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ARTICLE

G_4-Quartet Hydrogels from 5′-Hydrazino-Guanosine for Non-Covalent and Covalent Remediation of Contaminants from Water

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Creation of supramolecular hydrogels from relatively simple building blocks demonstrates the power of molecular self-assembly to make functional materials. G_4-quartet hydrogels are appealing for a number of applications, including environmental remediation of pollutants in water. We find that the guanosine analog, 5′-deoxy-5′-hydrazinoguanosine (HG **2**) self-assembles into a self-standing hydrogel in the presence of stoichiometric amounts (0.25 equiv) of KCl. The higher water solubility of HG **2** (14.5 mM), compared to the parent compound G **1** (2.1 mM), likely contributes to its enhanced gelation. The structural basis for this HG **2**•KCl hydrogel, confirmed by PXRD, IR and CD, is the G_4•K^+ quartet, which forms extended 1D ion-channel assemblies that entangle to give a stable and long-lived hydrogel. We also find that adding KCl to a saturated solution of HG **2** triggers generation of colloidal G_4•K^+ assemblies *in situ* that selectively and efficiently bind the anionic dye naphthol blue black (NBB) over a cationic dye. In addition to this non-covalent electrostatic binding of anions, the nucleophilic 5′-hydrazino group in the HG **2**•KCl hydrogel HG **2** enables efficient absorption of propionaldeyde from both the gas phase and from water solution, via formation of covalent hydrazone linkages with the gel matrix.

Introduction.

Supramolecular hydrogels, which consist mostly of water but are actually soft solids made by the molecular self-assembly of low molecular weight gelators (LMWG), have many applications in areas such as drug delivery, sensing, catalysis, materials science and environmental remediation.[1-3] The power of self-assembly can be used to create these supramolecular hydrogels from a rich variety of LMWGs, including synthetic compounds as well as natural products such as peptides, sugars, lipids and nucleosides/nucleotides.[4,5] Our group is especially interested in using supramolecular assemblies,[6] including hydrogels,[7] for separation of environmental contaminants in water. The significant surface area of hydrogel fibers can, ideally, be very useful for absorption and/or reaction with toxic compounds, enabling removal of pollutants from water via either non-covalent and/or covalent interactions.[3]

In this paper, we describe the preparation, characterization and applications of a supramolecular hydrogel/colloid made from a synthetic analog of guanosine (G **1**), namely the LMWG 5′-deoxy-5′-hydrazinoguanosine (HG **2**)[8] (Figure 1). This simple modification of the nucleoside's 5′-sidechain, from 5′-OH (G **1**) to 5′-$NHNH_2$ (HG **2**), provides a LMWG that self-assembles in the presence of KCl to form a transparent and stable supramolecular hydrogel. We then developed a method to generate *in situ* a colloidal suspension of this HG **2**•KCl assembly that enables the selective and rapid precipitation of an anionic dye from water (*vide infra*) (Figure 2A) We also show that the HG **2**•KCl hydrogel, with its high density of nucleophilic 5′-hydrazino groups, can efficiently remove an aldehyde from water by forming covalent bonds (Figure 2B).

Guanosine derivatives have long been known to form supramolecular hydrogels and other nanostructures.[9] In the past decade there has been a resurgence in the study of guanosine-based hydrogels, due in large part to their biocompatibility and myriad of useful functions.[10, 11] The structural basis for most of these hydrogels is the cation-templated G_4-quartet, wherein 4 guanine bases self-assemble into a hydrogen-bonded macrocycle with simultaneous coordination to an alkali(ne earth) metal cation.[9b,12] These individual G_4•M^+ quartets further stack to form extended 1D G_4-wires that can be hundreds of microns in length. These G_4-wires then entangle to give the 3D mesh that the hydrogel uses to entrap water (Figure 1).

We reasoned that the central cation channel within these G_4-wires would make G_4•M^+ hydrogels promising candidates for non-covalent, electrostatic binding of anionic species (Figure 2A).[13] The poorly-soluble guanosine (G **1**) is itself, however, not a good gelator. In the presence of KCl G **1** forms transient hydrogels that then crystallize (in minutes or hours), precluding the use of soft materials that are made only from G **1**. So, effort has been devoted to enhance the ability of G **1** and analogs to form longer-lived and functional hydrogels.[10]

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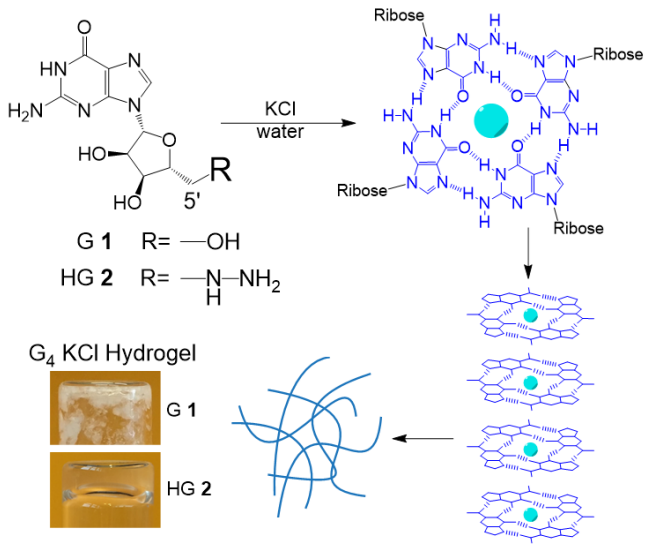


Figure 1. Hierarchical self-assembly that leads to formation of G_4-quartet based hydrogels. Hydrogen-bonded G_4-quartets, templated by K^+ cation, stack to give 1D G-quadruplexes, which then entangle to form a hydrogel. A transparent and long-lived hydrogel is formed with HG **2** (2 wt %, 68 mM) and 0.5 eq of KCl. In contrast, the parent nucleoside G **1** precipitates from solution in the presence of KCl.

One effective strategy for making stable G_4-quartet hydrogels, introduced by the McGown and Rowan groups,[14,15] is to use a binary mixture of different G analogs, which presumably adds enough disorder to the system so as to disfavor crystallization and maintain the hydrogel phase as the thermodynamically favored phase. We recently used this approach to prepare G_4-quartet hydrogels from KCl and a 1:1 mixture of 8-amino-guanosine (8-NH_2G) and G **1**.[7] These 8-NH_2G: G **1** hydrogels, kinetically stable when suspended in KCl solution, absorb anionic dyes from water into the gel matrix, effecting a "water-in-water" type of purification of these dye contaminants. However, one key drawback with the 8-NH_2G: G **1** gel was that this absorption of anionic dyes took a relatively long time (days) because of slow diffusion into the bulk hydrogel. Formation of colloidal G_4-quartet assemblies that aggregate in water to form functional phases, such as micelles and microgels, has recently been demonstrated by the Rivera group.[16] These G_4-quartet colloidal assemblies have been used to reversibly encapsulate drugs. We decided to take a similar approach by screening for conditions that might give rise to colloidal suspensions of G_4-quartet nanoparticles, rather than the dense 3D matrix of a hydrogel.[16] In this present study, we find that generation of a colloidal suspension of G_4-quartets in water at ambient temperature, done simply by mixing HG **2** and KCl, greatly increases the efficiency of the binding and separation of an anionic dye from water. We reason that the surface area of these colloidal G_4-quartet assemblies is larger than that of the bulk HG **2**•KCl hydrogel, so that non-covalent absorption of the anionic dye is no longer limited by slow diffusion.

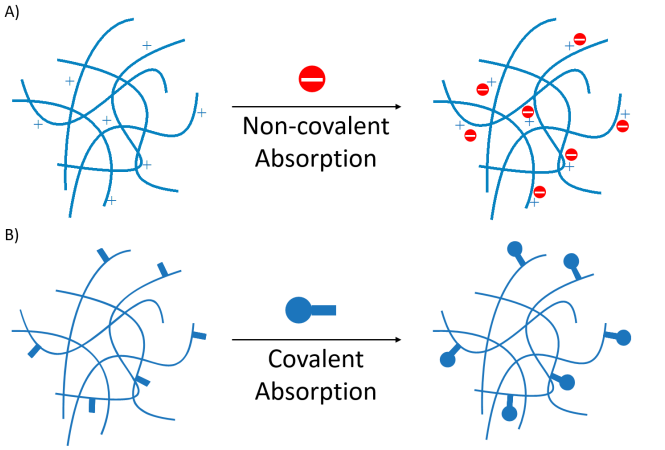


Figure 2. The HG **2**•KCl hydrogel can A) absorb anionic species via non-covalent interactions and B) trap aldehydes by forming covalent hydrazone bonds.

Another approach toward improving the hydrogelation properties of G **1** involves chemical modification of the nucleoside's sugar to enhance its water solubility. Whereas the unique hydrogen-bonding pattern of the G nucleobase is crucial for self-assembly to give a G_4•M^+ quartet structure, the ribose can be readily modified to change the properties of the LMWG and its corresponding hydrogel. For example, we have used borate ester chemistry to produce stable G_4-quartet hydrogels.[17] Borate esters, which form by reaction of cis-1,2-diols with borate, are anionic and this helps solubilize the intractable G **1**. These guanosine-borate hydrogels have recently been shown to have many interesting uses, including environmental remediation of cationic dyes,[18] as drug delivery vehicles,[19] as media for cell culture,[20] and as catalysts and sensors.[21]

Modification of the 5′-position of G **1** can also provide new guanosine analogs with enhanced solubility and better hydrogelation properties. The primary 5′-position of G **1** is relatively straightforward to chemically modify and typically such modifications do not impact formation of the key G_4-quartets since the 5′-position is remote from the hydrogen-bonding nucleobase. For example, Lehn and Sreenivasachary discovered a stimuli-responsive G_4-quartet hydrogel made from 5′-hydrazidoguanosine (LG **3**).[22] Importantly, the gel's 5′-hydrazido sidechain could form covalent acylhydrazone linkages with aromatic aldehydes. The Lehn group used this system, in a series of studies, to develop dynamic G_4-quartet hydrogels that enabled component selection in a dynamic combinatorial library.[22,23] Inspired by the utility of a compound such as LG **3**, and also motivated by our ongoing program in environmental remediation using G_4-quartet assemblies, we undertook a survey (and comparison with LG **3**) of the gelation properties of some other 5′-modified guanosines. At the outset, 3 major factors guided our choices in the 5′-modification of G **1**: 1) we wanted to find a LMWG that would give a self-standing hydrogel with low (ideally stoichiometric) equivalents of KCl template; 2) the functional group should be basic enough to be significantly protonated at neutral pH so that, in combination with its cationic G_4•K^+ core, the gel would favor non-covalent binding and extraction of anionic dyes;[24]

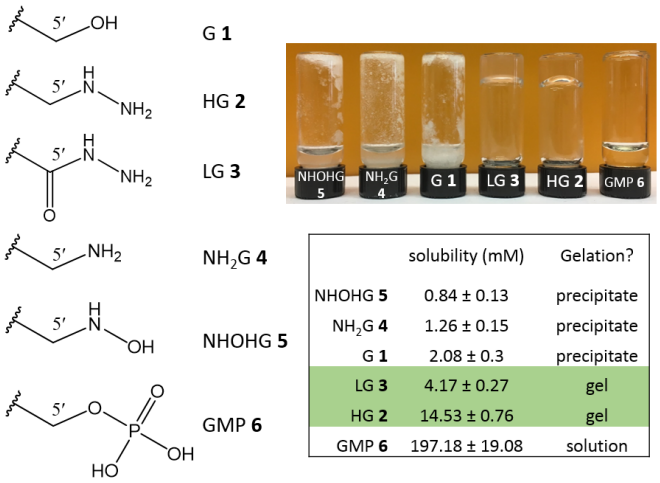
and 3) we wanted the 5′-group to be a good nucleophile, such as a hydrazino or hydroxylamino group with their potent α-effect,[25,26] so that the gel could be used to form covalent bonds with electrophiles such as aldehydes.[27] In short, we aimed to incorporate a basic and nucleophilic group into the 5′-position and have it function as a useful gelator for a range of environmental separations. Herein, we describe our initial studies on a G_4•KCl hydrogel made from 5′-hydrazino HG **2**. This G_4-quartet based system can be used to both non-covalently extract an anionic dye and also covalently absorb an aliphatic aldehyde from water.

Results and Discussion.

5′-Substituent influences the water solubility of guanosine derivative and its ability to form hydrogels in the presence of added KCl. As described below, during our ongoing studies on guanosine-based hydrogels we discovered that the known 5′-hydrazino HG **2** has some useful properties: 1) it forms transparent and self-standing G_4-quartet hydrogels upon addition of stoichiometric amounts of KCl; 2) these HG **2**•KCl hydrogels selectively bind anionic dyes (vs. cationic dyes); and 3) the HG **2**•KCl hydrogels, adorned with nucleophilic 5′-hydrazine sidechains, efficiently absorb propionaldehyde from both the gas phase and solution phase.

One property of HG **2** that we recognized was key for hydrogel formation was its enhanced water solubility, relative to poorly soluble G **1**. Smith and colleagues have shown that the water solubility of a LMWG can be crucial for hydrogelation.[28] As shown in Figure 3 we compared the hydrogelation properties and water solubility of 6 different G analogs that differed in their 5′-sidechain. These compounds included: the parent nucleoside, guanosine G **1**; this study's featured gelator, 5′-hydrazino HG **2**;[8] the 5′-hydrazido LG **3**;[22] the 5′-amino derivative, NH_2G **4**;[29] the 5′-hydroxylamino analog, NHOHG **5**;[8]and the free acid of guanosine 5′-monophosphate, GMP **6**. We purchased compounds **1** and **6** and we synthesized compounds **2**-**5** using known methods.

We first compared the ability of these different 5′-modified guanosines to gel water in the presence of 0.5 molar eq of KCl. We chose KCl as the salt to trigger self-assembly of G analogs **1-6** because of the well-established propensity of K^+ to template formation of G_4-quartets.[10] Thus, 2 wt % (ca. 68 mM) of each analog was added to water and heated until we obtained a clear solution. Then 0.5 eq of KCl (per monomer of **1**-**6**) was added and the solution was allowed to cool to rt. As shown in the inverted vials in Figure 3B, only the 5′-hydrazino HG **2** and 5′-hydrazido LG **3** formed self-standing hydrogels under these conditions. The NH_2G **4** and NHOHG **5** analogs precipitated while cooling. The parent G **1**, although it initially formed a hydrogel, turned into a precipitate within just 30 min. As expected, the highly soluble GMP **6** remained in solution, without any sign of hydrogelation or crystallization.



	solubility (mM)	Gelation?
NHOHG **5**	0.84 ± 0.13	precipitate
NH_2G **4**	1.26 ± 0.15	precipitate
G **1**	2.08 ± 0.3	precipitate
LG **3**	4.17 ± 0.27	gel
HG **2**	14.53 ± 0.76	gel
GMP **6**	197.18 ± 19.08	solution

Figure 3. A) Guanosine derivatives **1-6**, each with a different 5′-substituent**.** B) Vial-inversion test (after 1 day at rt) shows that only HG **2** and LG **3** form self-standing hydrogels. Each sample contains 2 wt % (ca 68 mM) nucleoside and 0.5 molar eq of KCl in H_2O. C) Molar solubilities of **1-6** at 22 °C in order of increasing order, as determined by UV measurements at λ= 254 nm. The green shading for 5′-hydrazido G (LG **3**) and for 5′-hydrazino G (HG **2**) highlight that these 2 derivatives, with "moderate" water solubility, form transparent and self-standing hydrogels.

We next determined the water solubility of G analogs **1-5** at 22 °C by adding 10 mg of each compound to 1 mL of deionized water. The suspensions were sonicated for 30 seconds and then stirred at 22 °C for 1 h before ultracentrifugation was performed to remove insoluble material. The supernatant was diluted and guanine's UV absorbance at λ= 254 nm was measured to calculate the solubility at 22 °C for **1**-**6** in water (see Figure S3). Figure 3C shows that the poorly soluble compounds (< 2 mM), namely G **1**, NH_2G **4** and NHOHG **5** do not form hydrogels with added KCl, but give copious precipitation. The 2 compounds that form hydrogels in the presence of KCl, HG **2** (14.53 mM) and LG **3** (4.17 mM), both have moderate solubility in water, when compared to analogs **1**, **4** and **5**. Most notably, substitution of 5′-OH in G **1** to the 5′-hydrazino group in HG **2** enhanced the nucleoside's aqueous solubility by 7-fold and resulted in efficient K^+ templated gelation of water instead of leading to precipitation.

These results, although based on a limited sample size, indicate a connection between a guanosine analog's solubility and its ability to form hydrogels in the presence of KCl; moderate solubility of the G monomer is needed for formation of G_4•K^+ hydrogels. This is not surprising since a proper balance of a hydrophobicity and hydrophilicity for the LMWG is often required to give a stable hydrogel.[28] If a monomer (like G **1**) is too insoluble in water, then the resulting G_4•K^+ structures are not well stabilized by hydration and the material crystallizes or precipitates. On the other hand, if the monomer is too soluble (such as GMP **6**) then the equilibrium favors high concentrations of soluble aggregates and the self-assembled fibers needed for gelation simply don't form in ample amount. While other factors (e.g. disorder, conformation, charge)

surely play a role in controlling gelation vs. precipitation/crystallization vs. dissolution, consideration of the relative solubility of G derivatives is probably a good start when designing, or screening, for new G_4-quartet hydrogels.

The HG 2•KCl hydrogel is made up of G_4-quartets. That addition of 0.5 equiv of KCl to a 2 wt % solution of HG **2** gave a transparent and self-standing hydrogel indicated that K^+ was likely responsible for templation of a G_4-quartet assembly. To confirm G_4-quartets on the molecular level we used data from powder X-ray diffraction (PXRD), infrared (IR) spectroscopy and circular dichroism (CD) analyses. The PXRD pattern of a lyophilized HG **2**•KCl gel showed characteristic diffraction peaks indicative of G-quadruplex formation: namely, the π-π stacking distance between individual G_4•K^+ quartets (d= 3.31 Å, 2θ= 26.9°) and the diameter of an individual G_4•K^+ quartet (d= 20.0 Å, 2θ= 4.4°) (Figure 4A).[30] IR spectroscopy also indicated that the HG **2**•KCl gel was composed of G_4-quartets. As shown in Figure 4B, the IR spectrum of a freeze-dried HG **2**•KCl hydrogel, when compared to that for "monomeric" HG **2**, showed a significant red shift of 45 cm^{-1} for the guanine's carbonyl stretching frequency after addition of KCl. This shift from 1724 cm^{-1} for the HG **2** monomer to 1679 cm^{-1} for the HG **2**•KCl gel is consistent with the carbonyl of a G_4-quartet being involved in strong intermolecular H-bonding and coordination to a central K^+ cation, as shown by Setnička, Lehn and colleagues in their IR study of hydrogels formed by hydrazide LG **3**.[31] Finally, CD spectroscopy also supported G_4-quartet formation by HG **2**. The CD signals in the 250-320 nm region arise due to stacking of G_4-quartets in a helical arrangement.[32] Figure 4C shows that the CD spectrum for a 2 wt % HG **2**•KCl hydrogel (68 mM **2**, 1.0 eq KCl) has a strong negative band near 270 nm and a strong positive band near 310 nm, which is diagnostic of the chiral stacking of individual G_4-quartets.[17,32] These pieces of information together, namely the PXRD, IR and CD data, support the proposal that the molecular structure of the HG **2**•KCl gel is based on stacks of G_4-quartets that form 1D nanowires containing a central K^+ ion channel.

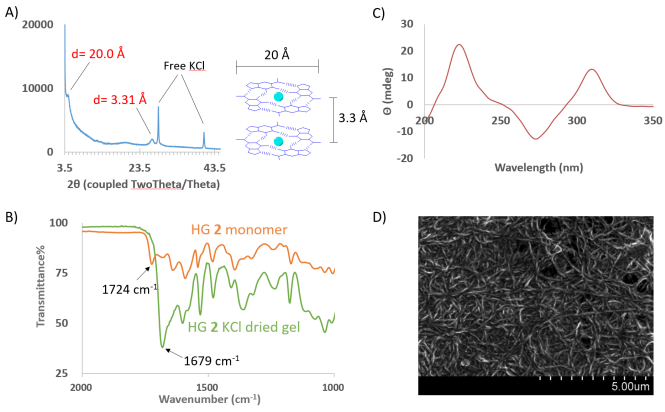


Figure 4. Characterization of G_4-quartet based HG **2**•KCl hydrogel. A) Powder X-ray diffraction pattern of a dried hydrogel shows evidence for G-quadruplex formation. B) Infrared spectrum of HG **2** monomer and the freeze-dried HG **2**•KCl gel. The stretching frequency of the guanine carbonyl showed a 45 cm^{-1} red shift. C) CD spectrum of the HG **2**•KCl hydrogel indicates a chiral G-quadruplex structure. D) A ESEM image of HG **2**•KCl hydrogel (2 wt%, 68 mM, 0.5 eq KCl) shows a fibrous and entangled network.

To gain a firmer understanding about the morphology of the HG **2**•KCl hydrogel we used scanning electron microscopy (SEM) to image the material. The SEM technique when used with an ionic liquid, increasingly being used to directly image supramolecular hydrogels,[33] enables one to observe the gel's porous network under wet conditions. Figure 4D shows an SEM photo obtained from a 2 wt % HG **2**•KCl hydrogel (68 mM, 0.5 eq KCl) that had been immersed in an ionic liquid. The SEM shows that this HG **2**•KCl hydrogel is a highly porous material with significant exposed surface area due to its mesh of entangled fibers. These fibers, which are 1-2 microns in diameter and hundreds of microns long, appear to be composed of bundles of strands (presumably G_4-nanowires) that wrap around one another to give a helical macrostructure.

The mechanical properties of the HG 2•KCl hydrogel can be modulated by K^+ concentration. Crystal structures of DNA G-quadruplexes and lipophilic G-quadruplexes always show K^+ ions sandwiched between every G_4-quartet.[34,35] Such an organization indicates that 0.25 eq of K^+ relative to G monomer is required to form the G-quadruplex ion channel (Figure 1). But, because of the poor water solubility of many G derivatives one typically needs excess KCl to form a G_4-quartet hydrogel. And, often, that G_4-quartet gel is only a kinetic product, as it crystallizes or precipitates over time. Because of the enhanced water solubility of HG **2**, we were hopeful that stoichiometric KCl (0.25 eq) might trigger the gelation of water by templating the formation of a G_4-quadruplex assembly. This was, indeed, the case. Figure 5A shows a series of inverted vials containing day-old hydrogels, each made with 2 wt% (68 mM) of HG **2** but with different amounts of KCl. The gel on the left, with 0.25 eq of KCl, is transparent, indicating complete dissolution of the gelator HG **2**. The curvature at the gel-air interface indicates that HG **2**•KCl gel made with 0.25 eq of KCl is more pliable than samples containing additional KCl (0.5-2.0 eq). Indeed, as more KCl was added, the HG **2**•KCl hydrogels became noticeably stiffer and hazier, perhaps due to formation of larger, insoluble HG **2**•K^+ assemblies.

In addition to these qualitative observations from the vial inversion tests we also used rheology to quantify the influence of KCl concentration on the macroscopic properties of the HG **2**•KCl hydrogel. Figure 5B shows rheology measurements on a series of HG **2**•KCl hydrogels, each 68 mM (2 wt %) in nucleoside but differing in their KCl concentration. After 1 day, the value of the storage (elastic) modulus (G'), which indicates a material's stiffness,[33] varied from ~30 Pa for a sample with 0.25 eq of KCl, to ~3000 Pa for a hydrogel that contained 2.0 eq of KCl. This spectrum of hydrogels with different mechanical properties (G' storage moduli that vary by over 2 orders of magnitude) indicates that simply changing KCl salt concentration is an easy and useful method to tune the properties of this G_4-quartet hydrogel. If one desires a flexible gel, perhaps to facilitate diffusion of compounds in and out of the matrix, simply use stoichiometric KCl. If one needs a tougher material to serve a specific purpose then one can add more KCl salt. In particular, gelation of water by guanosine

analogs such as HG **2** using stoichiometric concentrations of KCl would likely be beneficial for biological applications.

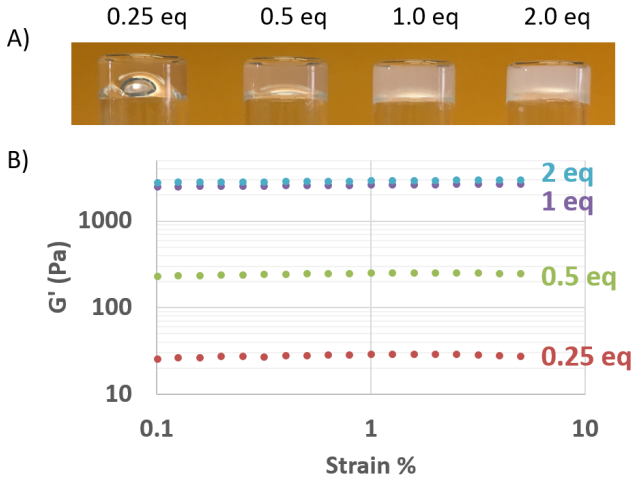


Figure 5. Changing KCl concentration influences HG **2** hydrogel properties. (A) Photos of HG **2**•KCl hydrogels (2 wt %, 68 mM) made with different amount of KCl 1 day after gel preparation. (B) Storage modulus (G') of HG **2**•KCl hydrogels increases with KCl concentration. Strain sweeps of HG **2** hydrogels were taken at a constant angular frequency of 10 rad/s 1 day after gel preparation.

Self-assembly of the cationic HG 2•KCl colloidal suspensions *in situ* is crucial for anionic dye removal. Previously, we have demonstrated the use of appropriately modified G_4-quartet hydrogels to remediate either anionic or cationic dyes via non-covalent, electrostatic interactions.[7,18] The slow diffusion of dye molecules into the bulk hydrogel limited, however, the efficiency and kinetics of dye uptake. In this paper, we show that insoluble $G_4•K^+$ assemblies,[16] which are colloidal suspensions and "gel-like" in their physical appearance, can be formed *in situ* simply by adding KCl to a saturated aqueous solution of HG **2**. This new phase for HG **2**, templated by the added KCl, can be used to bind and selectively precipitate anionic dyes (in the presence of cationic dyes). This technique, which relies on formation of a colloidal suspension of $G_4•K^+$ assemblies from HG **2**,represents a promising method for the easy and rapid remediation of water contaminated with anionic dyes. [36]

To test the ability of soluble HG **2** to form self-assembled aggregates in the presence of K^+, we first prepared a saturated solution of HG **2** (14.5 mM) at rt, after which aliquots of a KCl stock solution were added and the resulting solution was then well mixed. Instead of forming a continuous and self-standing hydrogel, a suspension of milky-white particles appeared immediately after addition of the KCl (Figure S5). As a control, a saturated solution of poorly soluble G **1** (2.1 mM) showed no sign of any new phase after addition of the same concentration of KCl (Figure S5). Subsequent ultracentrifugation enabled separation of this new insoluble material formed by addition of KCl to the saturated solution of HG **2**. IR spectra of this freeze-dried material showed the characteristic carbonyl shift (1680 cm^{-1}) for a G_4-quartet structure (see Figure S6), identical to that previously observed for the HG **2**•KCl hydrogel. This IR analysis confirms that this new phase arose from G_4-quartet self-assembly,[31] triggered by addition of KCl to the saturated solution of HG **2**.

Importantly, we also found that the concentration of added KCl could be used to control the amount of the new G_4-quartet colloidal suspension formed *in situ*. As shown in Figure S7, increasing concentrations of KCl were added to vials containing saturated solutions of HG **2** (14.5 mM), incubated for 1 h at rt and then subjected to ultracentrifugation to separate out the insoluble $G_4•K^+$ assemblies. Figure S7A shows that more white, "gel-like" material accumulates at the bottom of the vials with increasing amounts of added KCl. The concentration of HG **2** remaining in the supernatant 1 hour after addition of KCl was quantified by UV spectroscopy, again by monitoring the guanine absorption at λ=254 nm. The data in Figure S7B confirm that increasing concentrations of KCl lead to a significant decrease in the amount of HG **2** remaining in solution, as more insoluble $G_4•K^+$ assemblies are formed by self-assembly of HG **2** and the higher concentrations of KCl. This rapid and facile generation of insoluble $G_4•K^+$ assemblies at ambient temperature allows one to create a new phase that is easily separated from the aqueous phase. The central role of KCl in this self-assembly process is critical since it triggers the shift of equilibrium from the "monomeric" HG **2** form toward larger $G_4•KCl$ assemblies, creating a new phase that can then be used as a separation media.

As a first step toward using this *in situ* formation of insoluble $G_4•K^+$ material for environmental remediation, we confirmed the electrostatic nature of dye binding by the HG **2**•K^+ hydrogel itself. Thus, we found that this cationic hydrogel, with its central K^+ channel and its partially protonated 5'-hydrazino groups (pK_a of R-$^+NH_2NH_2$ is 7.9)[37], could maintain strong affinity for an anionic dye, as opposed to a cationic dye. We prepared 2 different solid cubes of the HG **2**•K^+ hydrogel (68 mM, 2 wt %, 2 eq KCl), with one sample containing the cationic dye crystal violet (CV), and the other sample of HG **2**•K^+ containing the anionic dye, naphthol blue black (NBB), a major pollutant generated by the textile industry (Figure 6A).[38] The two hydrogel samples were then added to a 5 mL solution of 155 mM KCl solution. As shown in Figure S8, the 2 gels had much different properties in terms of their ability to retain the 2 different dyes. Thus, the cationic CV dye diffused out of the HG **2**•K^+ hydrogel immediately to give a dark purple solution. In marked contrast, the anionic NBB dye remained bound within the HG **2**•K^+ hydrogel, showing no visible leakage under these same conditions, even after days. This experiment, which highlights the ability of the cationic HG **2**•K^+ hydrogel to selectively bind anionic vs. cationic dyes using non-covalent electrostatic interactions, boded well for our aim to develop a method to selectively extract anionic dyes from solution using K^+-templated self-assembly of HG **2**.

We next studied the charge preference of the aforementioned G_4-quartet assemblies, made by adding KCl to a saturated solution of HG **2**, to selectively remediate anionic dyes from water. Each of the vials shown in Figure 7B contained equal concentrations of NBB or CV dyes (100 μM). Vial 1 contained both of the components needed for self-assembly of G_4-quartets, namely saturated HG **2** (14.5 mM)

and KCl (100 mM). Vial 2 contained saturated HG **2** but no templating KCl. Vial 3 (dye and 100 mM KCl) and vial 4 (dye only) served as blank controls. After sufficient mixing and allowing to stand at rt for 1 hour, the samples were spun down by ultracentrifugation. While the solutions in vials 2, 3 and 4 remained blue (Figure 6B top picture), and showed no visible precipitate (indicating no electrostatic binding of NBB), vial 1 showed a dark-blue solid phase at the bottom of the vial and the supernatant was much more lightly colored than the other 3 samples. These experiments clearly indicate that the combination of gelator HG **2** and added KCl are able to form a new G_4-quartet-based phase *in situ* that can bind and co-precipitate the anionic contaminant NBB from solution. As a control, to demonstrate the system's electrostatic binding selectivity, we carried out similar experiments with the cationic dye CV (100 µM). In this case, the solutions in all 4 vials remained the same in color after mixing and ultracentrifugation, indicating the preference of the G_4-quartet assemblies to bind anionic dyes (Figure 6B middle picture).

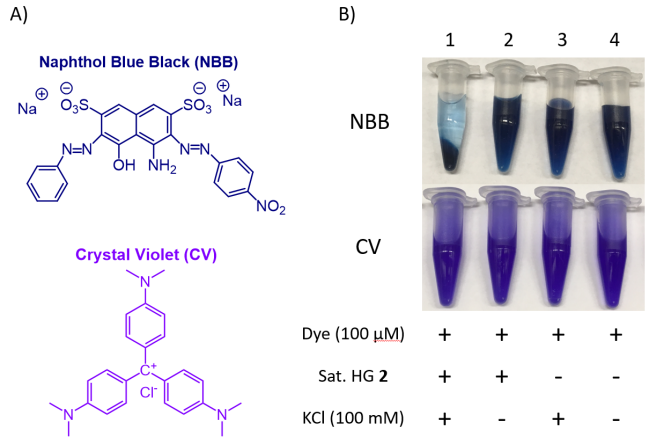


Figure 6. A) The structures of two dyes used. B) Self-assembly is crucial for anionic dye remediation: Addition of both saturated HG **2** and KCl followed by ultracentrifugation promotes self-assemblies of G_4 KCl that preferentially binds anionic NBB not cationic CV (all vials contain 1 mL solution with 100 µM dye).

We also studied the influence of KCl concentration on the efficiency of dye removal by self-assembly of HG **2**. We found that increasing the concentration of KCl, presumably by shifting the equilibrium toward formation of $G_4 \bullet K^+$ assemblies, increased the amount of visible precipitate and the efficiency of the anionic NBB absorption. After incubation and ultracentrifugation we used UV-vis spectroscopy to measure the amount of dyes, either anionic NBB or cationic CV, which remained in solution. Interestingly, with only 12.5 mM of KCl added, almost 70% of NBB was removed from solution (Figure 7). With more KCl added, the amount of NBB in the supernatant decreases exponentially. By contrast, less than 10% of the cationic CV was removed into the solid phase, even with the addition of 200 mM KCl. These experiments in Figure 7 once again demonstrate the selectivity of the HG **2**•KCl assemblies for binding anionic vs. cationic dyes. This strategy for anionic NBB removal utilizes the power of self-assembly with KCl serving as a trigger to promote formation of a cationic G_4-quartet based material from HG **2**. The system can potentially be expanded to the remediation of other water-soluble and toxic anions such as fluoroacetate, a potent inhibitor of the citrus acid cycle.[39] We are now pursuing such studies.

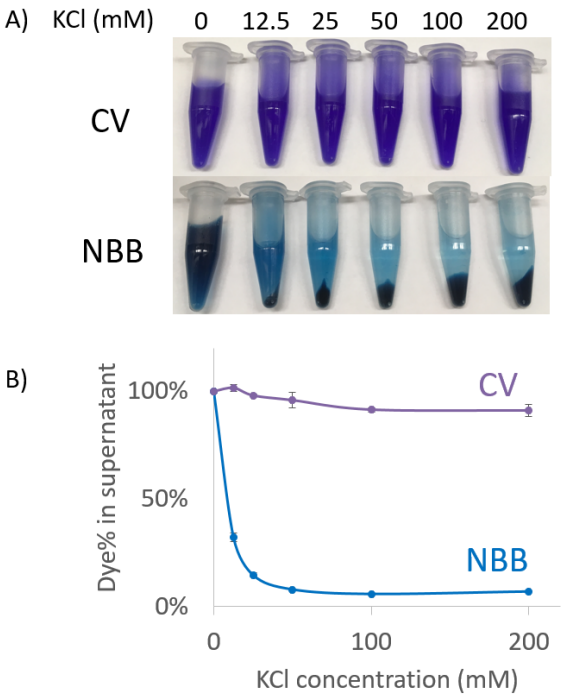


Figure 7: Increasing concentration of KCl, when added to saturated solutions of HG **2** (14.5 mM), increase the efficiency of precipitation of anionic dye NBB from solution. A) Qualitatively, addition of more KCl produces more blue colloids seen at the bottom of the vials for the anionic dye NBB, while no obvious change is observed for the cationic CV. All vials contain 1 mL solution with 100 µM dye. B) UV quantification of the amount of cationic CV and anionic NBB dyes remaining in solution as a function of added KCl concentration.

The nucleophilicity of the HG 2•KCl hydrogel enables remediation of propionaldehyde from both the gas phase and from solution. Being able to readily generate a stable HG 2•KCl hydrogel, whose surface is covered with nucleophilic 5'-hydrazino groups, should allow one to use this material to covalently trap aldehydes via hydrazone formation.[23-27] Aldehydes are significant environmental contaminants due to their use in many industrial and manufacturing processes and due to their electrophilic functionality, which makes them reactive with nucleophiles in biomolecules.[40] As a proof of concept we carried out initial uptake experiments with propionaldehyde, a volatile compound used extensively in the manufacture of polyvinyl and other plastics, in the synthesis of rubber chemicals, and as a preservative. Propionaldehyde was one of the 18 organic chemicals detected most frequently in the drinking water of 10 surveyed US cities and its remediation serves as an example of how supramolecular hydrogels like HG 2•KCl might be used to purify aldehyde-contaminated water.[41]

Based on the well-known chemistry of alkylhydrazines,[27] we expected that the HG **2**•KCl hydrogel would react with

propionaldehyde to form stable hydrazone adducts (Figure 8). As described below, we examined the covalent trapping of propionaldehyde by HG **2**•KCl hydrogel in both the gas phase and in aqeuous solution. One could imagine formulating this HG **2**•KCl hydrogel into thin films that would filter volatile aldehydes from the air that we breathe. Also, since the self-assembled HG **2**•KCl hydrogels are kinetically stable in solutions containing KCl (since the K^+ helps maintain the integrity of the gel fibers composed of G_4-quartet assemblies) we reasoned that one could covalently capture water-soluble aldehydes by soaking the HG **2**•KCl hydrogel in a salt solution containing these electrophiles.

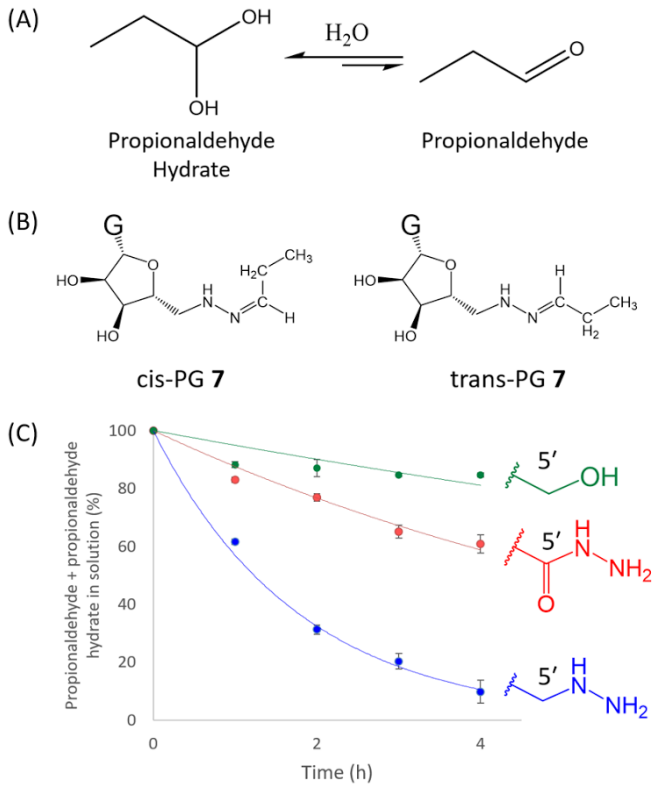


Figure 8: The HG **2**•KCl hydrogel can extract propionaldehyde from water (A) When dissolved in water propionaldehyde is in equilibrium with its hydrate. (B) The 5′-hydrazino HG **2** reacts with propionaldehyde to form a 1: 3.55 mixture of hydrazones, cis-PG **7** and trans-PG **7** . (C) The time-course for absorption of propionaldehyde (3.37 mM) from 155 mM KCl using different G_4•K^+ hydrogels (2 wt%, 68 mM). The amount of propionaldehyde and its hydrate in solution decreases after addition of hydrogels, as determined from ^{1}H NMR integration relative to an internal standard.

We first examined the gas phase absorption of propionaldehyde. Thus, we placed an uncapped vial containing a 0.5 mL portion of a 2 wt % HG **2**•KCl hydrogel (68 mM, 0.5 eq KCl) inside a larger vial. Then 1.0 eq of neat propionaldehyde was added to the large vial, after which we capped and tightly sealed the setup (see Fig S9). After allowing the system to incubate for 1 day at rt we lyophilized the sample and analyzed the freeze-dried powder using both ^{1}H and ^{13}C NMR spectroscopy. NMR analysis of the freeze-dried powder dissolved in DMSO-d_6 indicated that 2 new hydrazone compounds had been formed in > 95% yield from reaction of propionaldehyde and the HG **2**•KCl hydrogel. Both ^{1}H-^{1}H COSY and ^{1}H-^{13}C HSQC NMR analysis of the reaction products showed 2 separate sets of peaks, consistent with formation of the cis- and trans-hydrazones, cis-PG **7** and trans-PG **7** (see Figure 8B & Figures S10 & S11). The hydrazone diastereomers were assigned unambiguously from 1D NOE data (Figure S12) and the cis:trans ratio was determined to be 1 : 3.55, indicating a preference for the more stable trans isomer, trans-PG **7**. Electrospray ionization mass spectrometry of the lyophilized powder showed a single intense peak with m/z=338.12, again consistent with hydrazone formation from HG **2** and propionaldehyde to give cis/trans-PG **7** (Figure S13). These experiments demonstrate that a HG **2**•KCl hydrogel can be used to efficiently react with propionaldehyde in the gas phase so as to remove that volatile compound from the air. We are currently probing the use of thin films of the HG **2**•KCl hydrogel to react with a range of volatile aldehydes for their environmental remediation.

Having established that reaction occurs at rt between HG **2** and propionaldehyde in the gas phase, we next tested the ability of the HG **2**•KCl hydrogel (2 wt %, 68 mM, 2 equiv KCl) to absorb this same aldehyde from aqueous solution. We also prepared the G_4-quartet hydrogel made from G **1** and $KB(OH)_4$ (2 wt %, 0.5 eq $KB(OH)_4$)[17] and the LG **3**•KCl hydrogel (2 wt %, 2 eq KCl) made from the 5′-hydrazide LG **3**[22] so that we could compare their uptake of propionaldehyde with that of the HG **2**•KCl hydrogel (Fig. 8C). The various hydrogel cubes were each prepared using D_2O, allowed to cool in the mold for 1 h at rt and were placed into a 155 mM KCl solution of D_2O containing propionaldehyde (3.37 mM). The kinetics of propionaldehyde uptake from solution by the G_4-quartet based hydrogels was monitored over time by removing aliquots of the supernatant and analyzing them by ^{1}H NMR spectroscopy (Fig. 8C). At rt, propionaldehyde exists as an equilibrium mixture of the aldehyde and its hydrate. Signals for both the -CH_2- group of the aldehyde (δ 2.57 ppm) and the -CH_2- group of the hydrate (δ 1.60 ppm) decreased significantly when the solution of propionaldehyde was soaked in the presence of the HG **2**•KCl hydrogel. As shown in Figure 8C, the HG **2**•KCl hydrogel was the best of the 3 G_4-quartet based gels at reacting with propionaldehyde. Thus, after 4 h, the G **1**•$KB(OH)_4$ hydrogel showed minimal absorption (< 10%) of propionaldehyde whereas the LG **3**•KCl hydrogel containing the 5′-hydrazide (which Lehn and colleagues have shown reacts with a range of aldehydes)[22,23] demonstrated moderate uptake (~ 40%) of this aldehyde from solution after 4 h. The HG 2•KCl hydrogel, with its 5′-hydrazine, was the most effective in reaction with propionaldehyde, as ^{1}H NMR integration after 4 h showed that > 90 % of the compound had been removed from the supernatant that bathed the HG **2**•KCl hydrogel.

Conclusions

We have determined that by simply substituting the 5′-OH in G **1** for a 5′-$NHNH_2$ group gives a LMWG, HG **2**, whose water solubility is enhanced 7-fold and that gives a self-standing and stable G_4•KCl hydrogel even with stoichiometric concentrations of KCl. The role of KCl to promote G_4•KCl self-

assembly is further highlighted in dye remediation experiments, where the addition of KCl to a saturated solution of HG **2** solution creates cationic colloidal assemblies that can electrostatically bind to the anionic dye, NBB, and extract it from water. We have also demonstrated that the HG **2**•KCl hydrogel can be used as a "water-in-water" material to absorb dissolved propionaldehyde from both the gas and solution phases and sequester it in the gel's insoluble matrix. The ability of the supramolecular HG **2**•KCl hydrogel to remediate different classes of aldehydes could well be beneficial for broader environmental applications and purification of aldehyde-contaminated water. We are currently conducting such studies, with an eye toward environmental remediation.

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Conflicts of interest

There are no conflicts to declare.

Experimental

See electronic supporting information

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