

Supplementary Information

Heteropoly acids-driven assembly of glutathione into redox-responsive underwater adhesive

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1. Materials

Oxidized glutathione (GSSG) and reduced glutathione (GSH) were purchased from Aladdin GmbH and used without further purification. $\text{H}_4\text{SiW}_{12}\text{O}_{40}$ (HSiW, 2878.17 g/mol), $\text{H}_3\text{PW}_{12}\text{O}_{40}$ (HPW, 2880.05 g/mol), $\text{H}_4\text{SiMo}_{12}\text{O}_{40}$ (HSiMo, 1823.0 g/mol) were commercial sources from Sinopharm Chemical Reagent Co., Ltd and used without further purification. $\text{H}_6\text{P}_2\text{W}_{18}\text{O}_{62}$ (HP_2W_{18} , 4369.62 g/mol) was prepared in line with a reported method.¹ Tris(2-carboxyethyl) phosphine was purchased Aladdin GmbH. The solvent is deionized water, which was obtained from a reverse osmosis drinking water system at a resistivity of 18.25 M Ω ·cm.

[1] C. Kato, S. Nishihara, R. Tsunashima, Y. Tatewaki, S. Okada, X. M. Ren, K. Inoue, D. L. Long, L. Cronin, *Dalton Trans.* 2013, **42**, 11363.

2. Preparation of underwater adhesives

The synthetic procedure of adhesives is extremely simple that wet adhesives immediately formed in aqueous solution by mixing glutathione solution with heteropoly acids solution at room temperature.

GSSG/HPW adhesive: The underwater adhesive was fabricated through mixing GSSG (306.4 mg, 2 mL) with HPW (960 mg, 0.4 mL) in aqueous solution with the initial charge ratio of 1:1 between GSSG to HPW, then, the mixture was homogeneous mixed using vortex stirrer. The HPW is a strong inorganic acid and its aqueous solution shows very low pH value (pH < 1), thus the final pH of the mixed solution automatically locates at pH < 1.9. NaOH solution is utilized to precisely control the pH value at 1.9. Subsequently, the mixture was heated to 50 °C and cooled down to

room temperature (25 °C). The obtained yellow GSSG/HPW adhesive was washed with deionized water for three times and dried under vacuum. Anal. Calcd for GSSG/HPW: C, 9.93; H, 1.51; N, 3.45. Found: C, 9.88; H, 1.48; N, 3.38. Thermogravimetric analysis (TGA) suggests a mass loss of 1.74 % from 30 to 180 °C, which arises from crystal water in GSSG/HPW. Based on the results of TGA and elemental analysis, GSSG/HPW should correspond to an average formula: $H(C_{20}H_{32}N_6O_{12}S_2)_{1.5}PW_{12}O_{40}(H_2O)_4$.

GSSG/HSiW adhesive: The underwater adhesive was fabricated through mixing GSSG (306.4 mg, 2 mL) with HSiW (720 mg, 0.4 mL) in aqueous solution with the initial charge ratio of 1:1 between GSSG to HSiW, then, the mixture was homogeneous mixed using vortex stirrer. Subsequently, the mixture was heated to 50 °C and cooled down to room temperature (25 °C). The obtained yellow GSSG/HSiW adhesive was washed with deionized water for three times and dried under vacuum. Anal. Calcd for GSSG/HSiW: C, 10.72; H, 1.63; N, 3.72. Found: C, 10.77; H, 1.68; N, 3.77. Thermogravimetric analysis (TGA) suggests a mass loss of 2.68 % from 30 to 180 °C, which arises from crystal water in GSSG/HSiW. Based on the results of TGA and elemental analysis, GSSG/HSiW should correspond to an average formula: $H_{0.2}(C_{20}H_{32}N_6O_{12}S_2)_{1.9}SiW_{12}O_{40}(H_2O)_6$.

GSSG/HP₂W₁₈ adhesive: The underwater adhesive was fabricated through mixing GSSG (306.4 mg, 2 mL) with HP₂W₁₈ (383.2 mg, 0.4 mL) in aqueous solution with the initial charge ratio of 1:1 between GSSG to HP₂W₁₈, then, the mixture was homogeneous mixed using vortex stirrer. Subsequently, the mixture was heated to 50 °C and cooled down to room temperature (25 °C). The obtained yellow GSSG/HP₂W₁₈ adhesive was washed with deionized water for three times and dried under vacuum. Anal. Calcd for GSSG/HP₂W₁₈: C, 10.05; H, 1.66; N, 3.52. Found: C, 9.88; H, 1.60; N, 3.48. Thermogravimetric analysis (TGA) suggests a mass loss of 0.16 % from 30 to 180 °C, which arises from crystal water in GSSG/HP₂W₁₈. Based on the results of TGA and elemental analysis, GSSG/HP₂W₁₈ should correspond to an average formula: $H_{0.6}(C_{20}H_{32}N_6O_{12}S_2)_{2.6}P_2W_{18}O_{62}(H_2O)_2$.

GSSG/HSiMo adhesive: The underwater adhesive was fabricated through mixing GSSG (306.4 mg, 2 mL) with HSiMo (456 mg, 0.4 mL) in aqueous solution with the initial charge ratio of 1:1 between GSSG to HSiMo, then, the mixture was homogeneous mixed using vortex stirrer. Subsequently, the mixture was heated to 50 °C and cooled down to room temperature (25 °C). The

obtained yellow GSSG/HSiMo adhesive was washed with deionized water for three times and dried under vacuum. Anal. Calcd for GSSG/HSiMo: C, 15.57; H, 2.35; N, 5.45. Found: C, 15.88; H, 2.44; N, 5.89. Thermogravimetric analysis (TGA) suggests a mass loss of 1.12 % from 30 to 180 °C, which arises from crystal water in GSSG/HSiMo. Based on the results of TGA and elemental analysis, GSSG/HSiMo should correspond to an average formula: $H(C_{20}H_{32}N_6O_{12}S_2)_2SiMo_{12}O_{40}(H_2O)_4$.

GSH/HPW coacervate: The complex coacervate was fabricated through mixing GSH (307.4 mg, 2 mL) with HPW (960 mg, 0.4 mL) in aqueous solution with the initial charge ratio of 1:1 between GSH to HPW, then, the yellow mixture was homogeneous mixed using vortex stirrer. Subsequently, the mixture was heated to 50 °C and cooled down to room temperature (25 °C). The obtained yellow GSH/HPW coacervate was washed with deionized water for three times and dried under vacuum. Anal. Calcd for GSH/HPW: C, 9.93; H, 1.51; N, 3.45. Found: C, 9.88; H, 1.48; N, 3.38. Thermogravimetric analysis (TGA) suggests a mass loss of 1.85 % from 30 to 180 °C, which arises from crystal water in GSH/HPW. Based on the results of TGA and elemental analysis, GSH/HPW should correspond to an average formula: $H_{1.3}(C_{10}H_{17}N_3O_6S)_{1.7}PW_{12}O_{40}(H_2O)_4$.

The redox behavior of glutathione

Oxidation reaction: The complex coacervate was fabricated through mixing GSH (307.4 mg, 2 mL) with HPW (960 mg, 0.4 mL) in aqueous solution at room temperature, subsequently, gradually adding hydrogen peroxide (30 %, 226 mg) into complex coacervate. After ten minutes, faint yellow adhesive was obtained. The lyophilized faint yellow powder was further measured to investigate the formation of disulfide bonds.

Reduction reaction: The underwater adhesive was obtained through mixing GSSG (306.4 mg, 2 mL) with HPW (960 mg, 0.4 mL) in aqueous solution which the mixture was homogeneous mixed using vortex stirrer. Subsequently, the mixture was heated to 50 °C and cooled down to room temperature (25 °C), the yellow wet adhesive was obtained. The TCEP (Tris(2-carboxyethyl) phosphine) was added to adhesive which molar ratio of GSSG to TCEP was kept at 1 : 6, the complex transformed from yellow gel to faint yellow flow state under vortex stirrer after twenty minutes.

3. Measurements

Fourier Transform Infrared (FT-IR). FT-IR spectra of all the lyophilized powder samples were

measured on a Bruker Optics Vertex 80 V FT-IR spectrometer equipped with a DTGS detector (32 scans) with a resolution of 4 cm^{-1} using KBr pellets.

X-ray photoelectron spectroscopy (XPS). X-ray photoelectron spectroscopy was performed on an ESCACAB Mark (VG Company, UK) 250 spectrometer with a mono-chromic X-ray source (Al-Ka, 1489.6 eV) and the binding energy of C (1s) at 284.6 eV was taken as a standard.

Thermogravimetric analysis (TGA): TGA was performed on a Q500 Thermal Analyzer (New Castle TA Instruments) using high purity nitrogen as the carrier gas with a heating rate of $10\text{ }^{\circ}\text{C min}^{-1}$ in the temperature range of 30-900 $^{\circ}\text{C}$.

Elemental analysis (EA): Elemental analysis (C, H, N) was carried out on an Elementar vario MICRO cube (Germany). The final average formula was calculated on the basis of five replicate results.

Zeta potential measurements: The zeta potential values were measured using Malvern Zetasizer NanoZS instrument at room temperature. The diluted sample of the adhesive sample was utilized, and the measurements were repeated a minimum of three times.

Rheology measurement: The rheology properties of the GSSG/HPW adhesive were characterized at $25\text{ }^{\circ}\text{C}$ using a TA instrument AR2000 controlled-stress rheometer equipped with 8 mm stainless steel parallel plate geometry. A gap distance of 0.7 mm was used for all experiments. Oscillatory frequency sweep data were collected in the range from 0.1 to 100 rad/s, and the strain amplitude was fixed at 1%, which was based on the linear strain amplitude response regime. The self-healing property of the adhesive was monitored as follows: the network structure of GSSG/HPW was first broken using 100% strain amplitude, then tracking the recovery in G' over time by continuing to oscillate the adhesive at low (1%) strain amplitude.

Lap shear adhesion testing: The adhesion strength was performed on an Instron 5944 materials testing system. The adhesive samples were first compressed between two same substrates, such as glasses, titanium, polypropylene (PP) and polycarbonate (PC), to create a lap shear joint. Then, the adhesive application was performed by applying a compressive pressure of 35 kPa, and a contact time of 40 min. After that, the adherends were pulled apart immediately by a material testing system equipped with a deionized water holder. The force as a function of displacement was recorded using a 100 N load cell and a crosshead speed of 10 mm min^{-1} . The shear adhesion strength was obtained from the maximum force at joint failure divided by the overlapping contact

area. The GSSG/HPW sample was tested on the basis of five replicate measurements and averaged.

Nuclear magnetic resonance (NMR): ^1H NMR spectra were recorded on Bruker AVANCE 400 using 80 % H_2O +20 % D_2O as a solvent at 355 K. ^{31}P NMR spectra in deuterated methanol were carried out on Bruker AVANCE III 500 spectrometer at 161.8 MHz, with a 4 mm ZrO_2 rotor spinning at 15 kHz, 2048 scans, 9.0 μs pulse and 500 ms repetition time, at 25 °C. 85 % H_3PO_4 at 0 ppm was used as external standard for the chemical shift estimation.

Scanning electron microscopy (SEM): Scanning electron microscope (SEM) images were obtained from a JEOL FESEM 6700F electron microscope. The SEM specimens were prepared through depositing the heated adhesive aqueous solutions onto a silica wafer and then they were freeze-dried and air-dried, respectively.

Raman spectroscopy analysis: The Raman spectroscopy data were obtained using a Renishaw Raman system model 1000 spectrometer with a 532 nm excitation line. The solid power samples were used to investigate the transition between disulfide bonds and thiol.

Electrospray ionization mass spectra (ESI-MS): Electrospray ionization mass spectra (ESI-MS) data were acquired on a Bruker Daltonics Esquire 6000 spectrometer system. The solutions were injected directly into the evaporation chamber. The mass spectrometer was operated in the negative ion reflector mode.

4. Characterization



Fig. S1 Photo images of GSSG/HPW adhesive at hydrated and dehydrated state.



Fig. S2 Photographs of co-assembly of GSSG with H₆P₂W₁₈O₆₂ (HP₂W₁₈), H₄SiW₁₂O₄₀ (HSiW₁₂), H₄SiMo₁₂O₄₀ (HSiMo₁₂) at pH < 1, respectively.

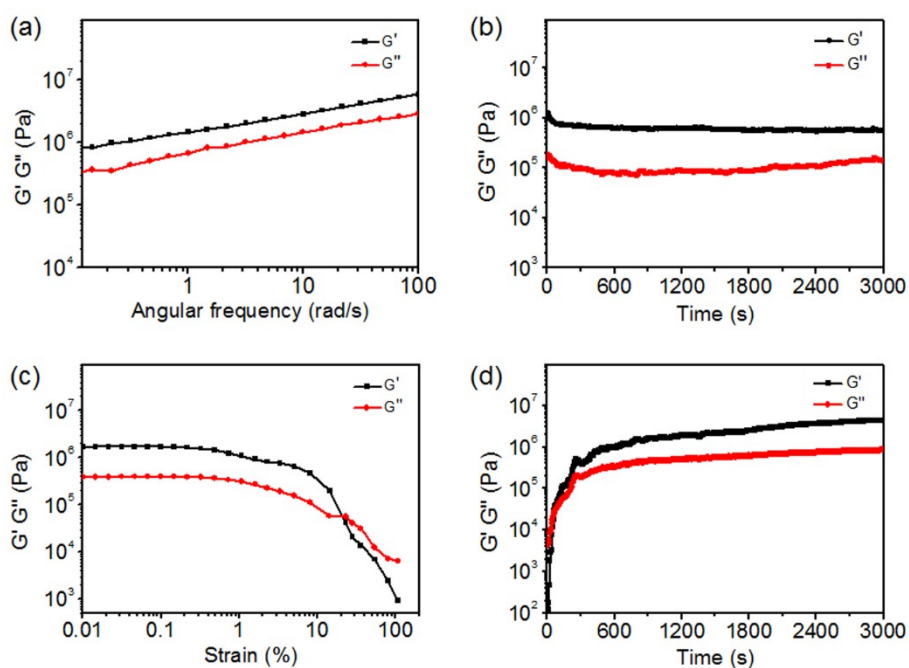


Fig. S3 (a) Frequency sweep analysis of GSSG/HPW adhesive at a constant strain of 1%. (It can be seen that the values of G' are greater than G'' over the entire frequency range, indicating the gel-like behaviour); (b) Dynamic time sweep of GSSG/HPW adhesive at 25 °C (frequency = 5 rad/s; strain = 1%, no substantial change was observed for the adhesive, indicating the good stability of the three-dimensional matrix of the adhesive); (c) Dynamic strain sweep of GSSG/HPW adhesive at the frequency of 5 rad/s; (d) Dynamic rheology data illustrating the self-healing properties of the GSSG/HPW adhesive. (The adhesive was first broken by applying a 100% strain through a strain amplitude sweep, which caused the moduli values to diminish drastically. The healing of the adhesive was then monitored over time by continuing to oscillate the adhesive at low (1%) strain amplitude, which was in the linear viscoelasticity region and did not disrupt the network. The dynamic time sweep experiment (Fig. S3b) revealed the good stability of the adhesive. We also investigated the self-healing property of the GSSG/HPW adhesive. The sample was first broken by applying a 100% strain, which caused the moduli values to diminish drastically (Fig. S3c). The healing behaviour of the adhesive was then monitored over time by continuing to oscillate the adhesive at low strain amplitude (1%). It was observed that the adhesive recovered its' original moduli values within 5 min.

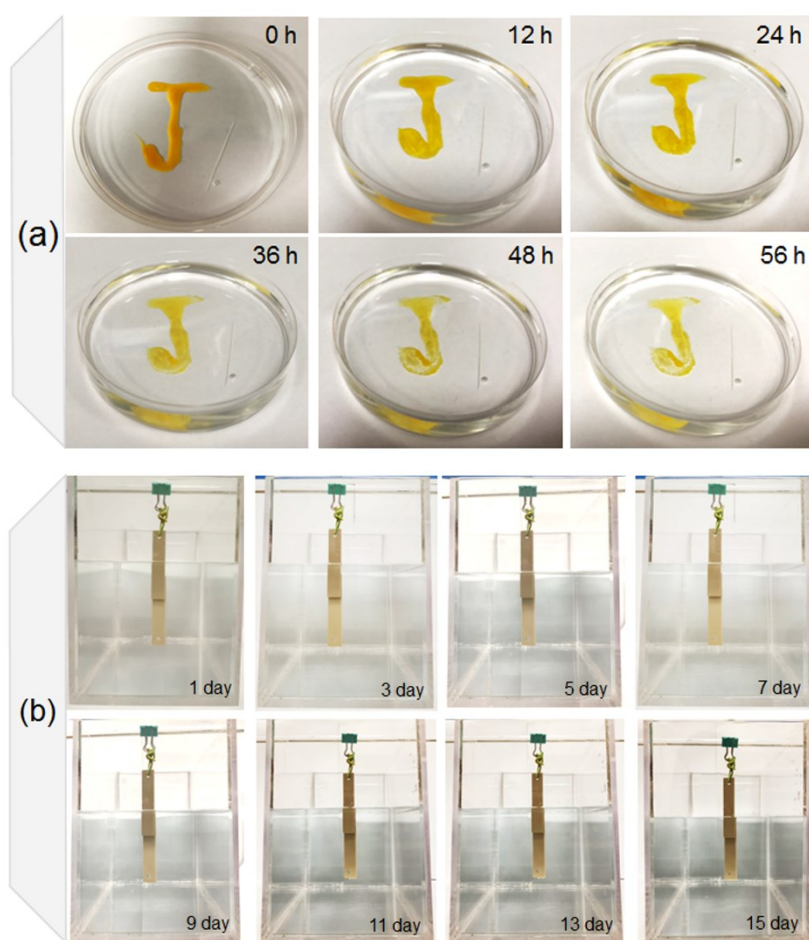


Fig. S4 (a) Digital images of the patterned GSSG/HPW coatings when exposing to aqueous solution (pH~6) with different aging time; (b) Digital images of the GSSG/HPW joint compressed between two polyether-ether-ketone substrates under water line (pH~6) with different aging time. The stability of the coating is poor when it was completely exposed to neutral aqueous solution. As shown in Fig. S4a, the morphology of the patterned adhesive coatings remained no change within 24 h under the water line (pH~6). However, gradual dissociation was observed (Fig. S4a) with further increasing the aging time. Surprisingly, the formed joint compressed between two substrates can keep 15 days in water (pH~6), implying good longevity (Fig. S4b).

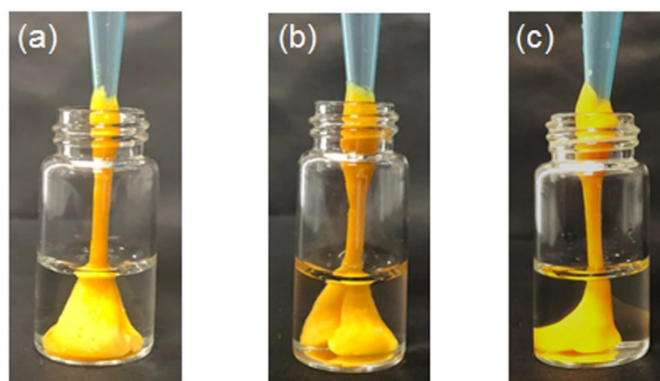


Fig. S5 Photographs of the as-prepared GSSG/HPW underwater adhesives at different NaCl concentrations: (a) 200 mM, (b) 500 mM, (c) 700 mM.

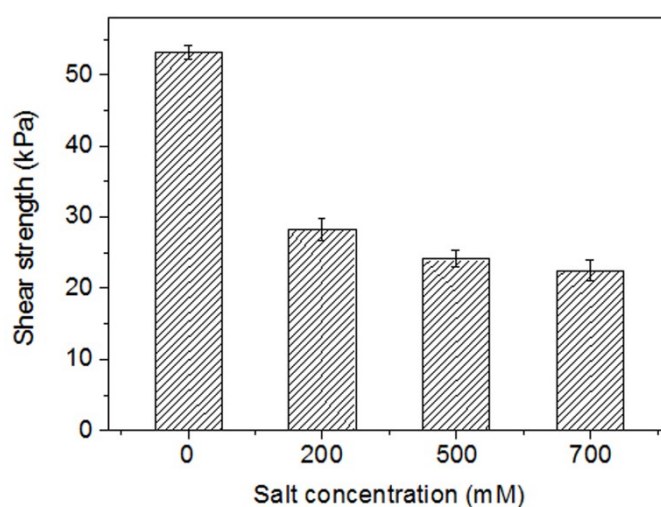


Fig. S6 Average shear strength of GSSG/HPW adhesive prepared at different NaCl concentrations (from 0 to 700 mM) bonded to titanium substrate.

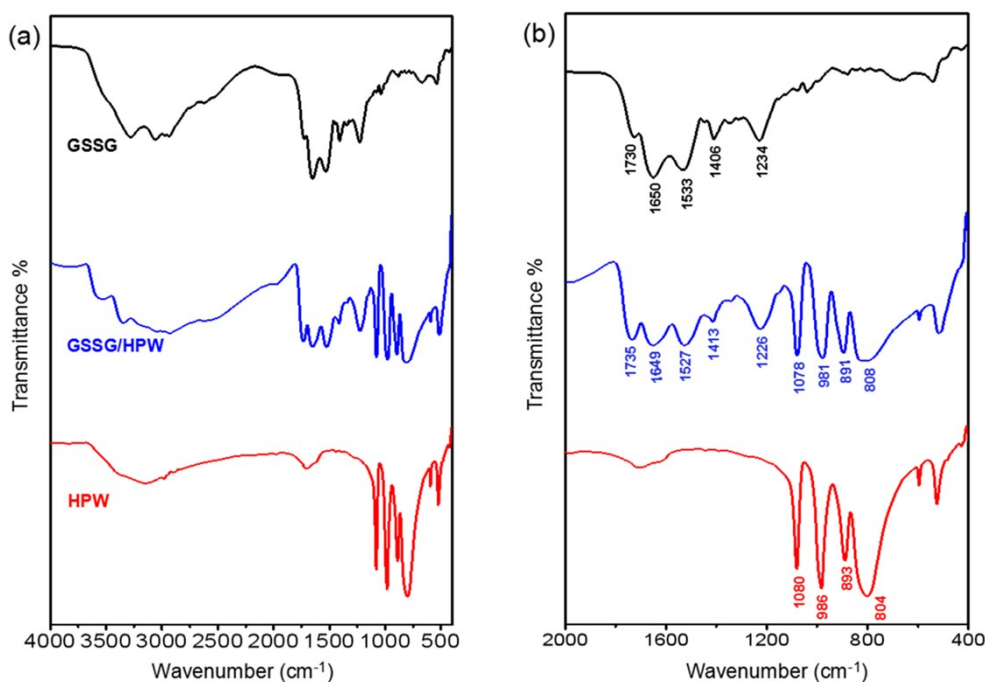


Fig. S7 (a) FT-IR spectra of HPW (red line), GSSG (black line) and the GSSG/HPW adhesive (blue line) in the range of 4000–400 cm⁻¹. (b) Enlarged FT-IR spectra in the range of 2000–400 cm⁻¹. The bands of HPW were identified by four characteristic absorption bands appearing at 1080 ($\nu_{\text{as}} \text{P-O}$), 986 ($\nu_{\text{as}} \text{W=O}$), 893 ($\nu_{\text{as}} \text{W-O}_b\text{-W}$) and 804 cm⁻¹ ($\nu_{\text{as}} \text{W-O}_c\text{-W}$), respectively. In the case of lyophilized GSSG/HPW adhesive, the stretching vibration modes of HPW shift to 1078, 981, 891, 804 cm⁻¹, respectively. Comparing with the amide I band (1650 cm⁻¹) of individual GSSG, the GSSG/HPW powder showed a similar band at 1649 cm⁻¹, demonstrating that GSSG molecules adapted random-coil conformation. The band shift arose from the electrostatic interactions between the protonated $\alpha\text{-NH}_2$ groups of GSSG and anionic HPW.

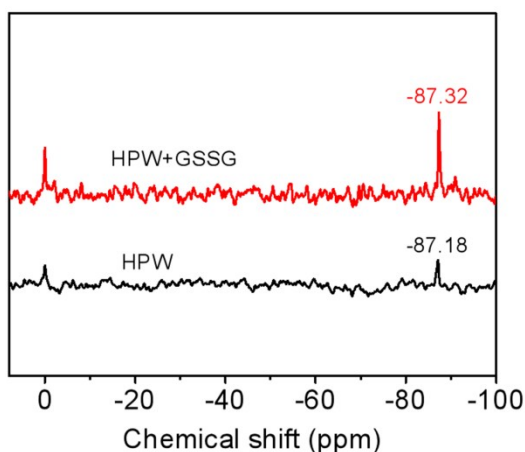


Fig. S8 ^{31}P NMR spectra of $\text{H}_3\text{PW}_{12}\text{O}_{40}$ (HPW) and GSSG/HPW adhesive in deuterated methanol. The HPW and GSSG/HPW have almost the same chemical shift in the same solvent, indicating that the topology structure of HPW keeps integrity during the ionic co-assembly process.

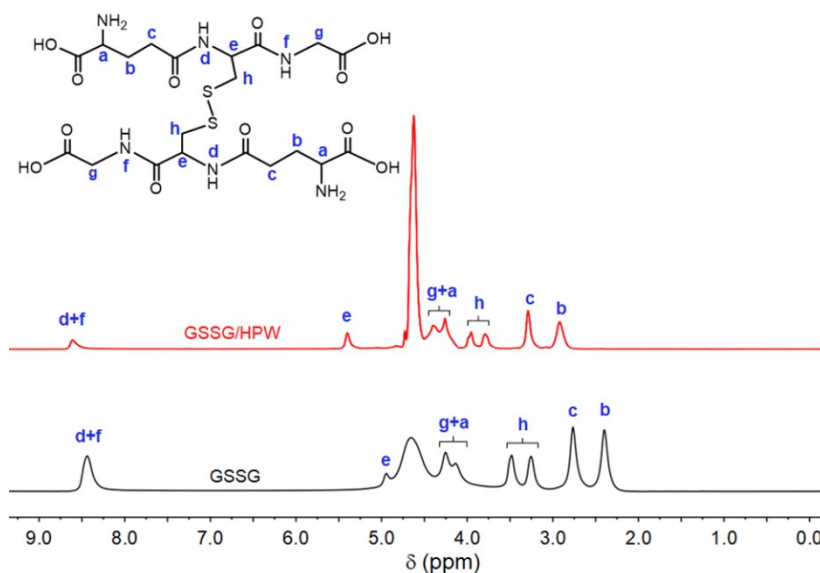


Fig. S9 ^1H NMR spectra of pure GSSG and GSSG/HPW adhesive in 80 % H_2O mixed with 20 % D_2O solvent at 355 K. The spectra revealed that the proton signals of GSSG/HPW were similar to those of individual GSSG, indicating that the chemical structure of GSSG molecule retains unchanged within the adhesive matrix.

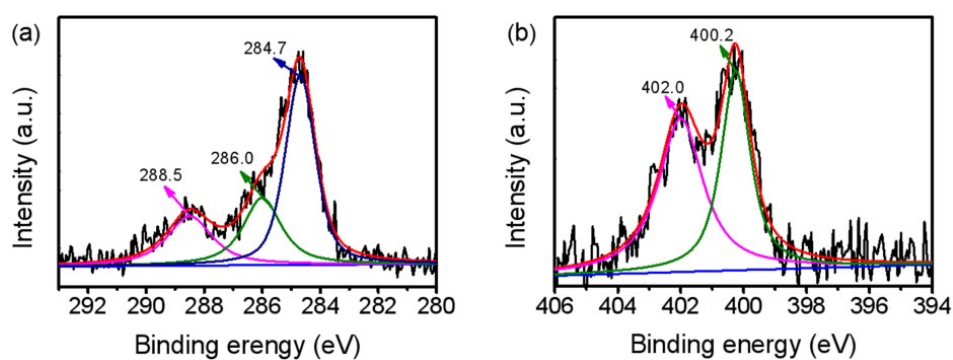


Fig. S10 XPS spectra of GSSG/HPW for the C1s level (left) and N1s level (right).

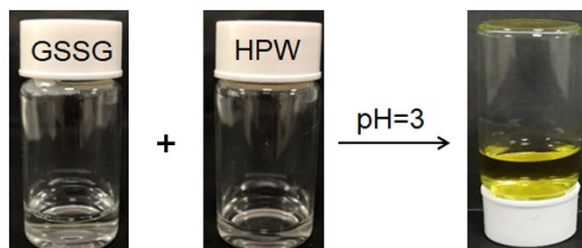


Fig. S11 Photographs of co-assembly of GSSG/HPW at pH = 3.

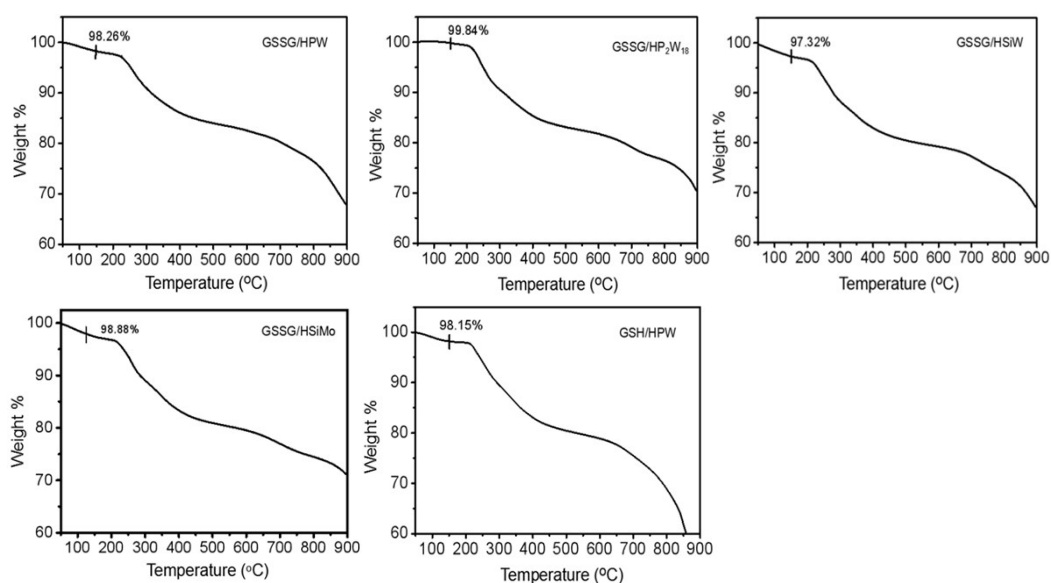


Fig. S12 TGA curves of the dried GSSG/HPW, GSSG/HP₂W₁₈, GSSG/HSiW and GSSG/HSiMo adhesives and GSH/HPW coacervate.

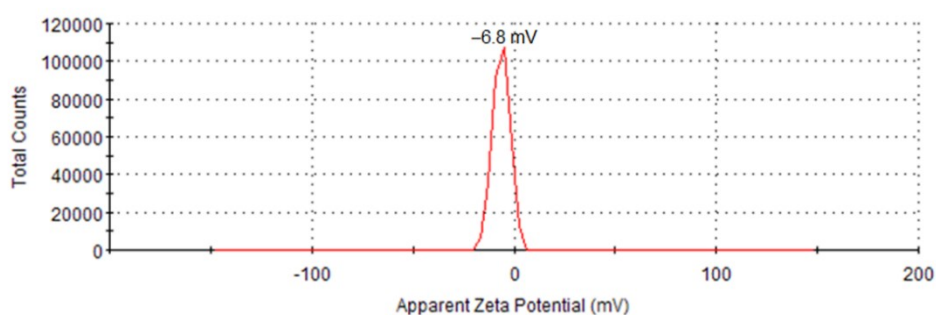


Fig. S13 Zeta potential of the diluted GSSG/HPW aqueous solution (pH = 1.9).

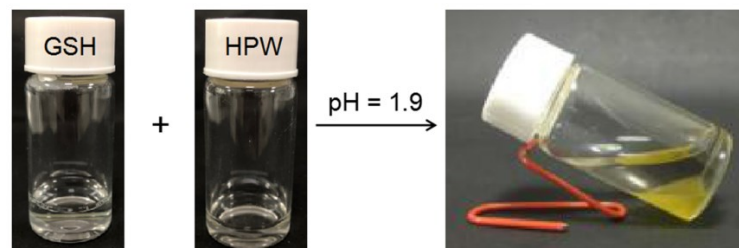


Fig. S14 Photographs of GSH/HPW coacervate at pH = 1.9

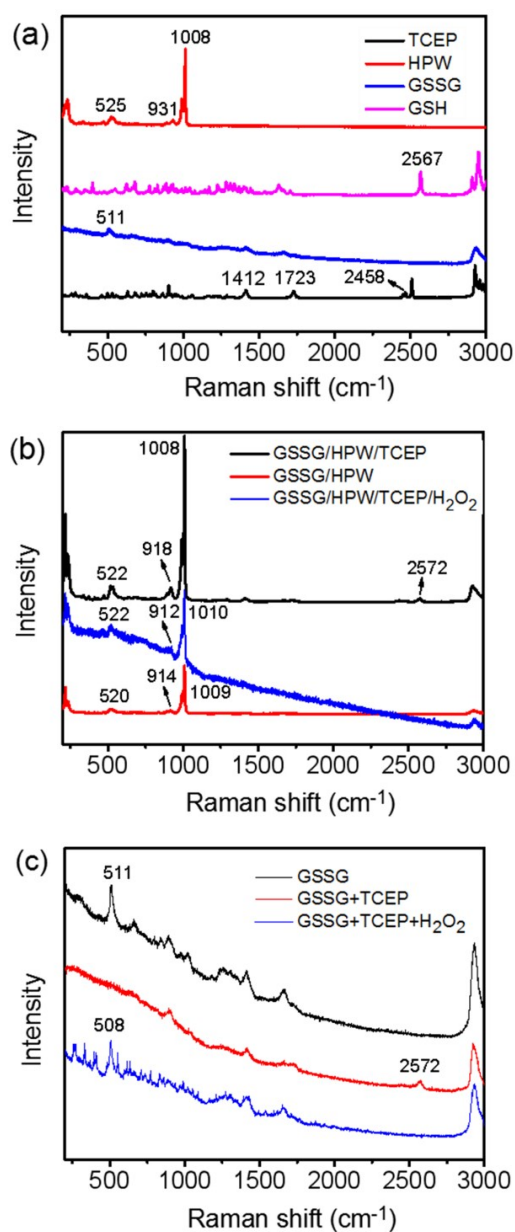


Fig. S15 Raman spectra of GSSG/HPW dynamic interactions. a) Raman spectra of alone TCEP, HPW, GSH, GSSG; b) Raman spectra of GSSG/HPW adhesive, GSSG/HPW adhesive

treated with TCEP, reduced GSSG/HPW sample treated with H_2O_2 ; c) Raman spectra of alone GSSG, after GSSG was mixed with TCEP, GSSG transformed into GSH and reduced GSSG was treated with H_2O_2 . Compared with (b), GSSG sample without HPW was treated with TCEP or H_2O_2 , we observed reversible transformation between thiol and disulfide bonds.

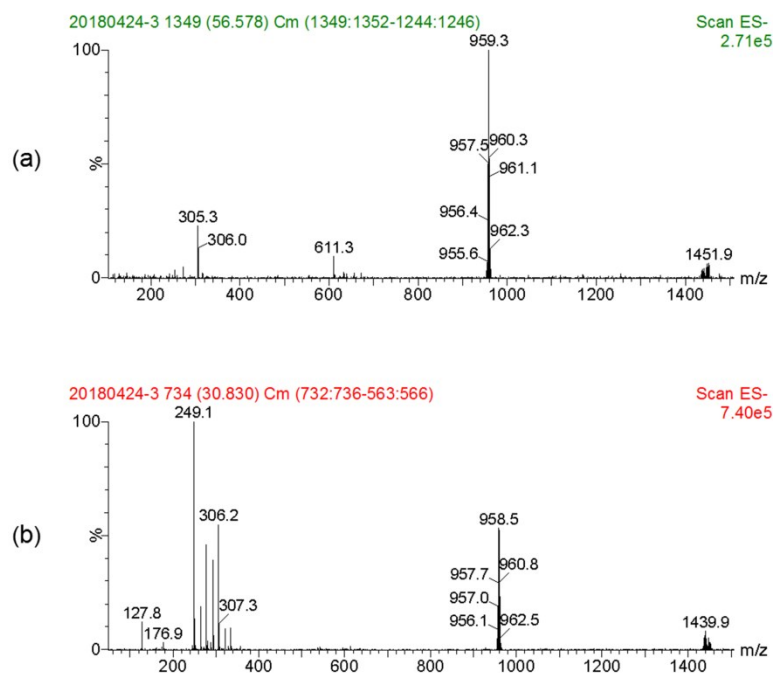


Fig. S16 ESI-MS spectra of GSSG/HPW (a) and GSSG/HPW/TCEP (b) samples with negative ion mode. (The samples were dissolved in $\text{H}_2\text{O}/\text{CH}_3\text{CH}_2\text{OH}$ mixed solution). The spectra of GSSG/HPW adhesive showed a peak at 611.3, ascribing to the mass-to-charge ratio of $[\text{GSSG}]^-$. This data indicates the presence of GSSG molecules within the adhesive matrix of GSSG/HPW. However, the peak corresponding to the GSSG (611.3) disappeared in the case of GSSG/HPW/TCEP. Instead, a peak at 306.2 corresponding to the mass-to-charge ratio of $[\text{GSH}]^-$ was observed, suggesting that the GSSG molecules were reduced to GSH by TCEP. The peaks located at 959.3 (958.5) and 1451.9 (1439.9) were ascribed to the anionic segments $[\text{PW}_{12}\text{O}_{40}]^{3-}$ and $[\text{HPW}_{12}\text{O}_{40}]^{2-}$, respectively.^[2]

[2] H. Sartzi, D.-L. Long, S. Sproules, L. Cronin, H. N. Miras, *Chem. Eur. J.* 2018, **24**, 4399-4411.