Supporting Information

For

Metalloprotein-Inspired Thermo-Gene for Thermogels

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Experimental Section

Materials

Folic acid hydrate (>98.0%) was purchased from Tokyo Chemical Industry Co. Ltd., and the chemical formula was determined by element analysis as $C_{19}H_{19}N_7O_6 \cdot 2H_2O$. Zinc nitrate hexahydrate ($Zn(NO_3)_2 \cdot 6H_2O$) and potassium hydroxide (KOH) were purchased from Xilong Chemicals with purity above 99.0%. Potassium Nitrate (KNO_3) were purchased from Beijing Tongguang Fine Chemicals Company with purity above 99.0%. Ethylene glycol (EG) and agarose were purchased from Sinopharm Chemical Reagent Co. Ltd. with A.R. grade and purity above 99.0%. Acrylamide (AM, >98.0%), Ammonium persulfate (APS, >99.0%) and Tetramethylethylenediamine (TEMED, >98.0%) was purchased from Tokyo Chemical Industry Co. Ltd. Polyvinyl alcohol (PVA, $[-CH_2CH(OH)-]_n$) was purchased from Alfa Aesar (by Thermo Fisher Scientific) with 98-99% hydrolyzed, average molecular weight 88,000-97,000. Water used in all experiments was purified by Milli-Q Advantage A10 ultrapure water system. All the hydrogel samples were freshly prepared immediately in each experiment.

Fabrication of folate-zinc ion hydrogel

In a typical procedure of folate-Zn^{2+} hydrogel preparation, folic acid was weighed into a vial and Milli-Q water was added to obtain desired folic acid concentration. Potassium hydroxide was then added to adjust pH above 10 and clear solution of folate was obtained. Next, certain amount of zinc nitrate was added into above folate solution. After 30 seconds to 1 minute's vortex, vial was allowed to stand still for 20 minutes to obtain the transparent folate/Zn^{2+} hydrogel. For folate-zinc ion gel in binary solvent, same fabrication procedure was employed except solvent was replaced by ethylene glycol – water (V/V=1/1) binary solvent.

Fabrication of double network gel

Three kinds of double network gel were prepared: PVA/folate-Zn^{2+} double network gel by freezing-thawing method, Agarose/folate-Zn^{2+} double network gel by heat-cooling method, and PAM/folate-Zn^{2+} double network gel by *in-situ* polymerization method. We use binary solvent ethylene glycol – water (V/V=1/1, EGW) in order to fabricate anti-freezing gel.

For PVA/folate-Zn^{2+} gel, an appropriate mass of polyvinyl alcohol (PVA, $[-CH_2CH(OH)-]_n$, purchased from Alfa Aesar with 98-99% hydrolyzed, average molecular weight 88,000-97,000) was first dissolved in EGW to desired concentration (1 wt% - 5 wt%) at 90 °C for 20 min to obtain transparent and homogenous PVA solution. Next, the homogenous PVA solution was allowed to cool to around 60 °C, then folate stock solution (100 mM in EGW) and zinc nitrate stock solution (100 mM in EGW) was added and mixed thoroughly by a magnetic stirrer. The typical concentration of folate and zinc nitrate is 15 mM (0.72 wt%) and 21 mM (0.62 wt%) respectively for 5 wt% PVA concentration, which means around folate-Zn^{2+} with around 25% of PVA concentration is sufficient to provide enough thermal protection to PVA. As for 3 wt% PVA gel, only 0.5 wt% folate-Zn^{2+} is sufficient for thermal protection, accounting for 15% of PVA concentration. In general, sufficient concentration of folate-Zn^{2+} is approximately 10-25% of PVA concentration. Then this viscous solution was cast into homemade molds and cooled at -20 °C for 30 min. After three cycle of freezing-thawing, the obtained PVA/folate-Zn^{2+} gel was prepared and different shapes of PVA/folate-Zn^{2+} gel can be achieved by different molds. For example, we use plastic cylinder molds with diameter of 1 cm for cylinder-shape gel preparation and flat molds composed of two flat glass plates (10 cm × 10 cm) sandwiching a 2 mm or 1 mm thick silicone gasket for thin film

gel preparation. For PVA single network gel as a control, a similar process was conducted without introduction of folate and zinc nitrate.

For agarose/folate-Zn^{2+} gel, an appropriate mass of agarose was first dissolved in EGW to desired concentration (1 wt% - 10 wt%) at 70 °C until solution become transparent and homogenous. Next the homogenous agarose solution was allowed to cool to around 50 °C, then folate stock solution (100 mM in EGW) and zinc nitrate stock solution (100 mM in EGW) was added and mixed thoroughly by a magnetic stirrer. The typical concentration of folate and zinc nitrate is 15 mM (0.72 wt%) and 21 mM (0.62 wt%) respectively for 10 wt% agarose concentration, which means around folate-Zn^{2+} with around 13% of agarose concentration is sufficient to provide enough thermal protection to agarose. Then the viscous solution was cast into homemade molds and cooled to room temperature. Similar molds was utilized to obtain cylinder or thin file agarose/folate-Zn^{2+} gel. For agarose single network gel as a control, a similar process was conducted without introduction of folate and zinc nitrate.

For PAM/folate-Zn^{2+} gel, an appropriate mass of acrylamide (AM) was weighed and added into EGW to desired concentration. Then Ammonium persulfate (APS) was added and fully dissolved into EGW. Next folate and zinc nitrate solution in EGW and tetramethylethylenediamine (TEMED) were added in sequence and mixed thoroughly. Then the solution was cast into homemade molds and after 10 – 20 minutes, the whole system was gelated. Typically, 29 uL 10 wt% APS was added into 2.7 mL 30 wt% acrylamide solution, then 600 uL folate (100 mM) and 840 uL zinc nitrate (100 mM) were added and mixed thoroughly, followed by addition of 3 uL TEMED. Final concentration of polyacrylamide was 10 wt% - 20 wt% with folate of 0.72 wt% and zinc nitrate of 0.62 wt%, meaning that folate-Zn^{2+} with only 6% - 13% of concentration of PAM is sufficient for thermal protection. Similar

molds was utilized to fabricate cylinder or thin file PAM/folate-Zn^{2+} gel. For PAM single network gel as a control, a similar process was conducted without introduction of folate and zinc nitrate.

Rheological measurement

The rheological properties of folate-Zn^{2+} hydrogels were measured with a ThermoHaake RS300 rheometer with a water bath. The folate/Zn^{2+} hydrogel samples were prepared and gently placed on the middle between a 35 mm diameter plate and a parallel plate rotor with the gap of 0.100 mm. A solvent trap was used to avoid water evaporation. Dynamic stress sweep spectra were carried out at frequency of 1 Hz. Dynamic frequency sweep spectra were recorded in the linear viscoelastic regime of the samples determined from dynamic stress sweep measurements (for example, at 1 Pa for hydrogel with [folate] = 15 mM, [folate]:[Zn^{2+}]=1:1.7). Dynamic oscillatory measurements were conducted at 1 Pa and 1 Hz at different temperature. Folate-Zn^{2+} hydrogels were fabricated and stabilized for at least 12 hours.

The rheological properties of double network gels were measured with Anton Paar model MCR-301 rheometer (Austria) at temperature range from – 30 °C to 80 °C. Dynamic oscillatory measurements for storage modulus (G') and loss modulus (G'') were measured with frequency of 1 Hz and strain of 0.1%. A solvent trap was used to avoid EGW evaporation. The samples tested here were disk-like cylinders made by PVA gel, folate-Zn^{2+} gel and PVA/folate-Zn^{2+} double network gel with 10 mm in diameter and 1 mm in height.

Characterization of conductivity

Conductivity of gels were first tested by incorporating gels into a circuit with a battery and a LED. As shown in Figure S5, folate-Zn^{2+} gels and PVA/folate-Zn^{2+} gels can

connect the circuit and make LED on in wide range of temperature between – 20 °C to 80 °C, while PVA single network gels can rarely conduct electricity.

Detailed resistivity measurement of PVA/folate-Zn^{2+} double network gels was conducted with a RTS-4 Four-Point Probes Resistivity Measurement System (made by 4PROBES Tech.). Measurement temperature is 25 °C and disk-like PVA/folate-Zn^{2+} double network gels with different amount of KNO_3 were measured.

Mechanical test for DNTG

Tensile analysis of the double network gels were tested on Instron 3365 machine. Dumbbell shaped gel samples with a length of 13 mm, thickness of 0.65 mm and width of 2 mm were used. The strain (%) was estimated as $\delta l/l_0$, where δl is a deformed length and l_0 is an original length. The tensile stress (σ) was measured as $\sigma=F/A_0$, where F is the force applied on the gel samples and A_0 is the original cross-section area of gel samples. The tensile rate of all tensile tests were 100 mm/min. The compression test were primarily conducted on ThermoHaake RS300 rheometer. The cylinder gel sample with a height of 1.5 cm and a diameter of 0.6 cm was placed on between a 35 mm diameter plate and a parallel plate. The sample and both paltes were heated to 80 °C, and parallel plate can be controlled to desired position with compression (lower position) and release (higher position) status of gels.

Setup of sensors based on PVA/folate-Zn^{2+} gels

PVA/folate-Zn^{2+} gel-based flexible sensors was prepared in following steps: (1) PVA/folate-Zn^{2+} gels were prepared as described before and were cut into stripe shape (about 3 cm ×1 cm). (2) Proper amount of silver conductive paint (05001-AB(SPI-PAINT)) was brushed onto both ends of PVA/folate-Zn^{2+} gel stripe. (3) One end of a silver wire was embedded into silver conductive paint and the other end was connected to Series 2400

SourceMeter (as a battery and a voltage/current meter). (4) Both ends of PVA/folate-Zn^{2+} gel stripe were placed on heater of 80 °C, and silver conductive paint will solidify and with aid of silver paint and silver wire, PVA/folate-Zn^{2+} gel was incorporated into circuit.

After fixing both ends of PVA/folate-Zn^{2+} gel stripe onto a finger, the bend of finger will cause stretch status of gel stripe. We control the voltage on the gel stripe as constant 1.0 V and monitor the current change when we repeatedly bend and release finger. Then the electronic resistance variation ($\Delta R/R_0$) can be calculated from change of current. Similarly, after the PVA/folate-Zn^{2+} gel stripe was attached onto a steel plate (the steel plate was covered by insulated transparent scotch tape to isolate steel and sensor, and the steel plate can be heated to 80 °C), the bend of steel plate will lead to stretch of gel leading to current change, which can be converted to electronic resistance change.

Structural characterization of folate-Zn^{2+}, PVA, and folate-Zn^{2+}/PVA gels

The microstructure of gels was characterized by scanning electron microscopy (SEM) and transmission electron microscopy (TEM). For sample preparation of SEM images, gels were placed on clean silicon wafer was freezed in liquid nitrogen, and further dried 24 h in vacuum. The samples were then observed with a field scanning electron microscope (Hitachi S-4800) at 2~4 kV voltage. The elemental mapping of zinc was obtained from freeze-dried gels by scanning electron microscope (Hitachi S4800) equipped with energy-dispersive X-ray spectroscopy (EDS). For sample preparation of TEM images, a slice of hydrogel was placed on 200 mesh copper grids coated with ultrathin carbon film. The copper grids were placed on top of a heating plate, which can control temperature at 25 °C or 80 °C, and the whole adsorption process occurred at constant temperature. A inverted watch glass was placed on the heating plate, to make copper grids and gels in a mositure atmosphere. After 10 minutes adsorption, hydrogel was removed and copper grids were allowed to dry in ambient

condition. Then TEM images were acquired using FEI Tecnai G2 T20 transmission electron microscope, operated at an acceleration voltage of 120 kV.

Circular dichroism (CD) spectroscopy was performed at different temperate controlled by a water bath with Folate-Zn^{2+} hydrogel ([folate] = 15 mM, [folate]: [Zn2+] =1:1.7.). The samples were placed into a quartz cell with 0.05 mm optical light path and CD spectra were recorded by a JASCO J-810 spectrometer at range of 220-550 nm with scanning speed of 200 nm/min, response time of 1 s, and bandwidth of 1 nm. At least two scans were accumulated for each trial.

1H-Nuclear Magnetic Resonance (1H-NMR) spectra were acquired on Bruker Avance III 500 MHz NMR at different temperature. The samples were prepared in deuterium oxide – ethylene glycol (V/V=1/1) binary solvent. Chemical shifts are reported in ppm relative to the residual solvent peak.

Small Angle X-ray Scattering (SAXS) spectra were performed with Ganesha system (SAXSLAB, U.S.) equipped with a multilayer focused Cu Kα radiation as the X-ray source (Genix3D Cu ULD) and a two-dimensional semiconductor detector (Pilatus 100 K, DECTRIS, Swiss). The scattering peak positions were calibrated with LaB6 for wide-angle region and silver behenate for small-angle region, respectively. Folate-Zn^{2+} hydrogel ([folate] = 15 mM, [folate]: [Zn^{2+}] =1:1.7.) was placed inside of a glass capillary and capillary was sealed in case of water evaporation at high temperature. A Linkam HFSX350-GI stage was utilized to control the temperature of sample. And the distance between the sample and the detector is 1050 mm.

Supplemental Figures

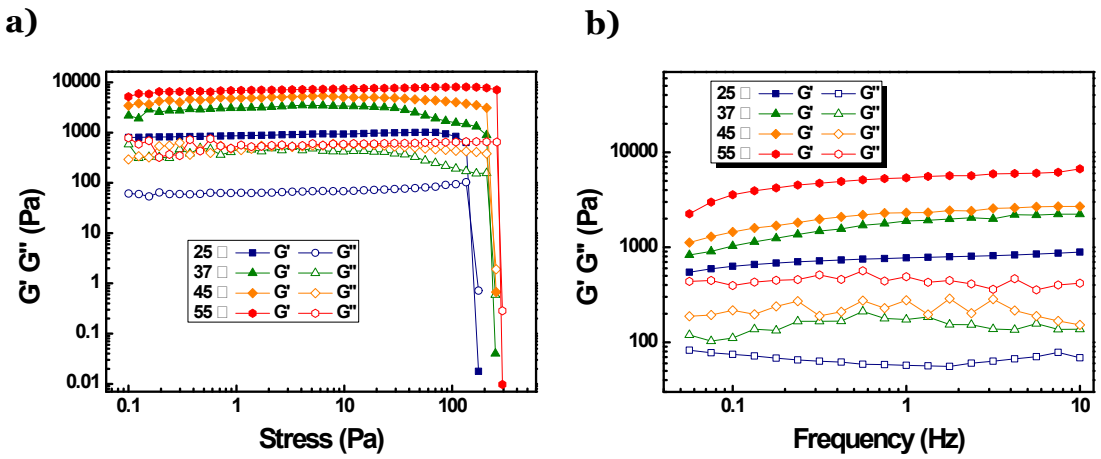

Figure S1. Stress-dependent (f = 1 Hz), and (f) frequency-dependent (stress = 1 Pa) oscillatory profiles of folate-zinc ion hydrogel with increasing temperature. [folate] = 15 mM, [folate]:[Zn^{2+}]=1:1.7

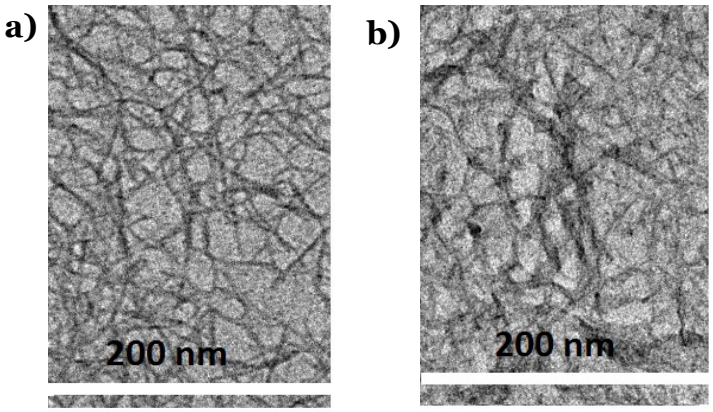

Figure S2. TEM images of Folate-Zn^{2+} hydrogel at 25 ℃ (a) and 80 ℃(b) . Both widths of (a) (b) are 200 nm.

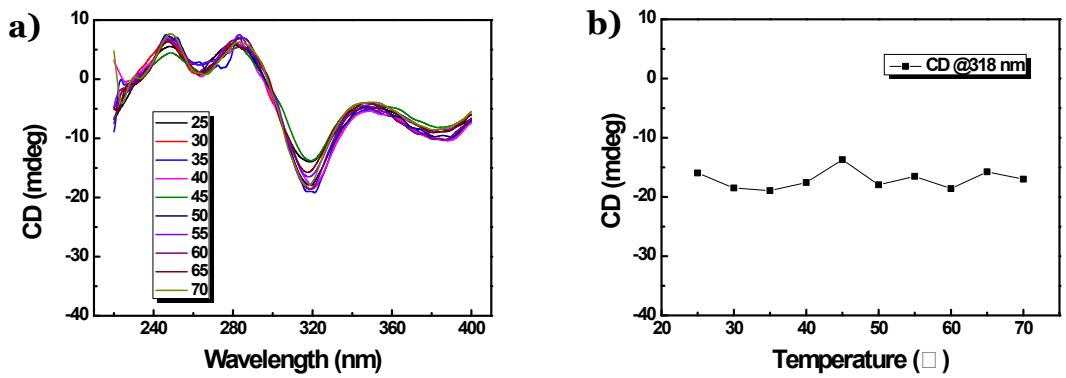

Figure S3. (a) CD spectra of Folate-Zn^{2+} gel in different temperature. (b) CD at 318 nm versus varied temperature.

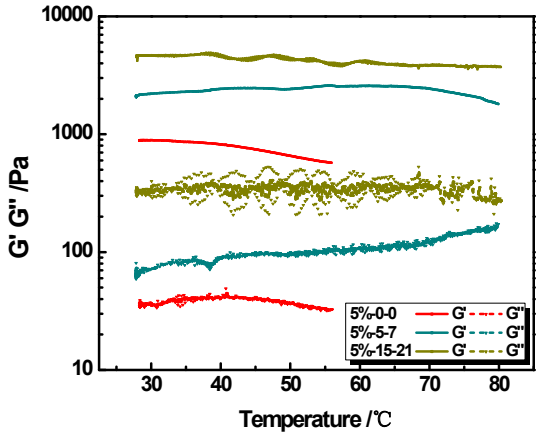

Figure S4. Rheological measurements of the DNTG with increased concentration of the thermo-gene folate-Zn^{2+} hydrogel. The shear moduli disappear at the temperature around 55℃, but addition the folate-Zn^{2+} thermo-gene enhances the heat resistance. [PVA]= 5% in all the systems, whereas [folate]-[Zn^{2+}]= 0 mM - 0 mM, 5mM - 7mM, 15Mm - 21mM, in lable 5%-0-0, 5%-5-7, 5%-15-21, respectively.

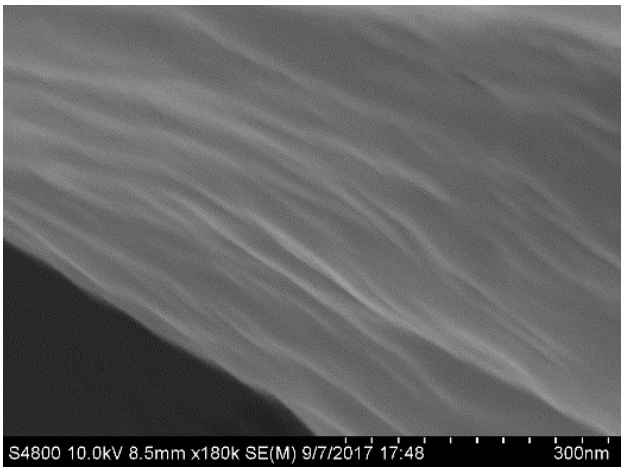

Figure S5. Magnified SEM image for the pore wall of the dried folate- Zn^{2+} hydrogel. Fibers are discernable on the wall.

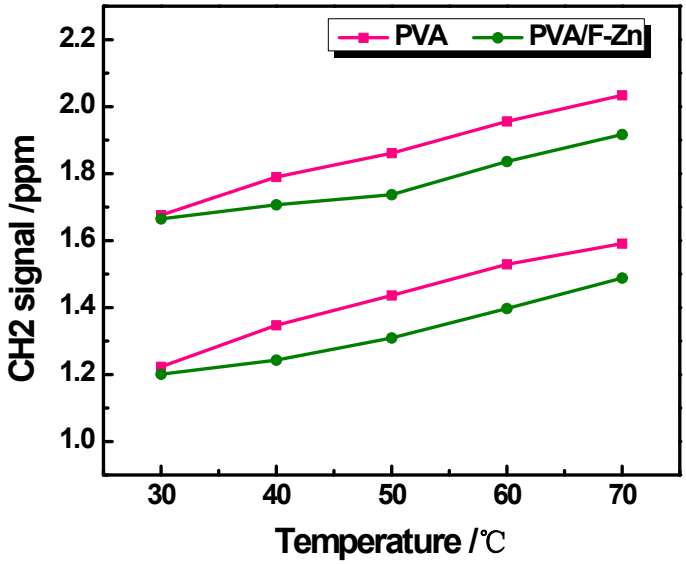

Figure S6. Variation of the chemical shifts for two groups of protons on the PVA chains in the PVA single hydrogel and PVA/F-Zn DNTG.

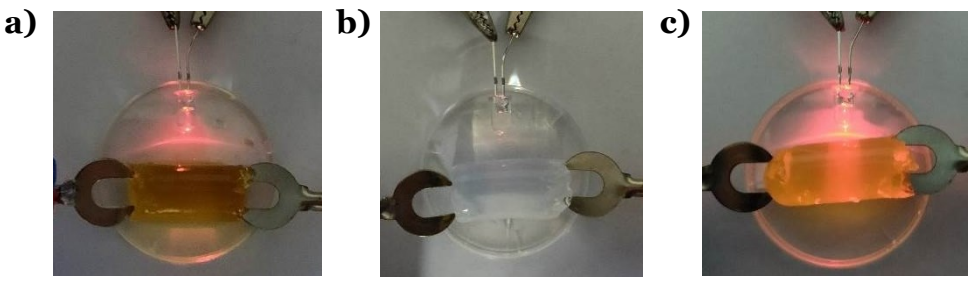

Figure S7. Conductivity demonstration with gel as conductor and LED as indicator in one circuit. Folate-Zn^{2+} gel (a), PVA gel at -20 ℃ (b), PVA/Folate-Zn^{2+} DNTG (c). T= 25 ℃.

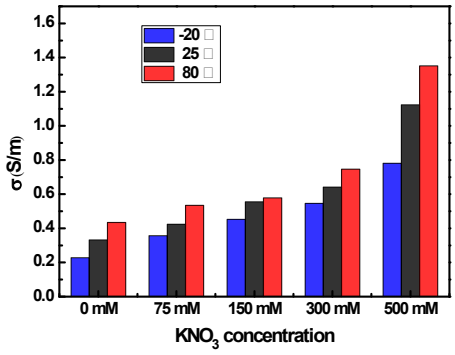

Figure S8. Conductivity of PVA/Folate-Zn^{2+} double network gel with different amount KNO3 at various temperatures.

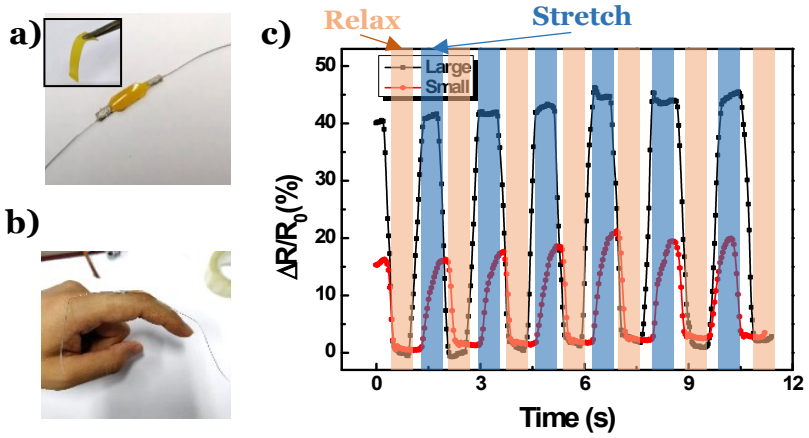

Figure S9. (a) The stress sensor made with a stripe of the DNTG film. The inset is the photo of the film stripe used in the sensor. (b) Sensing the finger movement by attaching the sensor to a human finger. (c) Relative change of the electrical resistance of the sensor under different (large and small) bending amplitude.

Figure S10. Disk-like folate-Zn^{2+} gel (a), PVA gel (b) and PVA/Folate-Zn^{2+} double network gel (c).

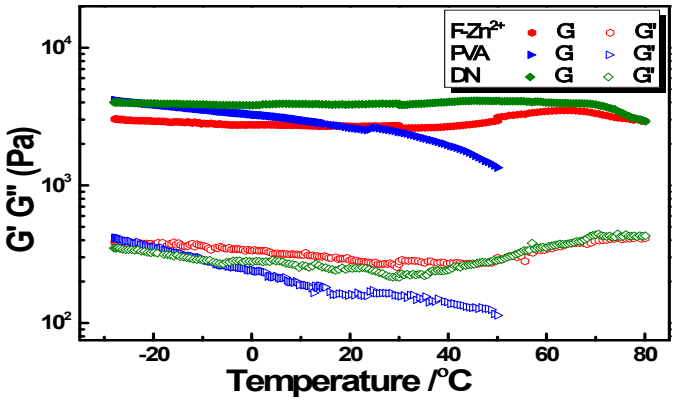

Figure S11. Rheological measurements of folate-Zn^{2+} gel(F-Zn^{2+}), PVA gel, and PVA/folate-Zn^{2+} DNTG in the temperature range of -30°C to 80 °C. The weight fractions of the thermal gene folate-Zn^{2+} in all the three gels are all 10%.