**Supplementary Information**

# Self-Assembly of Guanosine and Deoxy-Guanosine into Hydrogels: Monovalent Cation Guided Modulation of Gelation, Morphology and Self-Healing Properties

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**Preparation of hydrogel.**

In a glass vial (inner diameter 1.4 cm, height 5 cm) required amount of guanosine (G) or deoxy-guanosine (dG) and metal salt (NaCl/KCl/RbCl/$NH_4Cl$/$Tl_2SO_4$) were taken. Then required amount of water was taken into the vial and the mixture was heated and shaken until these components dissolved in water. When the mixture was cooled using water trap a self-supporting thermo-reversible hydrogel was formed. For thermo-irreversible gels, metal salts (HgCl/$AgNO_3$/AuCl) and G/dG were dissolved individually into water using heating and these hot solutions were mixed together and shaken well and then cooled down to obtain gels. For $Au^+$, gel was formed during strong heating of the mixture. *In situ* formation $Au^+$ by the reduction of $Au^{3+}$ within the mixture of G/dG and $AuCl_3$ can assist the formation of gel. The formation of hydrogel was confirmed by vial inversion method. Minimum gelation concentration (mgc) of the hydrogels was calculated only in terms of amount of the G/dG present in a gel. G/dG hydrogels were prepared by mixing metal salts into G/dG with different metal ions concentrations starting from G/dG:M molar ratios of 1 : 1 to 1 : 8. To understand the effect of counter ion for metal ion gelation, we have tested the gelation abilities of $Na^+$ towards G in the presence of different counter ions including sodium chloride, sodium azide, sodium perchlorate, sodium sulfate, sodium nitrate, sodium thiosulfate and others. All of them form metastable gels but nature of the gel vary ranging from very transparent to opaque gel.

The effect of pH on gelation properties has been investigated using G gel in sodium phosphate buffer having pH in the range 2-10. It has been found that with increasing pH of the buffer solution transparency of the gel decreases. G forms transparent viscous solution at pH <4.

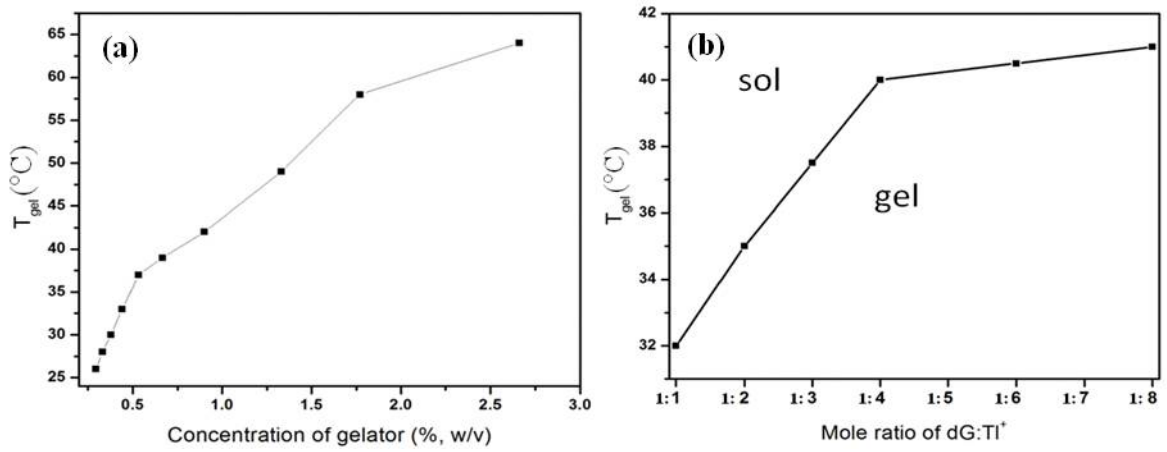


**Fig. S1** (a) The change in $T_{gel}$ with respect to gelator concentration for $Tl^+$ induced dG gel at a fixed mole ration of 1:4 (dG: $Tl^+$). (b) The change in $T_{gel}$ with respect to the metal ion concentration at a fixed gelator (dG) concentration (0.665%, w/v).

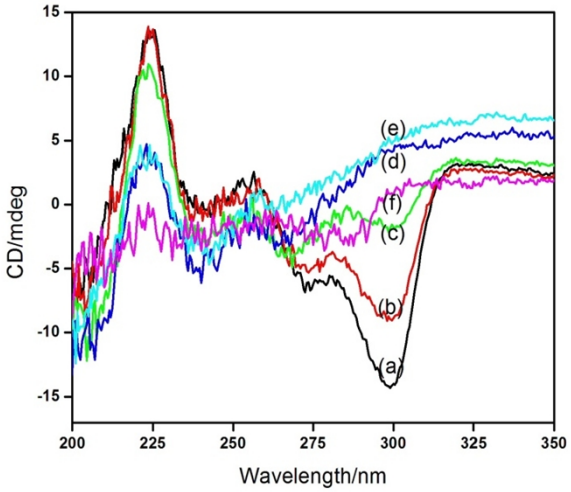


**Fig. S2** Temperature dependent CD spectra of KCl induced G gel in the temperature range 10-80°C : (a) 10°C, (b) 20°C, (c) 30°C, (d) 40°C, (e) 50°C and (f) 80°C.

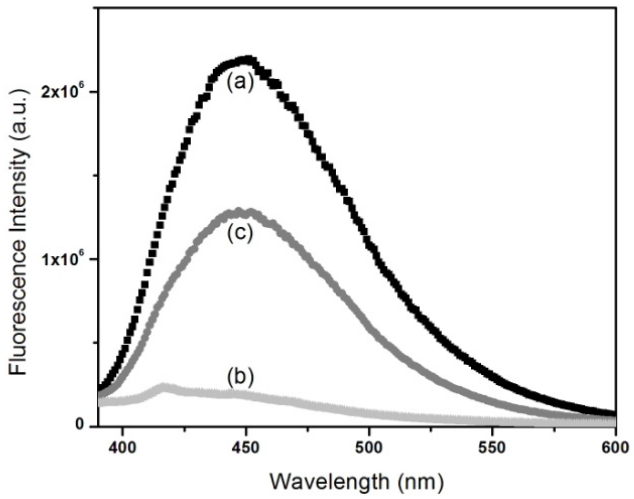


**Fig. S3** (a) Fluorescence spectra of G gel (a), dG gel (b), and co-gel (c) (excitation at 367 nm).

**Table S1** (a) G′ and G″ values (at an angular frequency 10 rad/s) of the G-gels for different metal ions at a fixed concentration of G (1.42 %,w/v) and a constant metal ratio (1:2 mole ratio for G:M). (b) G′ and G″ values (at an angular frequency 10 rad/s) of the dG-$Tl^+$ gel (at a fixed concentration 1.42 %,w/v of dG) for varying metal ion ratios.

TableS1(a):

| metal ions | G′ (Pa) | G″ (Pa) |
|---|---|---|
| $Na^+$ | 750 | 232 |
| $K^+$ | 4000 | 312 |
| $Rb^+$ | 3950 | 1140 |
| $NH_4^+$ | 58 | 30 |
| $Tl^+$ | 28690 | 1354 |
| $Hg^+$ | 650 | 210 |
| $Ag^+$ | 8106 | 720 |
| $Au^+$ | 51 | 28 |

TableS1(b):

| metal ions ratio | G′ (Pa) | G″ (Pa) |
|---|---|---|
| 1:0.5 | 1183 | 127 |
| 1:1 | 3479 | 423 |
| 1:2 | 4661 | 878 |
| 1:4 | 5092 | 1071 |
| 1:8 | 6510 | 1110 |

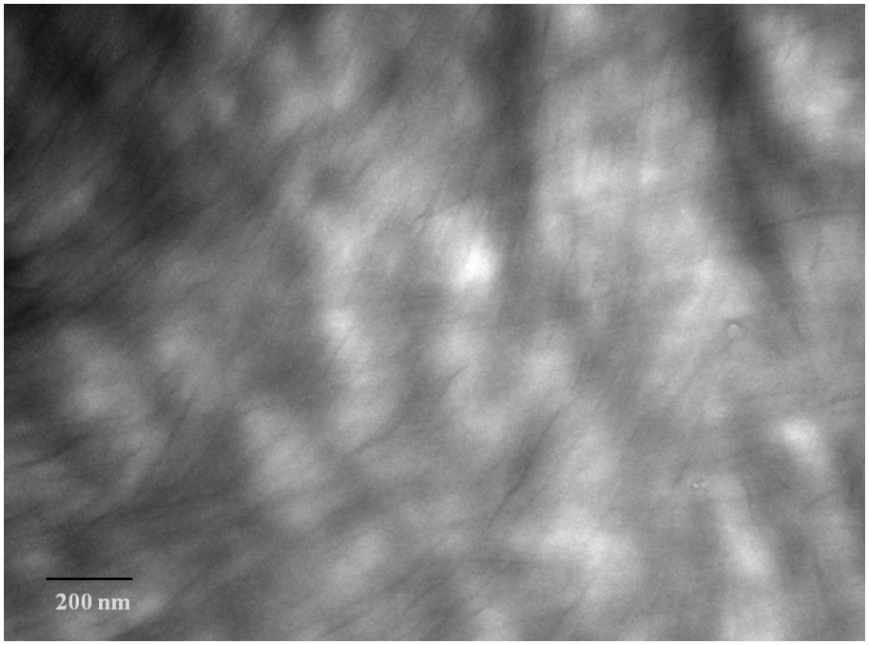


**Fig. S4** TEM image of $Ag^{+}$ ion induced G gel.

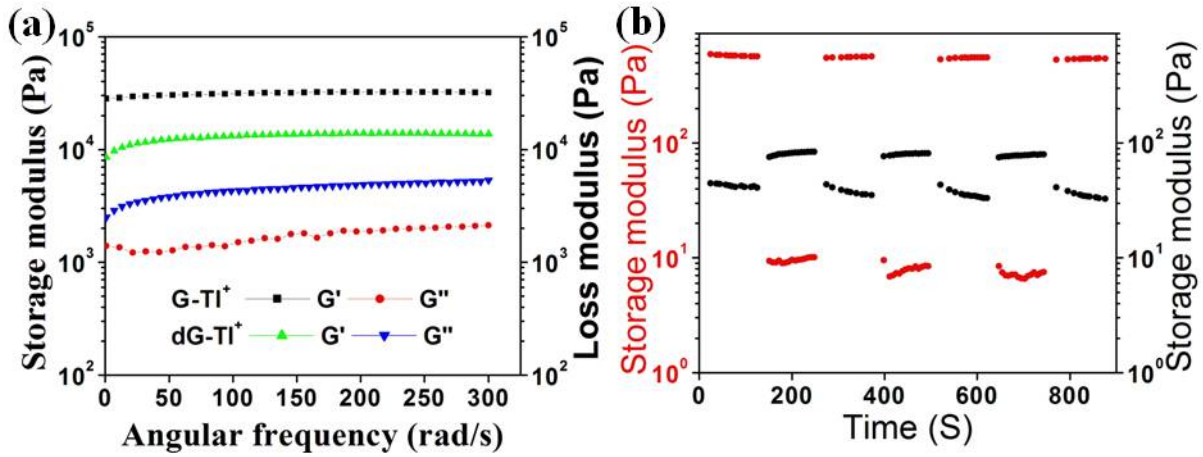


**Fig. S5** (a) Frequency dependence of the storage modulus (G′) and loss modulus (G″) of $Tl^{+}$ induced G and dG gels at a fixed concentration of G/dG (1.42 %,w/v) and a constant metal ion stoichiometry (1:2 mole ratio for G:M); (b) Continuous step strain measurements for co-gel, which was subjected to alternative 100% and 5% strain for breaking and recovery of gel respectively and four cycles were done. The entire study was performed by maintaining a constant angular frequency of 10 rad/s. Red and black indicate storage modulus (G′) and loss modulus (G″) respectively. Experiment was performed with co-gel having a concentration 1.42 % w/v, (1:2 metal ratio) and environmental temperature at 15 ºC.

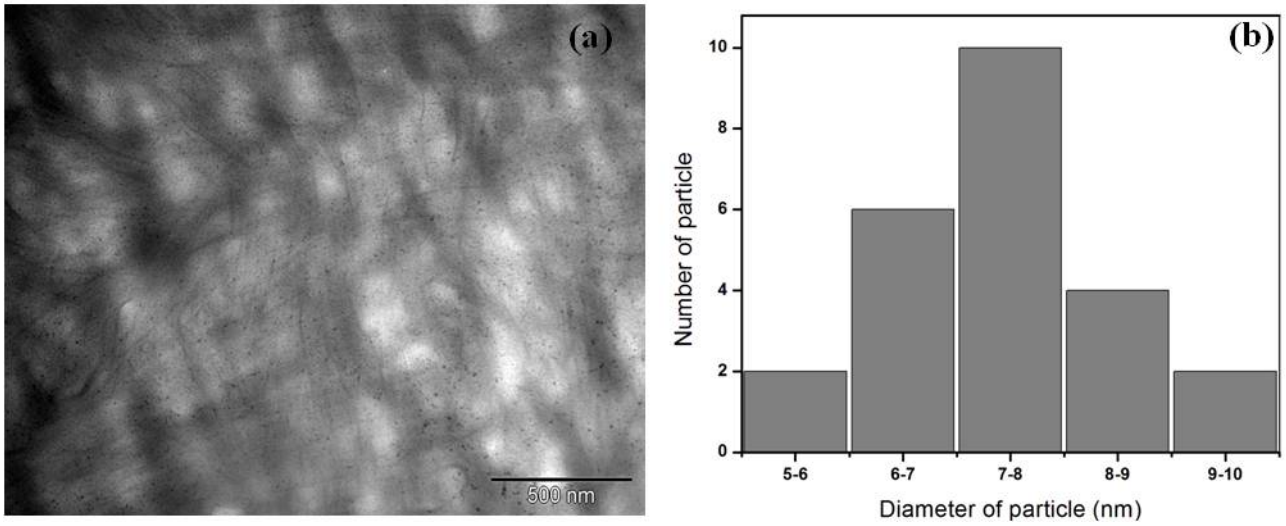


**Fig. S6** (a) TEM image of silver nanoparticles (AgNPs) containing hybrid G gel and (b) Particle size distribution of AgNPs suggesting they have uniform distribution.

**Drug Release Study.**

Hydrogel holds great potential for controlled drug delivery and tissue engineering. Over the years, numerous polymeric gels have been developed for this purpose.[S1] More recently, supramolecular hydrogel obtained from LMWG has sparked interest in the field of drug delivery due to their low cytotoxicity to cells.[S2] There are basically two approaches for therapeutic/drug delivery with hydrogel. Therapeutic agent can be physically entrapped within the voids of a self-assembled hydrogel. The encapsulated therapeutic agent can then be released from the gel via diffusion or enzymatic degradation of the gel.[S3] However, drug release by diffusion suffers from non-quantitative release. In another approach, therapeutic agent can be covalently bonded to hydro-gelator via hydrolyzable bond and then therapeutics can be released via hydrolysis.[S4] In this study, we report an approach for a quantitative and controlled delivery of therapeutic (vitamin B12) via the slow decomposition of the gelator from the therapeutic encapsulated hydrogel matrix ($K^+$ induced G gel). We have used only biologically relevant $K^+$ ion induced G gel for the current drug release study, excluding other metal ions induced gel as they are not biologically important and toxic. Release of the payload is virtually quantitative and spontaneous without triggering by enzyme, light, pH or temperature.

We examined the release properties of the G and dG gels for the controlled release of therapeutics using a model vitamin B2. For this purpose, potassium ion induced G and dG based hydrogels (2.35%,w/v) were prepared in potassium phosphate buffer at pH 7.4. The G gel in potassium phosphate buffer (with or without encapsulation of vitamin B2)

experiences a spontaneous and slow decomposition of gel upon standing over a period of time 50 hours at 37°C. In contrast, the corresponding dG gel is stable for long time period. It can be mentioned that both G and dG gels are stable for long time at fridge (-2 °C) with or without encapsulation of vitamin B2. PBS solution (pH 7.46) was covered upon the vitamin B2 encapsulated G and dG hydrogel separately and these vials were kept at 37 °C in an incubator. Vitamin molecules come out from hydrogel matrix into the PBS buffer, presumably by diffusion (Fig. S7). For the dG hydrogel, our experimental results indicate release of vitamin B2 from the gel matrix of up to ~75% within 70 hours

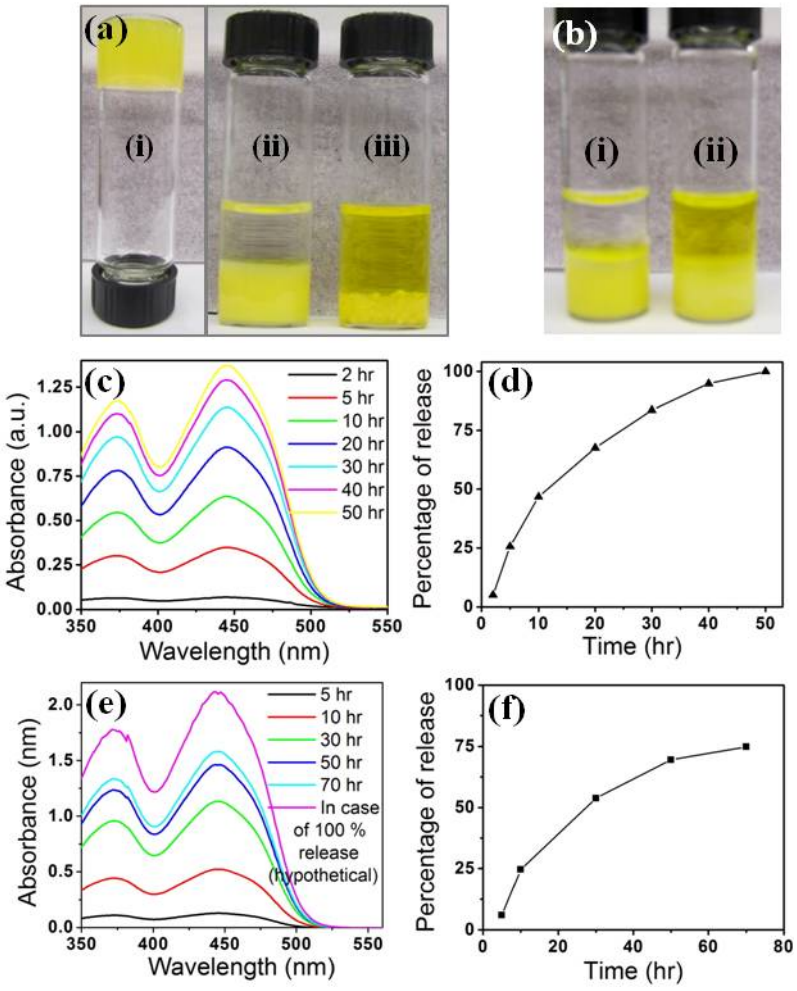


**Fig. S7** (a-i) Photograph of vitamin B2 encapsulated G gel (in potassium phosphate buffer, pH 7.4); Release study of vitamin B2 from G gel into PBS solution: (a-ii) before release showing gel at the bottom and PBS at the top of the vial and (a-iii) after complete release of vitamin B2 with concomitant decomposition of G gel; (b) Release study of vitamin B2 from dG gel into PBS solution: (b-i) before release showing gel at the bottom and PBS at the top of the vial and (b-ii) release of vitamin B2 after 70 hour. Time dependent UV-Vis study for the release of vitamin B2 from G gel (c) and dG gel (e). Release profile diagram of vitamin B2 from G gel (d) and dG gel (f).

(Fig. S7). Surprisingly, almost 100 % vitamin molecules released from the hydrogel matrix into PBS buffer in case of the G hydrogel (Fig. S7) within 50 hours. In this case, vitamin B2 is released into PBS buffer while simultaneous decomposition of the gel ensures complete release of vitamine B2. Though there are some reports of metastable gels, it has not been used yet for delivery purpose.[S5]

Therefore in this study our G hydrogel can be use a most efficient cargo than dG gel for the sustain release of vitamin. This is due to the difference in mechanism for drug release for two gels. It can be mentioned that we can regulate the drug release rate by changing the gelator and metal ion concentrations (at fixed environmental temperature and pH) as they can tune the gel strength. There is some advantage of our gels (a) drug encapsulated G gel is stable for long time at fridge and it release (quantitatively) the drug at 37°C through decomposition of gel, (b) potassium ion induced G gel is expected to be biocompatible, (c) G gel is stable at 37°C temperature and pH 7.4, (d) being a biomolecules, gelators do not need to synthesize at cost stand point.

***Experimental section for vitamin release study.***

For controlled release purpose, potassium ion induced G and dG based hydrogels (2.35%,w/v) were prepared in potassium phosphate buffer at pH 7.4. We used this gel due to the following reasons: (a) The G gel in potassium phosphate buffer experiences a spontaneous and slow crystallization concomitant with the precipitation of gelator upon standing over a period of time 50 hours at 37°C. This might be useful for complete release of the drug. In contrast, KCl induced G gel in water (only) collapses over a shorter period of time that is not useful for controlled release purpose. (b) Here, drug release study is possible at pH 7.4 (physiological pH).

In a typical experiment, 28.2 mg G was dissolved into 1 ml potassium phosphate buffer (pH 7.4, 200 mmol) with heating and then on cooling it gives opaque hydrogel. 0.2 ml aqueous solution of vitamin B2 (1 mg/5 ml) was added into the hydrogel and using heating-cooling method to obtain vitamin B2 encapsulated stable hydrogel (2.35%,w/v). Then, 2 ml PBS solution (strength 1X, pH 7.46) was covered upon the vitamin B2 encapsulated G and dG hydrogel separately and these vials were kept at 37 °C in an incubator. The dG hydrogel is stable in the presence of PBS in vial over a long period of time without disruption of gel but G hydrogel is slowly crystallize and precipitated out from the medium and the lifetime stability is 50 hours. It had been noticed that the PBS transformed into color due to diffusion of vitamin. A small aliquot of the buffer solution was taken at different time intervals and it was subjected to a UV/visible spectroscopy study.

**References:**

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