Supplementary Information

Chirality control to multi-stimuli responsive and self-healing supramolecular metallo-hydrogels

Bhagwati Sharma[a,c], Ashmeet Singh[a], Tridib K. Sarma[b], Neha Sardana[a,d] and Asish Pal*[a]*

[a]Institute of Nano Science and Technology

Habitat Centre, Phase X, Sector 64, Mohali, Punjab 160062, India.

E-mail: apal@inst.ac.in; nehasardana@iitj.ac.in

[b]Discipline of Chemistry, Indian Institute of Technology Indore,

Simrol, Khandwa Road, Indore- 453552, India.

[c]Presently at Department of Chemistry, Rewa Engineering College, Rewa, Madhya Pradesh - 486002, India.

[d]Presently at Indian Institute of Technology Jodhpur, N. H. 65, Nagaur Road, Karwar, Jodhpur, Rajasthan - 342037, India.

Materials and Methods:

Materials

L-Histidine, L-Arginine, L-Lysine, D-Histidine and Manganese chloride were purchased from Sisco Research Laboratories (SRL), India. Zinc nitrate hexahydrate, Zinc acetate dihydrate, Zinc chloride, Calcium chloride dihydrate, Magnesium sulfate, Iron(III) nitrate nonahydrate, Cobalt chloride hexahydrate, Nickel chloride hexahydrate, Copper sulfate hexahydrate, Cadmium nitrate terahydrate, Sodium hydroxide, ammonia solution, Ethylenediaminetetraacetic acid (EDTA), Trifluoroacetic acid, Sodium sulfide, Nitric acid, Acetonitrile and Methanol were purchased from Merck, India. Silver nitrate, Gold(III) chloride trihydrate and Glutamic acid monosodium hydrate were purchased from Sigma Aldrich. All the chemicals were of analytical grade and were used without any further purification. Milli-Q water was used throughout the experiments.

Instrumentation

FTIR spectra were recorded in KBr pellet using a Cary 600 series spectrometer from Agilent technologies. Elemental analysis was carried out on a Thermo Scientific Flash 2000 analyzer. Thermogravimetric analysis was performed using a Mettler Toledo thermal analysis system at a heating rate of 5 $^{\circ}$C per minute under nitrogen atmosphere. Field emission scanning electron microscopy images were recorded on a JEOL 7600F microscope after gold coating. Transmission electron microscopy images were recorded on a JEOL JEM-2100 instrument at an accelerating voltage of 200 kV. Circular dichroism spectra were recorded using a JASCO J-1500 spectrometer. Rheological measurements were performed using an Anton Paar Physica MCR 302 rheometer.

Preparation of Zn-histidine metallogel

A solution of L- or D-histidine (100 mM) was prepared in water by the dropwise addition of 1M NaOH solution, until the pH of the solution reached to 12.3. Similarly, a solution of $Zn(NO_3)_2.6H_2O$, $Zn(CH_3COO)_2.2H_2O$ or $ZnCl_2$ (100 mM) were prepared in water. 1.0 mL of the zinc salt was added to the above prepared histidine solution, which led to the spontaneous formation of self-standing metallo-hydrogel.

Interaction of L-histidine with Zn^{2+} ions in mixed solvent systems

For the interaction of Zn^{2+} ions with histidine in mixed solvent systems, L-histidine solution (100 mM) was prepared in water and a solution of $Zn(NO_3)_2$ (100 mM) was prepared in methanol or acetonitrile. The interaction was studied by adding the Zn^{2+} solution (0.8 mL) (in MeOH or CH_3CN) to an aqueous solution (0.8 mL) of L-histidine, which resulted in the formation of a white precipitate.

Stimuli responsive studies of the Zn-histidine metallogel

Stimuli responsive nature of the Zn-histidine metallogel was studied by subjecting the gel to different stimuli. All the stimuli responsive studies were performed using 1.4 mL of the Zn-L-histidine gel.

The thermo-responsive nature of the gel was studied by heating the vial containing the gel at a temperature of 135 ^oC for 20 minutes, when the gel transformed into a sol.

The reversible pH responsive nature of the gel was studied by adding 100 µL of 1M HNO_3 to the gel, which led to the formation of a clear solution within 5 minutes. To this clear solution, an equivalent amount of 1M NaOH solution was added, which led to the recovery of the gel.

The chemoresponsive nature of the gel was studied by addition of EDTA, Na_2S and TFA to the gel taken in a vial prepared by the addition of 0.7 mL of $Zn(NO_3)_2$ to 0.7 mL of L-histidine.

20.45 mg of EDTA was spread over the gel in the vial, which led to the formation of clear solution within 2 minutes. Similarly, 5.45 mg of Na_2S was added to the gel, which led to the collapse of the gel. The addition of 100 µL of 1M TFA to the gel immediately led to a clear solution. The addition of an equivalent amount of NH_3 to this clear solution led to the reformation of the gel state.

Transmission electron microscopic studies

Samples for electron microscopic studies were prepared by taking a small amount of the gel in an eppendorf tube. The gel (15 mM) was diluted using water. Then the sample was drop-casted on a carbon coated copper grid for TEM analysis a followed by room temperature drying. EDAX spectrum was recorded using a Bruker X Flash 6130 EDAX instrument attached to the TEM

Circular dichroism studies

Circular dichroism spectra were recorded using a quartz cell of 1 mm pathlength and data pitch 0.1 nm with a scanning speed of 50 nm/min. The concentrations of the amino acids were 2.5 mM. For the CD studies of the gels, a weak gel with a concentration of 25 mM (both the reactants) was first prepared and then diluted ten times in water to get a concentration of 2.5 mM. Each spectrum is the average of two consecutive scans.

Job's plot was obtained by following the CD signal of the Zn-L-histidine complex at 217 nm. L-histidine and $Zn(NO_3)_2$ with a concentration of 2.5 mM were prepared and mixed with varying mole fractions of both the reactants such that the final volume of the solution was 1.0 mL.

Rheological studies

Rheological investigations were performed on the Zn-histidine metallogel by using parallel plate geometry of diameter 50 mm (PP-50). The hydrogel was placed on the plate of the rheometer using a microspatula. The temperature was maintained at 25 ^{o}C using a peltier temperature controller attached with Julabo chillar. The dynamic strain sweep experiments were performed using a constant frequency of 10 rad s^{-1}. The frequency sweep experiments were performed using a constant strain of 0.1% in the frequency range from 0.05-200 rad s^{-1}. For time sweep experiment, Zn and histidine were mixed onto rheometer plate and G', G'' were monitored with time using a constant strain of 0.1% and angular frequency 10 rad s^{-1}. Thixotropic oscillatory rheology for self-healing behavior was performed by applying alternating cycles of 0.1% and 100% strain with an angular frequency of 10 rad s^{-1}.

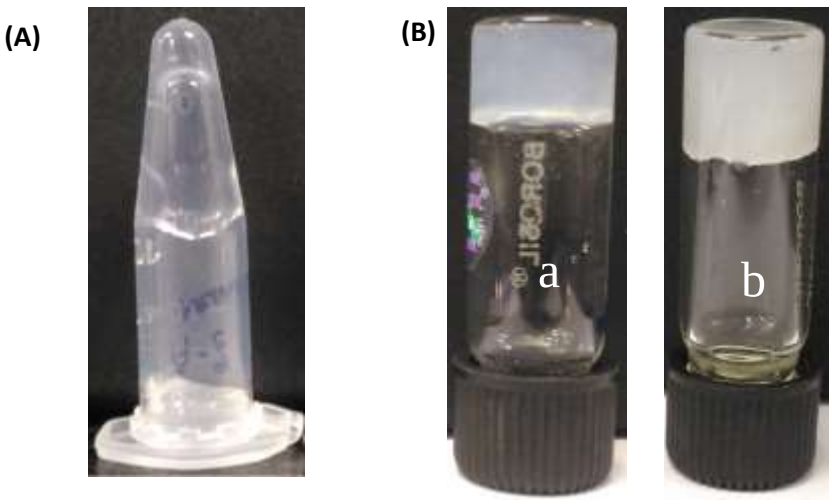

Figure S1. (A) Digital image of the clear solution obtained upon the addition of $Zn(NO_3)_2$ to an aqueous neutral solution of L-histidine. (B) Digital images of the hydrogel formed from $Zn(NO_3)_2$ and an alkaline solution of L-histidine prepared using (a) NaOH and (b) NH_3, showing that change of base does not affect gelation.

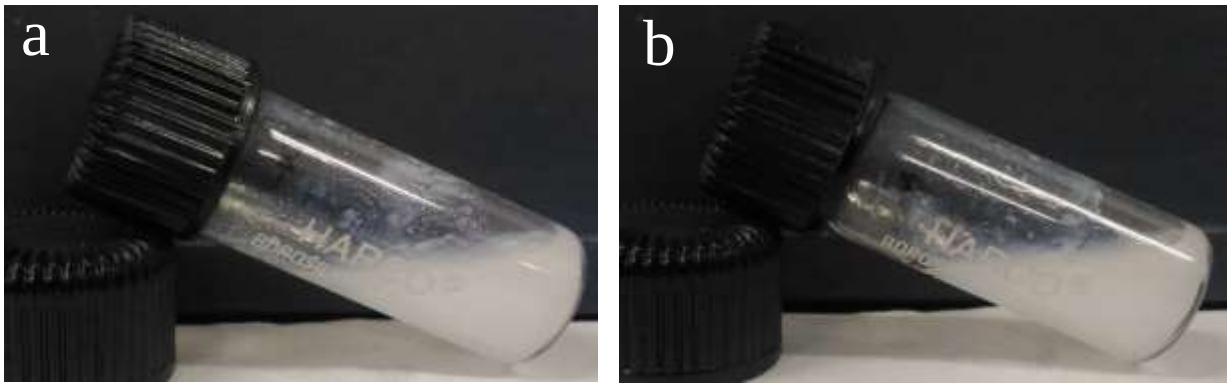

Figure S2. Digital images of the precipitate obtained upon the addition of $Zn(NO_3)_2$ in (a) methanol and (b) acetonitrile to an aqueous alkaline solution of *L*-histidine.

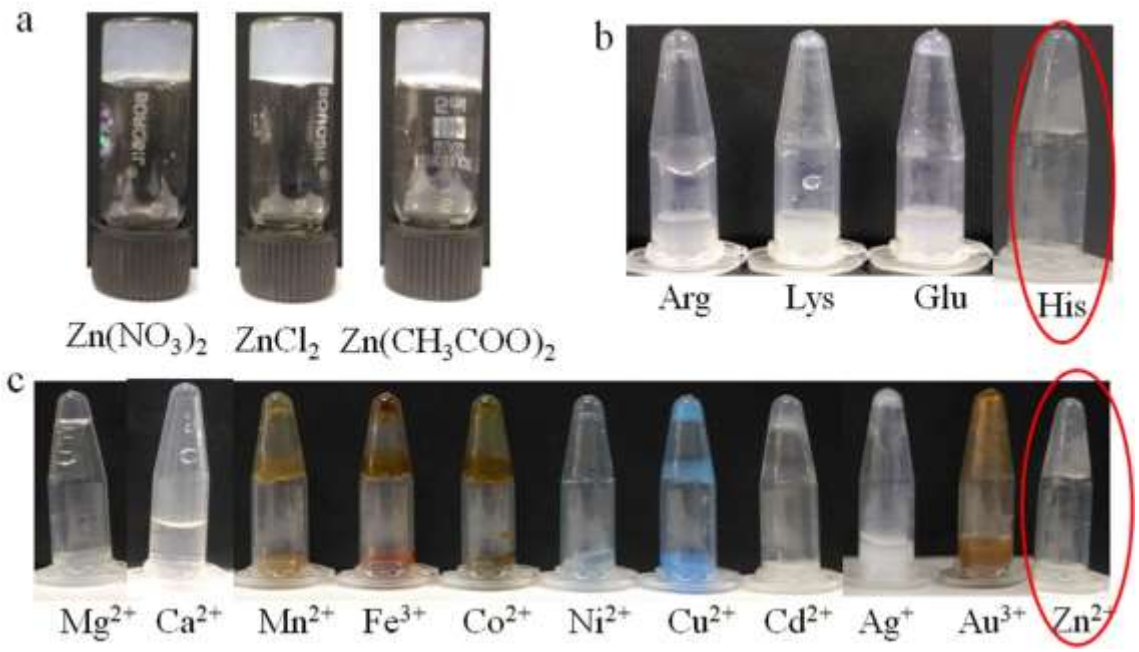

Figure S3. Digital images showing (a) the formation of stable gels upon changing of metal counterions, (b) the formation of precipitates upon the interaction of arginine, lysine and glutamic acid with Zn^{2+} ions and (c) the specificity of Zn^{2+} ions towards the formation of hydrogel upon interaction with histidine.

Table S1. Interaction of *L*-histidine with Zn^{2+} ions with varying molar ratio of metal ion: ligand.

Histidine (M)	**$Zn(NO_3)_2$ (M)**	**Result**
0.1	0.1	Stable gel
0.1	0.05	Slightly turbid solution
0.1	0.025	Clear solution
0.1	0.01	Clear solution
0.05	0.1	Weak gel
0.025	0.1	Clear solution
0.01	0.1	Clear solution
0.05	0.05	Stable gel

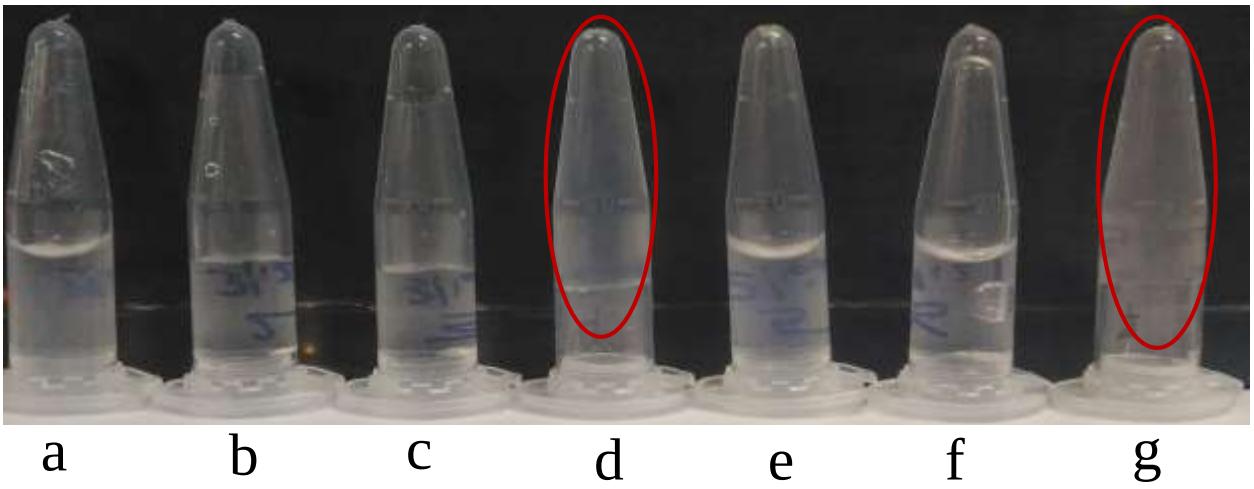

Figure S4. Digital images of the turbid and clear solutions as well as gels formed with varying molar ratios of L-histidine:Zn^{2+} ions, (a) 0.1M: 0.05M, (b) 0.1M: 0.025M, (c) 0.1M: 0.01M, (d) 0.05M: 0.1M, (e) 0.025M: 0.1M, (f) 0.01M: 0.1M, (g) 0.05M: 0.05M. (Red circles indicate the concentrations at which gel formation occurred).

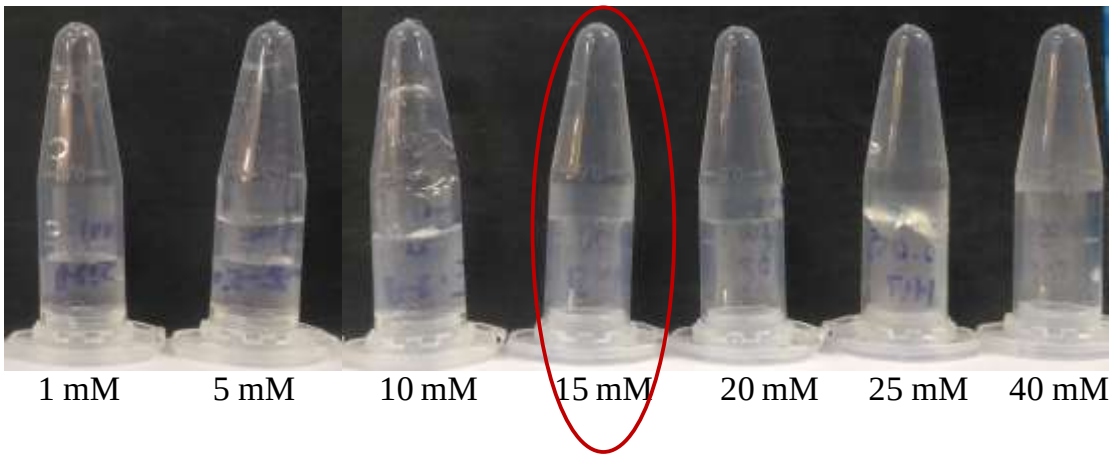

Figure S5. Digital images showing the interaction of Zn^{2+} with *L*-histidine at different molar concentrations of the reactants. The minimum gelation concentration was found to be 15 mM.

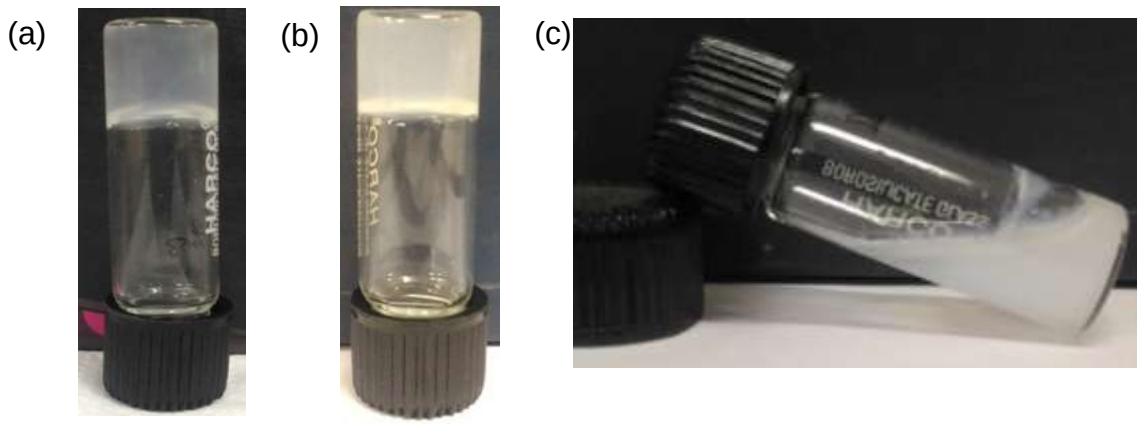

Figure S6. Digital image of the hydrogel and turbid sol formed upon addition of Zn^{2+} ions to (a) *L*-Histidine, (b) *D*-Histidine and (c) a 1:1 racemic mixture of histidine (*DL*-histidine).

Figure S7. Digital images of the precipitates and hydrogels formed by histidine and Zn^{2+} ions with varying ee % of *D*-histidine. Partial gel formed upon addition of Zn^{2+} ions to a 30% ee solution of *D*-histidine.

Table S2. Control of gelation by varying the enantiomeric excess of *L*- or *D*- forms of histidine. Final concentration was 100 mM of Zn(II) and 100 mM of Histidine

L- Histidine (0.2M)	**D-Histidine (0.2M)**	**$Zn(NO_3)_{2.6}H_2O$ (0.2M)**	**ee (%)**	**Result**
0.5 ml	0 mL	0.5 mL	100 (L)	Gel
0.437 mL	0.063 mL	0.5 mL	75 (L)	Gel
0.375 mL	0.125 mL	0.5 mL	50 (L)	Weak gel
0.312 mL	0.188 mL	0.5 mL	25 (L)	Turbid Sol
0.275 mL	0.225 mL	0.5 mL	10 (L)	Turbid Sol
0 ml	0.5 mL	0.5 mL	100 (D)	Gel
0.063 mL	0.437 mL	0.5 mL	75 (D)	Gel
0.125 mL	0.375 mL	0.5 mL	50 (D)	Weak gel
0.188 mL	0.312 mL	0.5 mL	25 (D)	Turbid Sol
0.225 mL	0.275 mL	0.5 mL	10 (D)	Turbid Sol

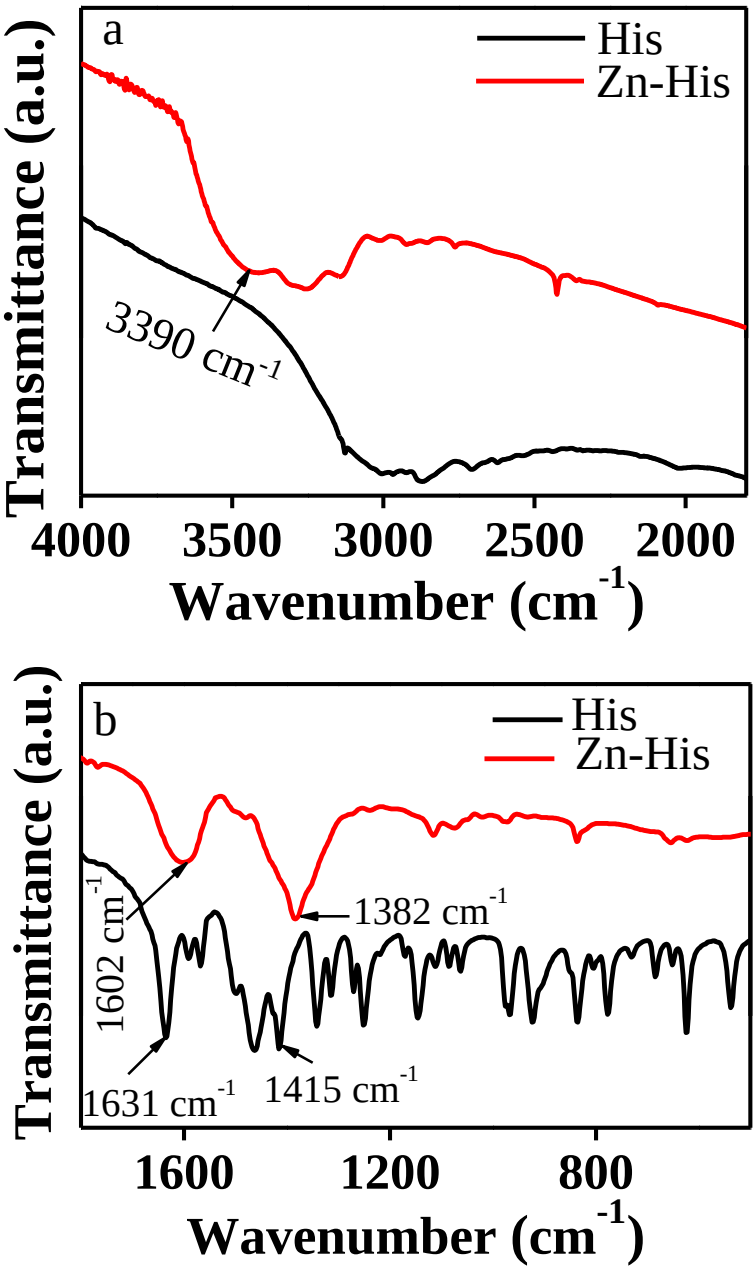

Figure S8. FTIR spectrum of *L*-histidine and Zn-*L*-histidine xerogel in (a) 4000-1800 cm^{-1} region and (b) 1800-400 cm^{-1} region respectively.

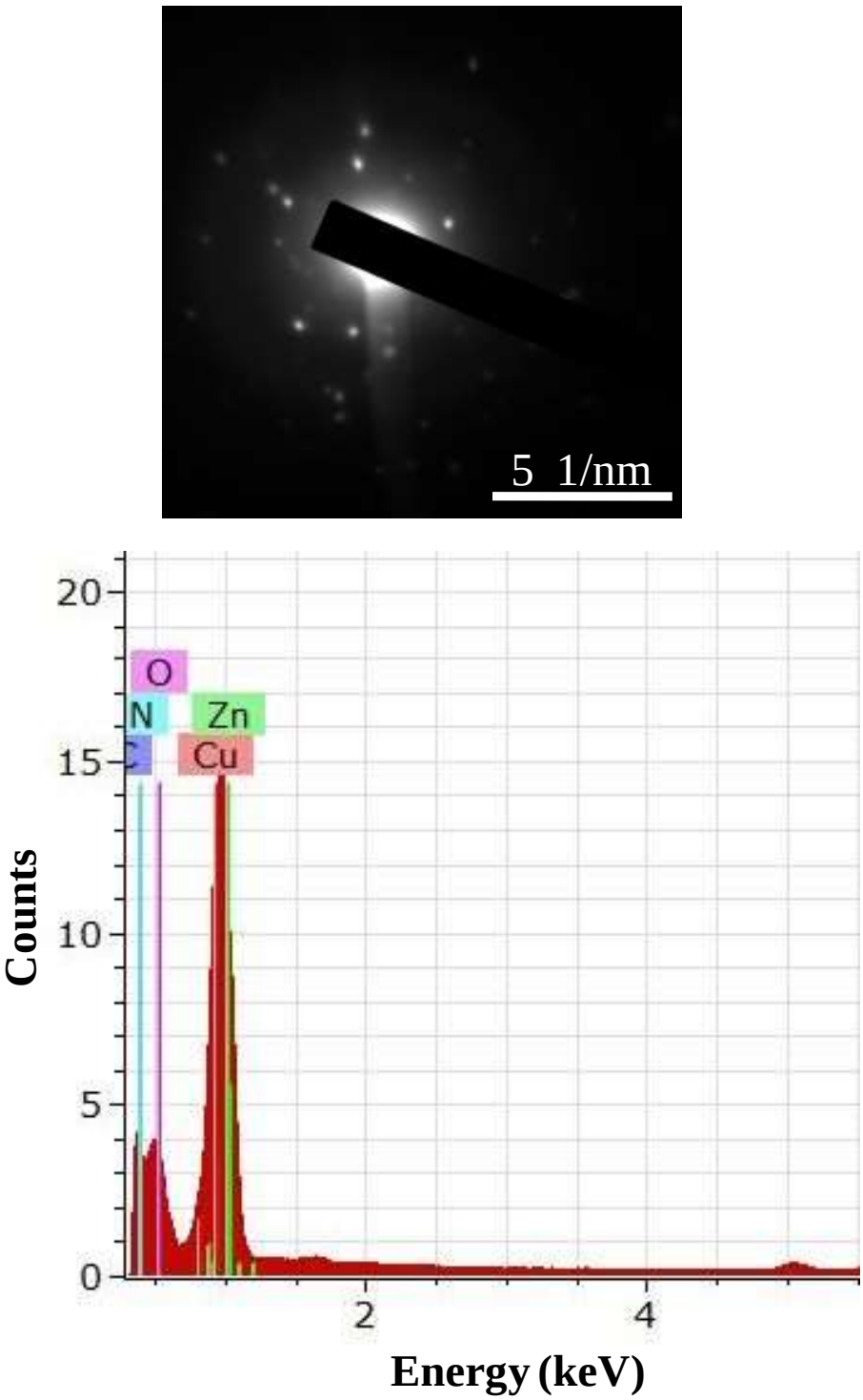

Figure S9. (A) Selected area electron diffraction (SAED) pattern of the Zn-L-histidine gel fibers. (B) EDX spectrum of the Zn-histidine hydrogel, showing the presence of C, N, O and Zn. (Cu indicated in the spectrum is due to the copper TEM grid).

Table S3. Experimental and theoretical elemental composition of the [Zn(His)(H_2O)$_3$(OH)] complex formed upon the interaction of Zn^{2+} ions to aqueous alkaline histidine solution.

	C (%)	N (%)	H (%)
Experimental	25.06	14.56	3.94
Theoretical	24.80	14.47	5.11

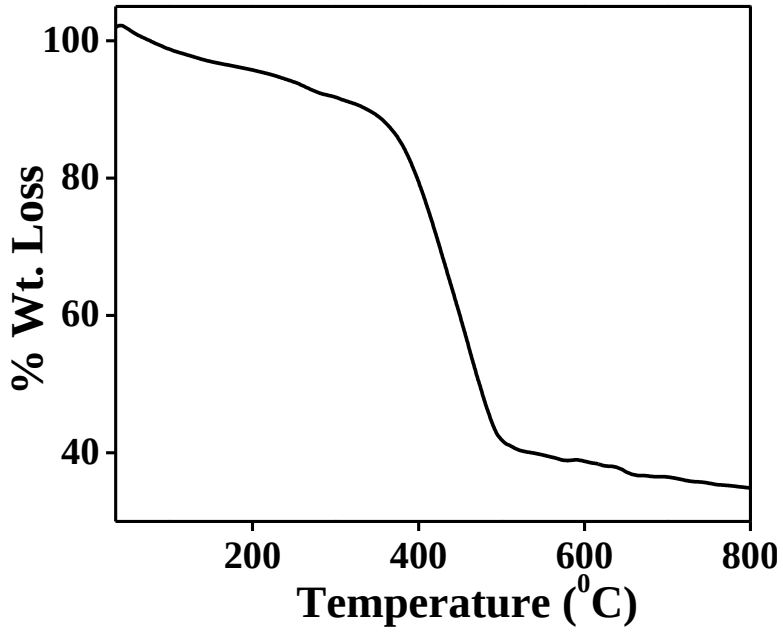

Figure S10. TGA analysis of the freeze dried Zn-*L*-Histidine metallogel.

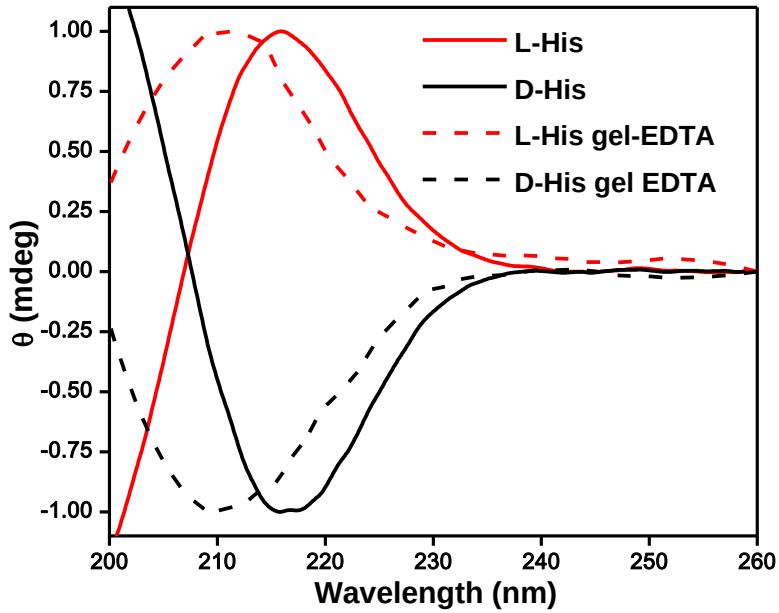

Figure S11. CD spectrum of the sol obtained after addition of EDTA to *L*-histidine-Zn gel and *D*-histidine-Zn gel, showing signals similar to pure *L*- and *D*-histidine respectively with slight blue shift.

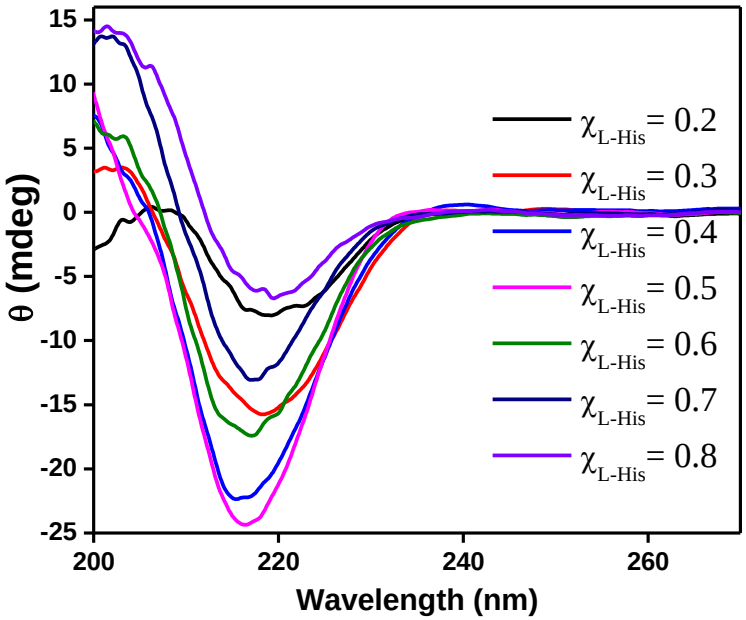

Figure S12. CD spectra of the supramolecular assembly with varying mole fraction of L-histidine to form Zn-*L*-Histidine.

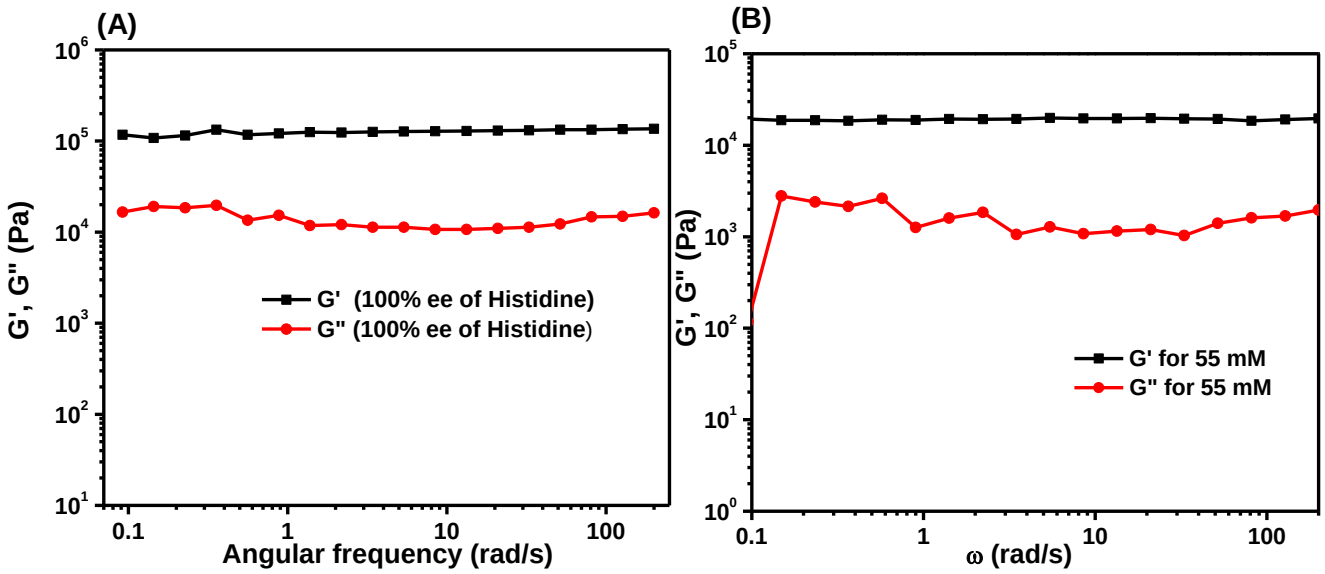

Figure S13. Dynamic frequency sweep rheological investigation of the Zn-*L*-histidine metallogel (1:1 complex) at a constant strain of 0.1% (A) 100 mM) (B) 55 mM.

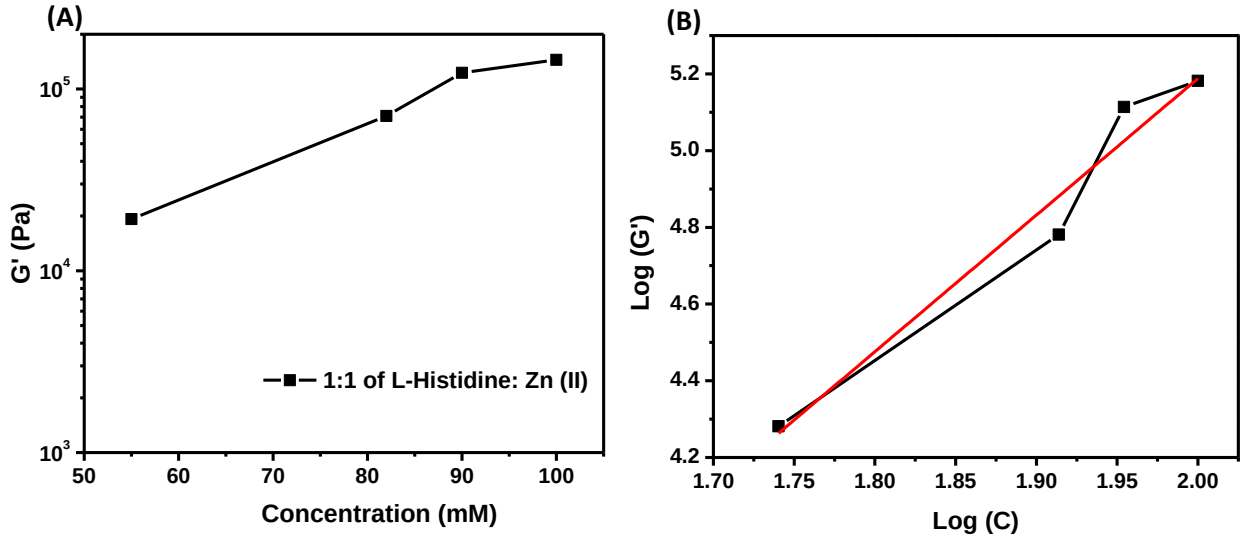

Figure S14. (A) Plot of G' with concentration of Zn-*L*-Histidine in 1:1 ratio for 100, 90, 82, 55 mM. (B) Logarithmic plot of Log G' and Log C with linear fit having slope of 3.5.

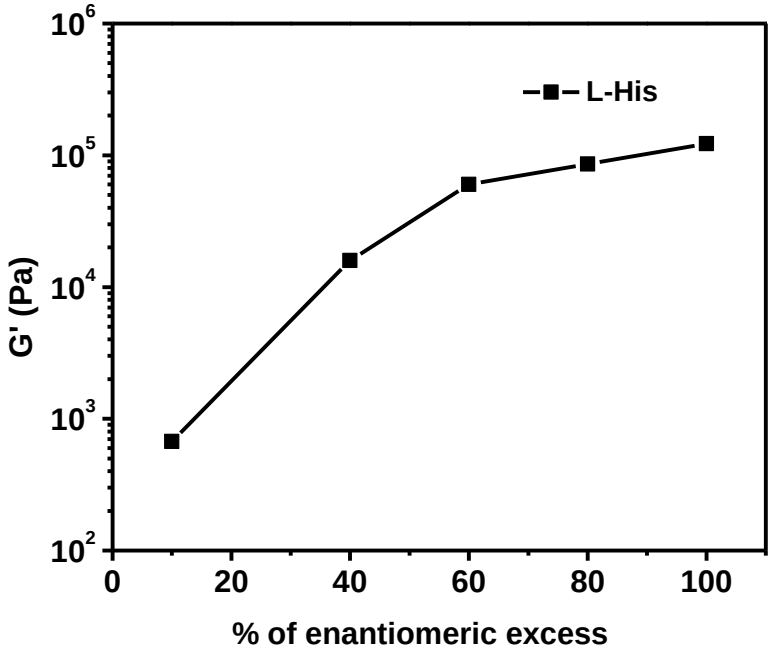

Figure S15. Plot of G' with enantiomeric excess of 1:1 ratio of Zn-*L*-Histidine (100 mM of Zn and mixture of *L*-Histidine and *D*-Histidine).

Figure S16. Digital image of the self-healed single block of Zn-histidine prepared from four single gel blocks held horizontally.

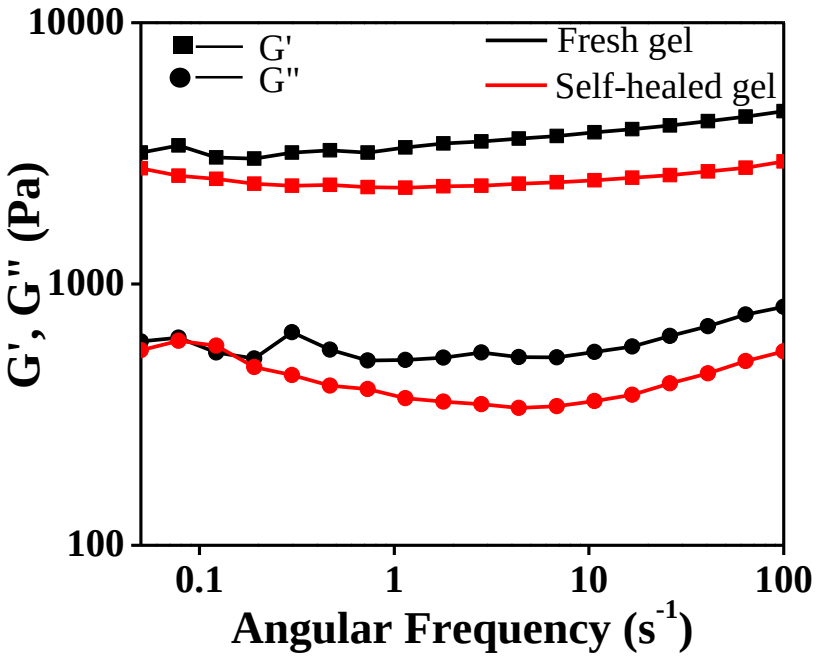

Figure S17. Frequency sweep rheological investigation of fresh Zn-*L*-His gel and self-healed Zn-*L*-His gel.