Electronic Supplementary Material (ESI) for ChemComm.

Electronic supplementary information

Self-healing metal coordinated hydrogels using nucleotide ligands

Hao Liang,*[a,b] Zijie Zhang, [b] Qipeng Yuan[a] and Juewen Liu* [b]

[a] State key Laboratory of Chemical Resource Engineering, Beijing University of Chemical Technology, Beijing, P.R. China. E-mail: lianghao@mail.buct.edu.cn

[b] Department of Chemistry and Waterloo Institute for Nanotechnology, University of Waterloo, Waterloo, Canada. E-mail: liujw@uwaterloo.ca; Fax: +1 519 7460435; Tel: +1 519 8884567 ext. 38919

Materials and methods

Chemicals. Adenine, adenosine, adenosine 5′-monophosphate (AMP) disodium salt, adenosine 5′-triphosphate (ATP) disodium salt hydrate, guanosine 5′-monophosphate (GMP) disodium salt hydrate, cytidine 5′-monophosphate (CMP) disodium salt, fluorescein, rhodamine B, rhodamine 6G and albumin-fluorescein isothiocyanate conjugate (F-BSA), zinc chloride, copper dichloride, ferric chloride, aluminum chloride, nickel dichloride, cobalt dichloride, cadmium dichloride, manganese dichloride, magnesium chloride, and calcium chloride were from Sigma-Aldrich. 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES) was from Mandel Scientific (Guelph, ON). Milli-Q water was used to prepare all the buffers and solution. All other reagents and solvents were of analytical grade and used as received.

Titration of Zn^{2+} into Nucleotides. First, 1 mL of 2 mM nucleotides (AMP, GMP or CMP) in HEPES (pH 7.4, 10 mM) was placed into a quartz cuvette. Then 20 μL of $ZnCl_2$ (10 mM in Milli-Q water) was added into the cuvette at each time, and the UV-vis extinction at 550 nm was recorded using an Agilent 8453A spectrophotometer.

Preparation of Metal and Nucleotide Coordinated Complexes. The nucleotides/Zn^{2+} complexes were prepared by adding 200 μL of $ZnCl_2$ (10 mM in Milli-Q water) into 800 μL of 2 mM nucleotides in HEPES buffer (10 mM, pH 7.4) to produce the 1:1 molar ratio complex. Precipitants were observed within several minutes. The samples were then centrifuged at 9,000 rpm for 5 min and washed with Milli-Q water to remove the remaining chemicals. Other metal ions were also reacted with AMP at the same concentration using the method mentioned above.

DLS. The coordination complexes were re-dispersed into Milli-Q water by vortex mixing for DLS measurement (Zetasizer Nano 90, Malvern) at 25 °C. ζ-potential was measured using the dip-cell set-up under the same condition.

TEM. TEM was performed on a Philips CM10 transmission electron microscope. The sample was prepared by pipetting a drop of the aqueous solution of the coordination complexes onto a 230 mesh holy carbon copper grid. The sample was dried on a filter paper.

Sol–gel phase transition behavior. The tube inverting method was used to observe the

flowing sol and non-flowing gel state of the Zn/AMP complex in aqueous medium. Each sample was prepared at the same concentration (5 mM) by the above method. The pH was adjusted from 4 to 13 with 0.25 M NaOH and 0.25 M HCl. The samples were allowed to incubate for 2 h. For the temperature effect, the temperature was increased at intervals of 25 °C, and the tubes were maintained at a constant temperature for 30 min. All tubes were centrifuged at 9,000 rpm for 5 min, and then the sol-gel phase was determined by inverting the tube for 1 min to judge whether a gel is formed. The formed gel was weighed on a balance to quantify its amount.

Rheometry. Rheological experiments were conducted on a Carri-Med CSL500 controlled-stress rheometer. A cone-plate geometry with a cone diameter of 25 mm was used. The temperature was controlled by the bottom Peltier plate. All tests were performed immediately after transferring 0.5 mL of a Zn/AMP hydrogel (5 mM) onto the sample stage. Frequency sweep was obtained with a strain of 0.4%. Strains sweep was obtained with a frequency of 0.1 Hz.

Incorporation of Dyes or Protein into Zn/AMP Gel. 200 μL of 25 mM AMP in HEPES (10 mM, pH 7.4) and 2 μL dyes (5 mM) were added into 700 μL of HEPES buffer (10 mM, pH 7.4), and the solution was mixed. Dye-doped nucleotide/metal complexes were prepared by adding $ZnCl_2$ (50 mM, 100μL). The amount of dyes incorporated in complexes was determined by measuring the absorption intensity of dyes in the supernatants. Immobilization of protein within Zn/AMP gel was performed by mixing 200 μL of 25 mM AMP in HEPES (10 mM, pH 7.4), 10 μL albumin–fluorescein isothiocyanate conjugate (F-BSA, 5 mg/mL) and 700 μL HEPES buffer (10 mM, pH 7.4). Then $ZnCl_2$ (50 mM, 100μL) was added. The amounts of protein incorporated into the Zn/AMP gel were calculated by absorption intensities of the supernatant solutions compared to aqueous solutions of the untreated protein in HEPES buffer.

Self-Healing Experiment. For ease of visualization, Zn/AMP gels containing F-BSA and rhodamine B were first prepared separately by the method mentioned above. Then, the Zn/AMP gel containing rhodamine B was broken by vortex mixing, and the broken gel was taken out and put on the top of Zn/AMP gel containing F-BSA. The mixed sample was centrifuged at 9,000 rpm for 5 min.

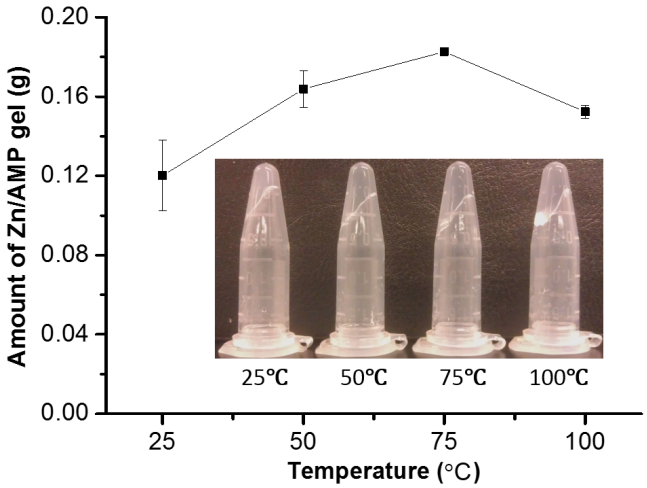

Figure S1. Effect of temperature on the Zn/AMP gel formation.

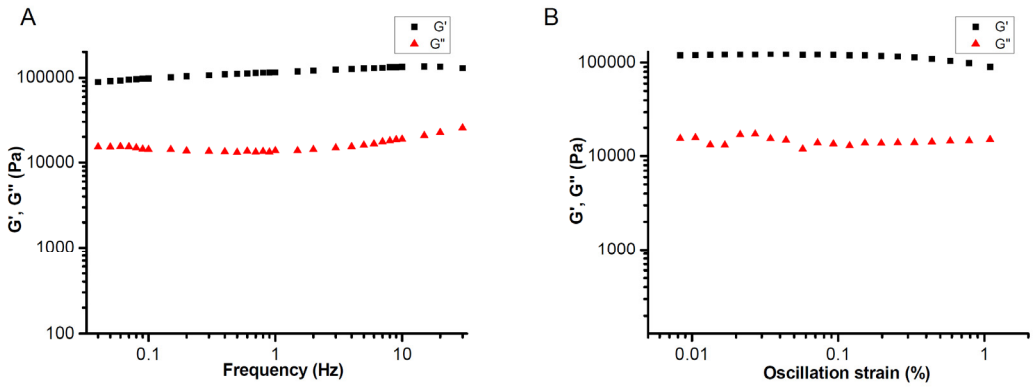

Figure S2. (A) Dynamic frequency sweep rheometry data for Zn/AMP gel at 25 °C (strain kept at 0.4 %). (B) Strain sweep rheometry data (storage modulus G' and loss modulus G" vs. strain) for Zn/AMP gel at 25 ℃ (frequency: 1 Hz).

The viscoelastic property of the Zn/AMP hydrogel was confirmed by rheometry. All tested samples were prepared by 5 mM $ZnCl_2$ and 5 mM AMP in HEPES buffer. 0.5 mL of Zn/AMP hydrogel was transferred onto the sample stage for measurement. A dynamic oscillatory frequency sweep experiment was measured under 0.4 % strain at 25 °C. In the frequency sweep experiment, storage modulus (G') values were much higher than those of loss modulus (G") with a linear response observed over a wide range of frequencies (0.03-30Hz), as shown in Figure S2A. The changes G' and G" under shear strain were recorded at a constant

frequency of 1 Hz. The strain sweep showed a linear regime between 0.01 and 1% (Figure S2B). These results show the elastic character of the hydrogel.[S1] It is consistent with other reported metal-organic coordinated hydrogels.[S2]

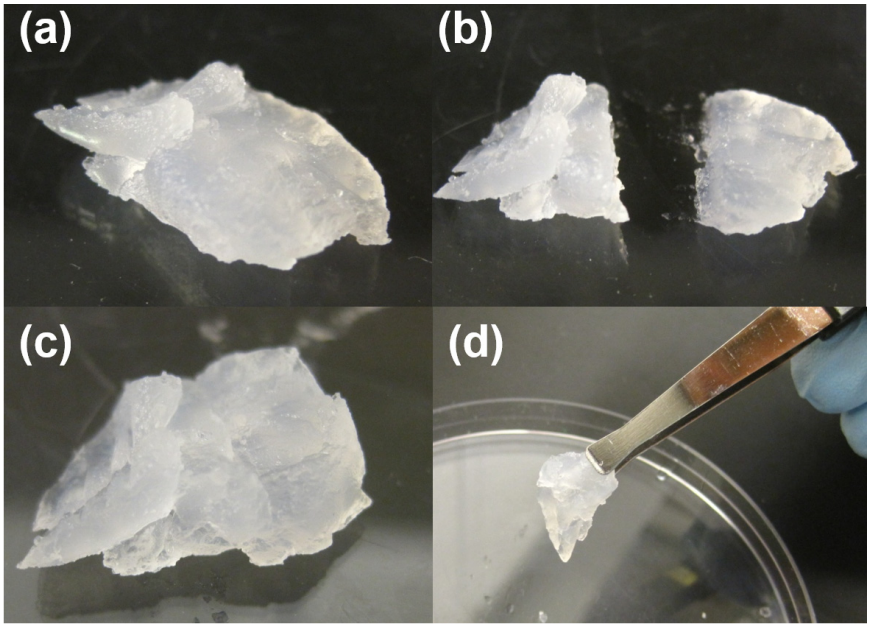

Figure S3. Self-heating test. The Zn/AMP hydrogels (5 mM) were prepared in several tubes to obtain sufficient amount of samples. Then, all the formed gels were pooled together on a petri dish (a). This new combined gel was cut in two halves (b). Then, the two pieces were brought back together by a mild press and the self-healing process occurred instantaneously (c). The gel integrity was maintained by holding one of the pieces (d). This also indicates the self-healing property of such gels.

Additional References

S1. (a) Jiang, Y.; Zeng, F.; Gong, R.; Guo, Z.; Chen, C.-F.; Wan, X., A multi-stimuli responsive organogel based on a tetrapeptide-dithienylcyclopentene conjugate. *Soft Matter* **2013,** *9* (31), 7538-7544; (b) Das, P.; Yuran, S.; Yan, J.; Lee, P. S.; Reches, M., Sticky tubes and magnetic hydrogels co-assembled by a short peptide and melanin-like nanoparticles. *Chemical Communications* **2015,** *51* (25), 5432-5435.

S2. (a) Foster, J. A.; Parker, R. M.; Belenguer, A. M.; Kishi, N.; Sutton, S.; Abell, C.; Nitschke, J. R., Differentially Addressable Cavities within Metal–Organic Cage-Cross-Linked Polymeric Hydrogels. *Journal of the American Chemical Society* **2015,** *137* (30), 9722-9729; (b) Saha, S.; Bachl, J.; Kundu, T.; Diaz Diaz, D.; Banerjee, R., Amino acid-based multiresponsive low-molecular weight metallohydrogels with load-bearing and rapid self-healing abilities. *Chemical Communications* **2014,** *50* (23), 3004-3006.