Supporting Information

Mussel-mimetic Hydrogels with Defined Cross-linkers Achieved by Controlled Catechol Dimerization Exhibiting Tough Adhesion for Wet Biological Tissues

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EXPERIMENTAL SECTION

Materials. Unless otherwise specified, the chemicals used in the current work were used without further purification. Dopamine hydrochloride, ammonium persulfate (APS), and N, N, N', N'-tetramethylethylenediamine (TEMED) were purchased from J&K Scientific. Sodium borate, sodium bicarbonate ($NaHCO_3$), sodium phosphate dibasic (Na_2HPO_4), sodium phosphate monobasic (NaH_2PO_4), and methacrylate anhydride were purchased from Sigma-Aldrich. Acrylamide and sodium nitrite ($NaNO_2$) were purchased from Aladdin. 3-acrylamido phenylboronic acid (AAPBA) was purchased from Frontier Scientific, Inc. Dimethyl sulfoxide (DMSO), sulfuric acid (98%), tetrahydrofuran (THF), methanol, ethyl acetate, and hexane were purchased from RCI Labscan.

Synthesis of Nitro-Dopamine, DMA and nDMA. Nitro-Dopamine and dopamine methacrylamide (DMA) were synthesized following published protocols, respectively.[1-2] Nitrodopamine methacrylamide (nDMA) was synthesized according to the reported method of DMA with some modification.[2] Briefly, 20 g of sodium borate and 8 g of $NaHCO_3$ were added to 200 ml of deionized water and bubbled with nitrogen for 30 min. 0.8 g of Nitro-dopamine sulfate (2.70 mmol) was then added, followed by the dropwise addition of 0.6 ml of methacrylate anhydride (4.05 mmol) in 10 ml of THF. The reaction mixture was then stirred for 18 h at room temperature. The aqueous mixture was washed twice with 100 ml of ethyl acetate and then the pH of the aqueous solution was reduced to less than 2 by 2 M aqueous hydrochloric acid solution. After extraction with ethyl acetate, the organic layers were reduced and dropped to hexane with vigorous stirring. The product was recrystallized from hexane and dried to yield yellow solid. ^1H NMR spectroscopy of nDMA (400 MHz, DMSO-d_6): 7.49-7.25 (d, 1H), 6.70-6.54 (d, 1H), 5.59 (s, 1H), 5.30 (s, 1H), 3.37 (m, 2H), 2.98 (t, 2H), 1.82 (s, 3H).

ITC Test. The isothermal titration calorimetry (ITC) experiments were carried out on a MicroCal iTC200 isothermal titration calorimeter (ITC) at 20.00 ± 0.01 °C. 1 mM nDMA or DMA was prepared in phosphate buffered saline (PBS, pH 7.4) containing 0.5 wt% DMSO under nitrogen protection and loaded in the sample cell (200 μL), respectively. AAPBA at a 15-fold higher concentration (15 mM) was also prepared in the same buffer and loaded in the syringe. A titration consisted of 19 consecutive injections of 2 μL with 120 s intervals between injections. An analogous blank titration was run in which the same titrant AAPBA solution was titrated into the PBS buffer (0.5 wt% DMSO). These heats of dilution were subtracted from the peak area of the experiment groups prior to data analysis with Origin 7.0 software.

Hydrogel Formation. The preparation of hydrogels is briefly described here. First, 0.8 M stock solutions of nDMA, AAPBA, DMA were prepared in DMSO. The dual-crosslinked nDMA/AAPBA-X hydrogels were then readily prepared by the APS/TEMED-induced polymerization of precursor solutions containing nDMA (20 mM, 1 mol%), AAPBA (20 mM, 1mol%) and acrylamide (2 M) in 0.2 M phosphate buffer (pH = 7.4, room temperature) under nitrogen protection. X refers to the same concentration of APS and TEMED, and two different concentrations (20 mM, and 30 mM) were investigated, respectively. The control hydrogels prepared with DMA/AAPBA (DMA/AAPBA-20), nDMA alone (nDMA-20), and DMA alone (DMA-20) were also fabricated with the identical acrylamide concentration and buffer condition.

Mechanical Test. Mechanical testing was performed with samples as prepared. First, rectangular hydrogels ($n \geq 3$) were cast using teflon molds. Samples were then secured in custom clamps, and subjected to extension at room temperature at deformation rate of 60 mm min^{-1}, (Kinexus rheometer, Malvern, 20 N load cell). Fracture strain, and fracture stress were measured. Toughness of rectangular nDMA/AAPBA-20 hydrogel (25 mm (L)×8 mm (W)×1.2 mm (T)) was determined by pure shear test according to the previous report.[3]

Compression tests were performed on a universal mechanical testing machine (Weizheng Instruments Co. Ltd., Shenzhen, China). This machine is equipped with a load cell of 0.2 kN. Uniaxial compressive tests were conducted on cylindrical samples ($n \geq 3$) at room temperature at 5 mm min^{-1} after applying a pre-load of 0.01 N for determination of compressive moduli (slope from 10-20% strain), fracture strain, and fracture stress.

Adhesion Energy Measurement. The adhesion energy was measured with 180°-peeling test. Ribbons of the hydrogels (10x3x50 mm^3) were in situ formed onto porcine skin with one end open, forming bilayer hybrids. The back surfaces of the hydrogels were bonded to stiff polyethylene terephthalate (PETE) films by Krazy Glue, which limited the deformation of hydrogels along the peeling direction, and thus the adhesion energy was two times the measured plateau value of the peeling force per unit width. The free ends of hydrogels and the porcine skins were secured in custom clamps, and subjected to unidirectional extension at room temperature at deformation rate of 100 mm min^{-1}, (Kinexus rheometer, Malvern, 20 N load cell).

Rheological Test. The rheological tests of the hydrogels were performed on an Kinexus rheometer (Malvern) at room temperature. For oscillatory frequency sweep experiments, the constant strain was fixed at 1%. Continuous step-strain measurement of the nDMA/AAPBA-20 hydrogel was performed at high-amplitude oscillatory parameters (γ = 400%, ω = 1 Hz) and low-amplitude oscillatory parameters (γ = 1%, ω = 1 Hz). For the frequency sweeps of swelling nDMA/AAPBA-20 hydrogels, cylindrical nDMA/AAPBA-20 hydrogels were first equilibrated in 50 mL of either the acidic solution (pH 2) or PBS (pH 7.4) buffer for 20 h. Besides, the swelling ratio was calculated through the swollen masses divided by as-prepared masses.

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2. Lee, H.; Lee, B. P.; Messersmith, P. B., A reversible wet/dry adhesive inspired by mussels and geckos. *Nature* **2007,** *448* (7151), 338-341.

3. Liu, J.; Tan, C. S. Y.; Yu, Z.; Lan, Y.; Abell, C.; Scherman, O. A., Biomimetic Supramolecular Polymer Networks Exhibiting both Toughness and Self-Recovery. *Advanced Materials* **2017,** *29* (10).

SUPPORTING RESULTS

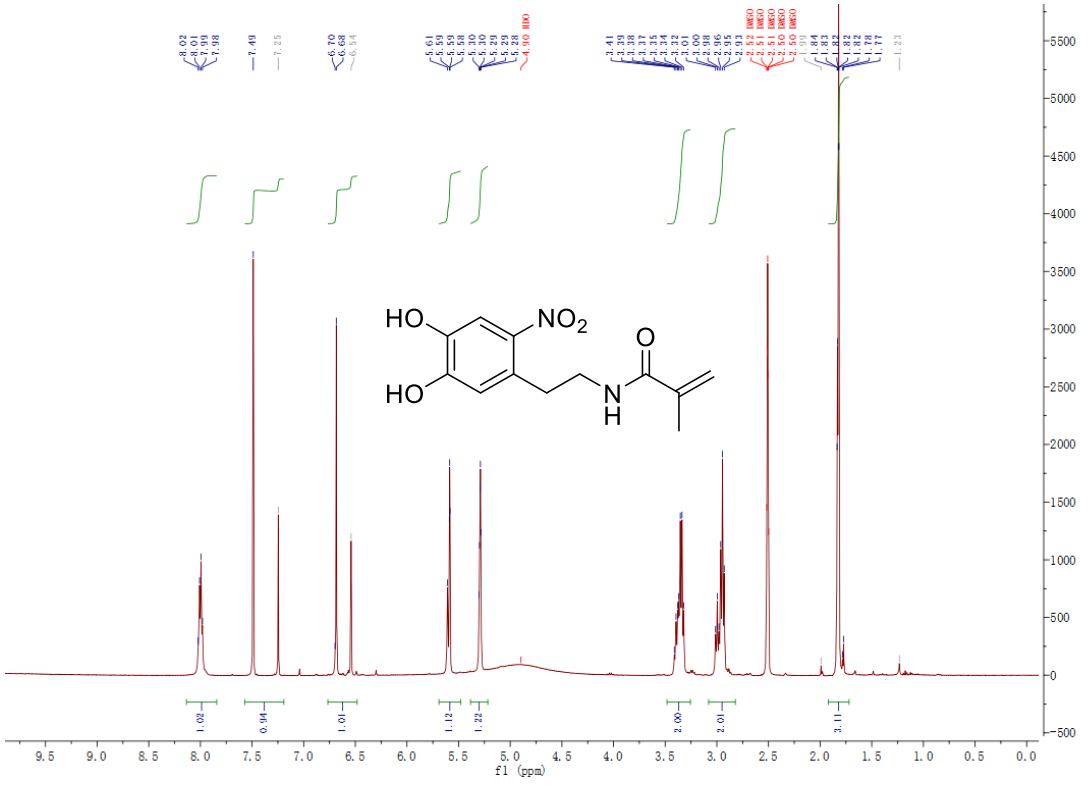

Figure S1. ^{1}H NMR of nDMA recorded in DMSO-d_6.

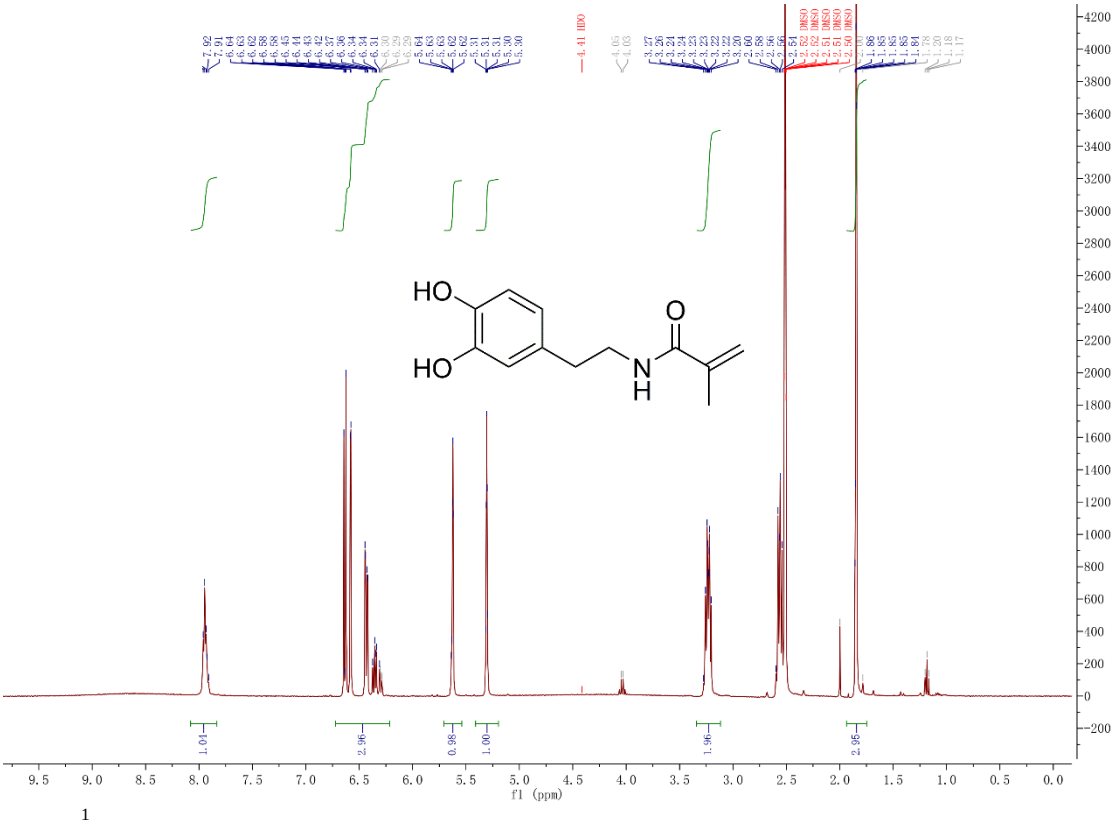

Figure S2. ^{1}H NMR of DMA recorded in DMSO-d_6.

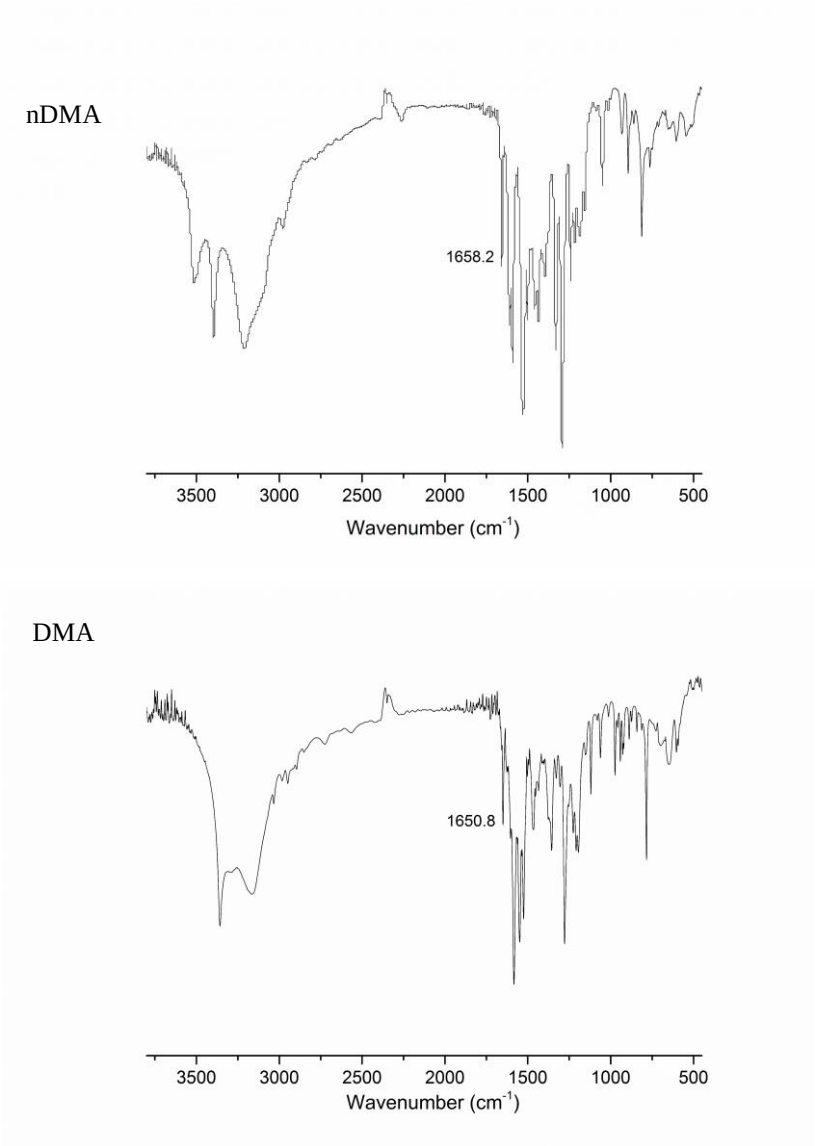

Figure S3. The FTIR absorbance of nDMA (Up) and DMA (Down), respectively. Fourier Transform Infrared Spectrometry (FTIR) was conducted on an IRTracer-100 (Shimadzu, Tyoto, Japan). If the catechols have been oxidized into quinones, the adjacent diketone groups will have a strong absorption peak among 1730-1710 cm^{-1}. As shown in the above figures, the FTIR results show the successful synthesis of both DMA and nDMA.

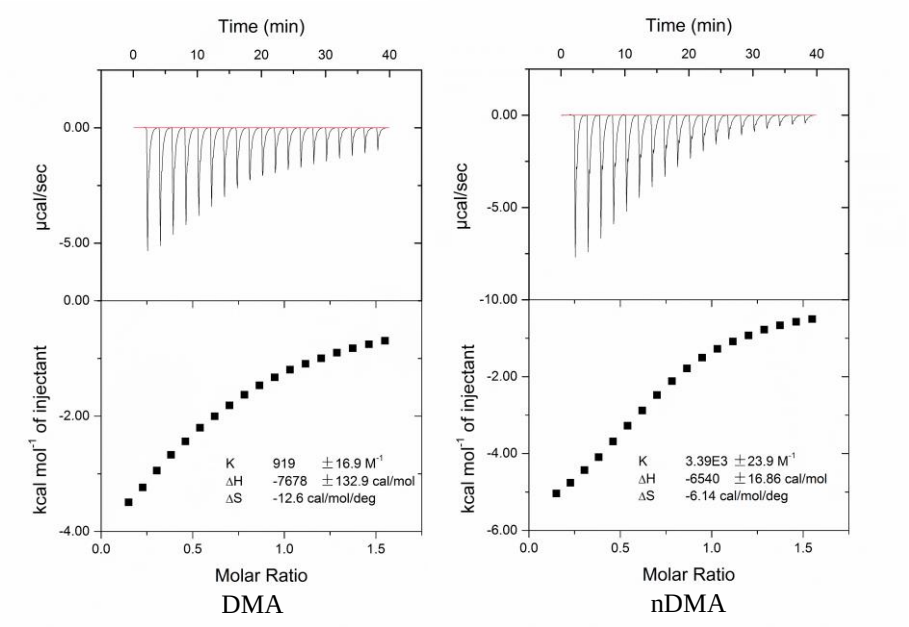

Figure S4. ITC trace for the curve of AAPBA solution into DMA or nDMA solution.

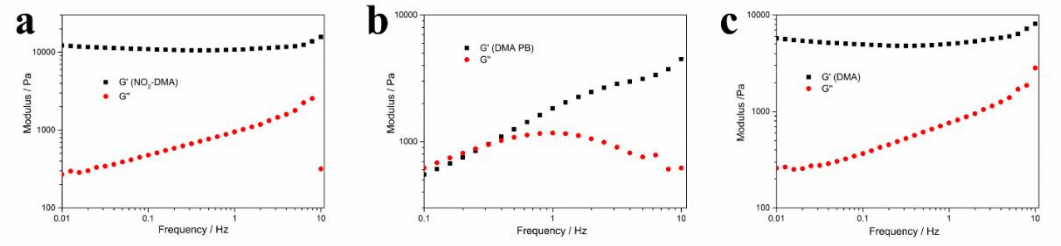

Figure S5. Frequency sweep of a) nDMA-20 hydrogel; b) DMA/AAPBA-20 hydrogel; c) DMA-20 hydrogel. The storage modulus (G') of the single-crosslinked nDMA-20 or DMA-20 hydrogels exhibit nearly no frequency-dependence because of the lack of dynamic crosslinking.

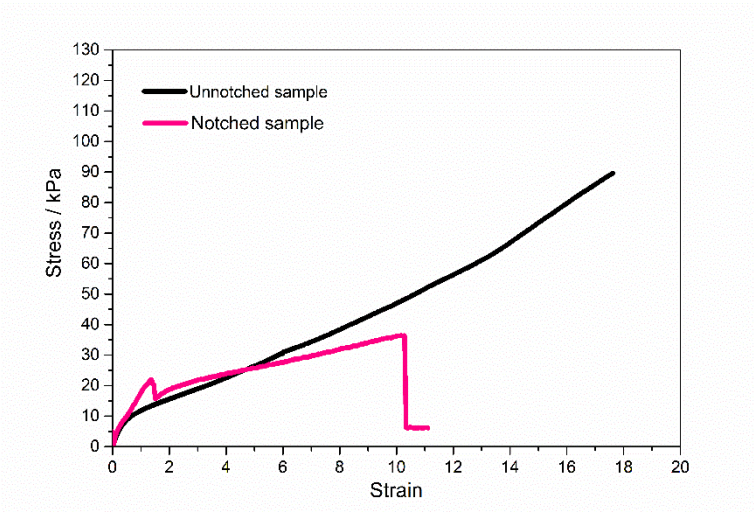

Figure S6. Representative force-extension curve of pure shear test for the unnotched (black) and notched (pink) nDMA/AAPBA-20 hydrogel.

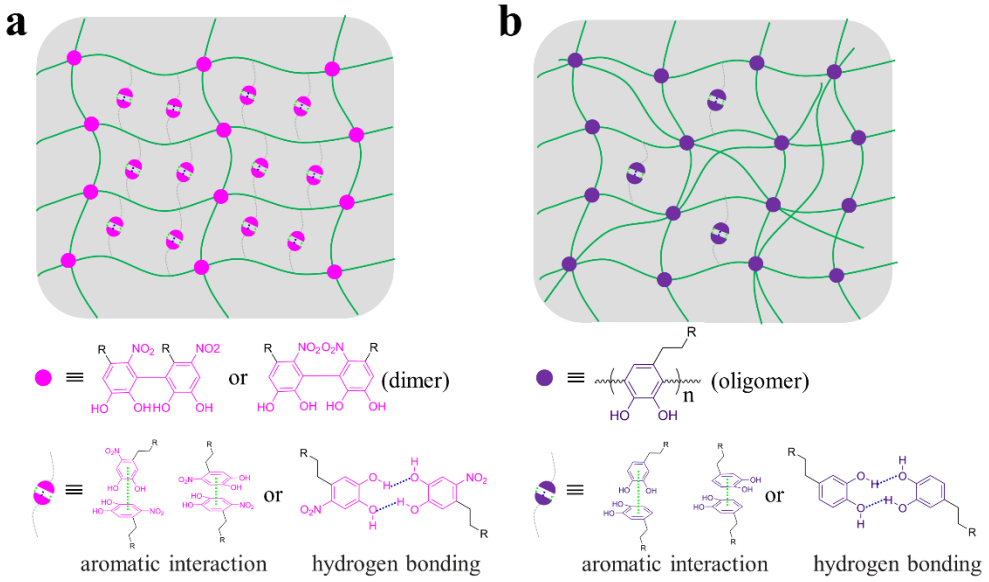

Figure S7. Schematic illustration of a) nDMA-20 hydrogel, and b) DMA-20 hydrogel.

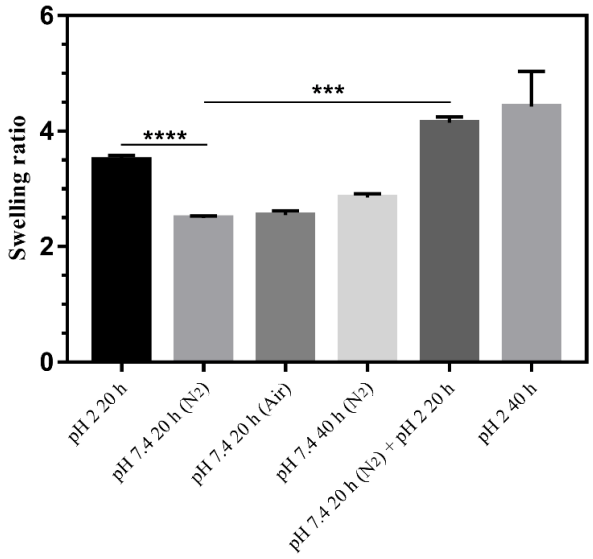

Figure S8. Swelling ratios of the nDMA/AAPBA-20 hydrogels under different conditions. ****P < 0.0001, ***P < 0.001. As shown in Figure S8, when the dual-crosslinked hydrogels were first immersed in PBS buffer for 20 h and then immersed in pH 2 buffer for another 20 h, the swelling ratio of hydrogels was very similar to the hydrogels immersed in pH 2 buffer for 40 h, thereby further corroborating the stimuli-responsive property of nDMA/AAPBA complexes.

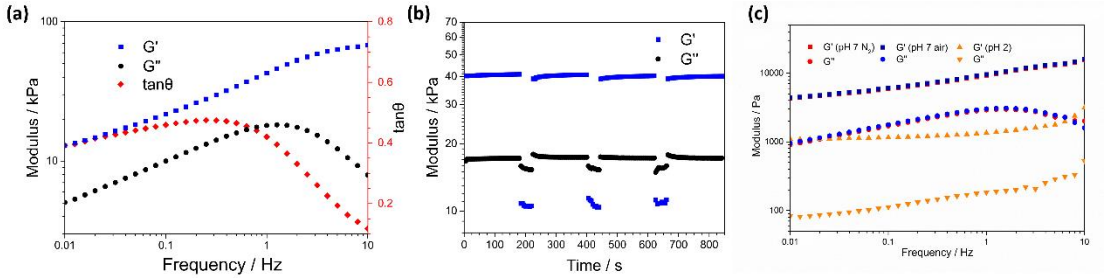

Figure S9. (a) G′, G″ and tanθ values of the dual-crosslinked nDMA/AAPBA-20 hydrogel from a room-temperature frequency sweep (from 0.01 to 10 Hz, 1% strain). (b) Continuous step-strain measurements of the nDMA/AAPBA-20 hydrogel at room temperature (high-amplitude oscillatory parameters: γ = 400%, f = 1 Hz; low-amplitude oscillatory parameters: γ = 1%, f = 1 Hz). (c) Frequency sweeps after equilibrating nDMA/AAPBA-20 hydrogels in the acidic buffer (pH 2), PBS buffer (pH 7.4, under nitrogen protection), and PBS buffer (pH 7.4, exposed to air) for 20 h, respectively.

To further characterize the dynamic mechanical behavior of the dual-crosslinked nDMA/AAPBA-20 hydrogels, we performed rheological frequency sweep analysis on the hydrogels. Due to the dynamic nature of nDMA/AAPBA complexes, the nDMA/AAPBA-20 hydrogels show frequency-dependent storage (G') and loss (G") moduli (Figure S9a). Besides, the DMA/AAPBA-20 hydrogels have much lower moduli than the nDMA/AAPBA-20 hydrogels (Figure S5), corroborating the poor DMA/AAPBA binding at physiological pH as elucidated by ITC (see Figure S4).

Rapid recovery of the mechanical properties of nDMA/AAPBA-20 hydrogels after a large-amplitude oscillatory breakdown was observed at 25 °C (Figure S9b). Upon application of a large-amplitude oscillatory shear strain (γ = 400 %, ω = 1 Hz), G' decreased from 40 kPa to nearly 10 kPa, resulting in a quasi-liquid state. However, when the strain amplitude decreased (γ = 1 %, ω = 10 Hz), G' immediately nearly recovered to its initial value. Additionally, because the complexation between nDMA and AAPBA is pH-dependent, we performed frequency sweeps on the nDMA/AAPBA-20 hydrogels after equilibrating hydrogels in either the acidic buffer (pH 2) or phosphate buffered saline (PBS, pH 7.4) for 20 h. The hydrogels equilibrated in PBS buffer (under nitrogen protection) have significantly higher moduli than the hydrogels equilibrated in pH 2 buffer. Besides, the hydrogels equilibrated in pH 2 buffer were not completely dissolved, indicating the formation of dual-crosslinked network (Figure S9c).