.4) in the fluorescence cell. To this solution 0.33, 0.50, 1.00, 2.00 and 3.00 equivalents of Eu(CF_3SO_3)$_3$ were gradually added. The solutions were left for 30 minutes after each metal addition to allow for the equilibration of the system (see Figure S15) and after this on each stage the CPL was recorded. In the case of hydrogels a small thin amount of the prepared gel sample was loaded into a concavity optical glass slide (BRAND® cavity slides 1.2-1.5 mm. model: BRAND, 475505) which was placed in contact with a second concavity slide and sealed with nail varnish to make a bubble chamber. CPL were recorded on a custom built spectrometer in Durham University, consisting of a laser driven light source (Energetiq EQ-99 LDLS, spectral range 170 to 2100 nm) coupled to an Acton SP2150 monochromator (600 g/nm, 300 nm Blaze), allowing excitation wavelengths to be selected with a 6 nm FWHM band-

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pass.[53] The collection of the emitted light was facilitated by a lock-in amplifier (Hinds Instruments Signaloc 2100) and Photoelastic Modulator (Hinds Series II/FS2AA). The differentiated light was focused onto an Actron SP2150 monochromator (1200 g/nm, 500 nm Blaze) equipped with a high sensitivity cooled Photo Multiplier Tube (Hamamatsu H10723-20 PhotoSensor red corrected). The detection of the CPL signal was achieved using the field modulation lock-in technique. The electron signal from the PMT was fed into the lock-in amplifier (Hinds Instrument Signaloc 2100). The reference signal for the lock-in detection was provided by the PEM control unit. The monochromators, PEM control unit and lock-in amplifier were interfaced with a desktop PC and controlled by Labview 2013 code. The CPL dissymmetry factor (g_{lum}) values were calculated using the following equation: $g_{lum}=(2(I_L-I_R))/(I_L+I_R)$, where I_L is intensity of the left and I_R is intensity of the right circularly polarized components of the emitted radiation (*i.e.* $I_L - I_R$) and the total luminescence intensity (*i.e.* $I_L + I_R$). Samples were measured in a custom-build scanning-monochromator CPL spectrometer, details of which can be found elsewhere.[26,53] The gel samples were excited at 340 nm and the self-assembly samples in the solution were excited at 295 nm. The respective excitation wavelength was selected after establishing maximised emission intensity as a function of excitation wavelength. Luminescence was collected between 570 and 720 nm at 0.5 nm increments, with 5 accumulated spectra averaged.

Spectrophotometric titrations and binding constants

The formation of the luminescent 1:1, 1:2 and 1:3 (M:L, where M = Eu(III) and L = **1**) species was ascertained by both UV-visible and luminescence titrations of a solution of **1** (8.84×10^{-5} M) with $Eu(CF_3SO_3)_3\cdot6H_2O$ ($0\rightarrow2$ equivalents). The data were fitted using the non-linear regression analysis program, SPECFIT®.[58]

pH titration experiments

The experiments were performed for aqueous solutions of **1** (c = 8.84×10^{-5} M) and Eu**1**$_3$(CF_3SO_3)$_3$ (c = 2.95×10^{-5} M) in the presence of 0.1 M NaCl as ionic strength. The pH of solutions was changed using HCl and NaOH aqueous solutions with different concentrations ranging from 0.01 to 1 M. In a typical experiment the solution was first acidified by adding a drop of HCl 37% to reach pH 2− 2.5 and then titrated with freshly prepared sodium hydroxide solutions at different concentrations (1, 0.1, and 0.01 M). After each addition of base, the pH of the solution was measured using a KCl-saturated electrode from Metrohm AG and UV−visible absorption and emission spectra were recorded. The reverse titration experiment was performed upon addition of HCl aqueous solution.

SUPPLEMENTAL INFORMATION

The content of the Supplemental Information includes experimental details and synthetic procedures along with the spectra recorded for the investigated systems such as NMR, UV, fluorescence, delayed luminescence spectra, non-linear regression analysis data, lifetimes and quantum yield values, CD, rheological, SEM and optical microscopy data.

ACKNOWLEDGMENTS

The authors declare no competing financial interest. The authors would like to thank Dr. Gary Hessman for mass spectrometry measurements, Prof. Eoin Scanlan for useful discussions, Dr. Jason M. Delente and Tómas Arnar Guðmundsson for additional SEM images, Dr. Benjamin Haffner and Steven Burke for help with rheology measurements, the Science Foundation Ireland (PI awards 13/IA/1865 to T. G. and 17/CDA/4704 to M. M.), Dr. Brendan Twamley for the help with optical microscopy and determining the unit cell parameters for the single crystals grown within **G4-2**, the Irish Research Council (IRC), the School of Chemistry, and Trinity College Dublin for the provided infrastructure and financial support. Also, authors would like to thank Prof. Seamas C. Donnelly for the support to Dr. Ciaran O'Reilly and Drs. Adam F. Henwood, Ena T. Luis and Helen M. O'Connor for proofreading the manuscript.

AUTHOR CONTRIBUTIONS

This is a peer-reviewed, accepted author manuscript of the following research article: Kotova, O., O'Reilly, C., Barwich, S. T., MacKenzie, L. E., Lynes, A. D., Savyasachi, A. J., Ruether, M., Pal, R., Möbius, M. E., & Gunnlaugsson, T. (2022). Lanthanide luminescence from supramolecular hydrogels consisting of bio-conjugated picolinic-acid-based guanosine quadruplexes. *Chem*. https://doi.org/10.1016/j.chempr.2022.01.015

C.O. carried out the synthesis of the ligand **1** and all the intermediates, O.K. carried out the synthesis of the hydrogels, all the thermogravimetry, photophysical and CD measurements along with spectrophotometric titrations including binding constants determination and pH titrations. A.D.L. and A.J.S. carried out microscopy studies of the gels, S.T.B. and M.E.M. carried out rheological analysis of the gel samples. M.R. performed NMR experiments, L.M. and R.P. recorded all chiral luminescence spectroscopy. O.K. and T.G. co-wrote the manuscript, C.O., L.E.M., R.P., S.T.B. and M.E.M contributed to the writing and correcting various parts of the manuscript, all research was performed under the guidance and supervision of T.G.

DECLARATION OF INTERESTS

The authors declare no competing interests.

SCHEMES AND FIGURES

Scheme 1. The chemical structures employed in this study

Schematic representation of K(I)-stabilized guanosine quadruplex (**G4**) functionalized with the ligand **1** (**G4-1**) and showing the binding site to Eu(III) ions.

Figure 1. Spectroscopic titration studies using Eu(III)

(A) The changes in the Eu(III)-centered emission spectrum of **1** ($c = 8.84 \times 10^{-5}$ M) upon addition of $Eu(CF_3SO_3)_3$ in DPBS (pH = 7.1, λ_{ex} = 285 nm); (B) CPL response of **1** ($c = 8.84 \times 10^{-5}$ M) upon addition of 0.33 equivalents of Eu(III) in PBS (pH = 7.4, λ_{ex} = 295 nm) at 22 °C.

Figure 2. Guanosine quadruplexes as building units for the hydrogel formation

Schematic representation of anticipated guanosine quadruplex formation within obtained hydrogels functionalized with **1** (**G4-1**; photograph of inverted and upright freshly made sample under day light), and Eu(III) in different metal to ligand ratios (**G4-2**, **G4-3**, **G4-4**; photographs of inverted freshly made samples of the resulting hydrogels under day and UV light).

Figure 3. Morphological investigations

(A-C) SEM images of **G4-2** (sample covered with Au/Pd) at different magnifications.

Figure 4. Oscillatory rheology measurements of G4-2 gel

(A) Frequency dependence of the storage modulus G' (black line) and loss modulus G'' (red line) at a strain amplitude of 1%. (B) The corresponding strain sweeps at f = 1 Hz. (C) The recovery test whereby applying alternating strain amplitudes of 100% and 1% at f = 1 Hz the response changes from liquid- to solid-like, respectively; the moduli in these regimes remain unchanged.

Figure 5. Photophysical evaluations of hydrogel G4-2

(A) Absorbance and fluorescence (λ_{ex} = 285 nm), (B) absorbance overlaid with CD, (C) fluorescence/total emission intensity and corresponding CPL spectra of **G4-2** (λ_{ex} = 340 nm).

TABLES

Table 1. CPL dissymmetry factors (g_{lum}) calculated for the self-assembly of 1 ($c = 8.84 \times 10^{-5}$ M) with 0.33 eq of Eu(III) in PBS (pH = 7.4), λ_{ex} = 295 nm

$^5D_0 \rightarrow {}^7F_J$	λ_{CPL}, nm	g_{lum} 1 + 0.33 eq Eu(III)
$^5D_0 \rightarrow {}^7F_1$	592.5	–0.022
$^5D_0 \rightarrow {}^7F_1$	597.0	–0.032
$^5D_0 \rightarrow {}^7F_2$	613.5	+0.016
$^5D_0 \rightarrow {}^7F_2$	615.0	+0.007
$^5D_0 \rightarrow {}^7F_2$	616.0	+0.006
$^5D_0 \rightarrow {}^7F_2$	619.0	–0.010

Table 2. CPL dissymmetry factors (g_{lum}) calculated for G4-2 hydrogel, λ_{ex} = 340 nm, and for the self-assembly of 1 ($c = 8.84 \times 10^{-5}$ M) with 1.00 eq of Eu(III) in PBS (pH = 7.4), λ_{ex} = 295 nm

$^5D_0 \rightarrow {}^7F_J$	λ_{CPL}, nm	g_{lum} G4-2	g_{lum} 1 + 1.00 eq Eu(III)

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$^5D_0 \rightarrow {}^7F_1$	592.5	–0.003	+0.001
$^5D_0 \rightarrow {}^7F_1$	597.0	+0.006	–0.026
$^5D_0 \rightarrow {}^7F_2$	613.5	–0.002	–0.0002
$^5D_0 \rightarrow {}^7F_2$	615.0	–0.001	+0.006
$^5D_0 \rightarrow {}^7F_2$	616.0	–0.003	+0.006
$^5D_0 \rightarrow {}^7F_2$	619.0	+0.001	+0.008

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