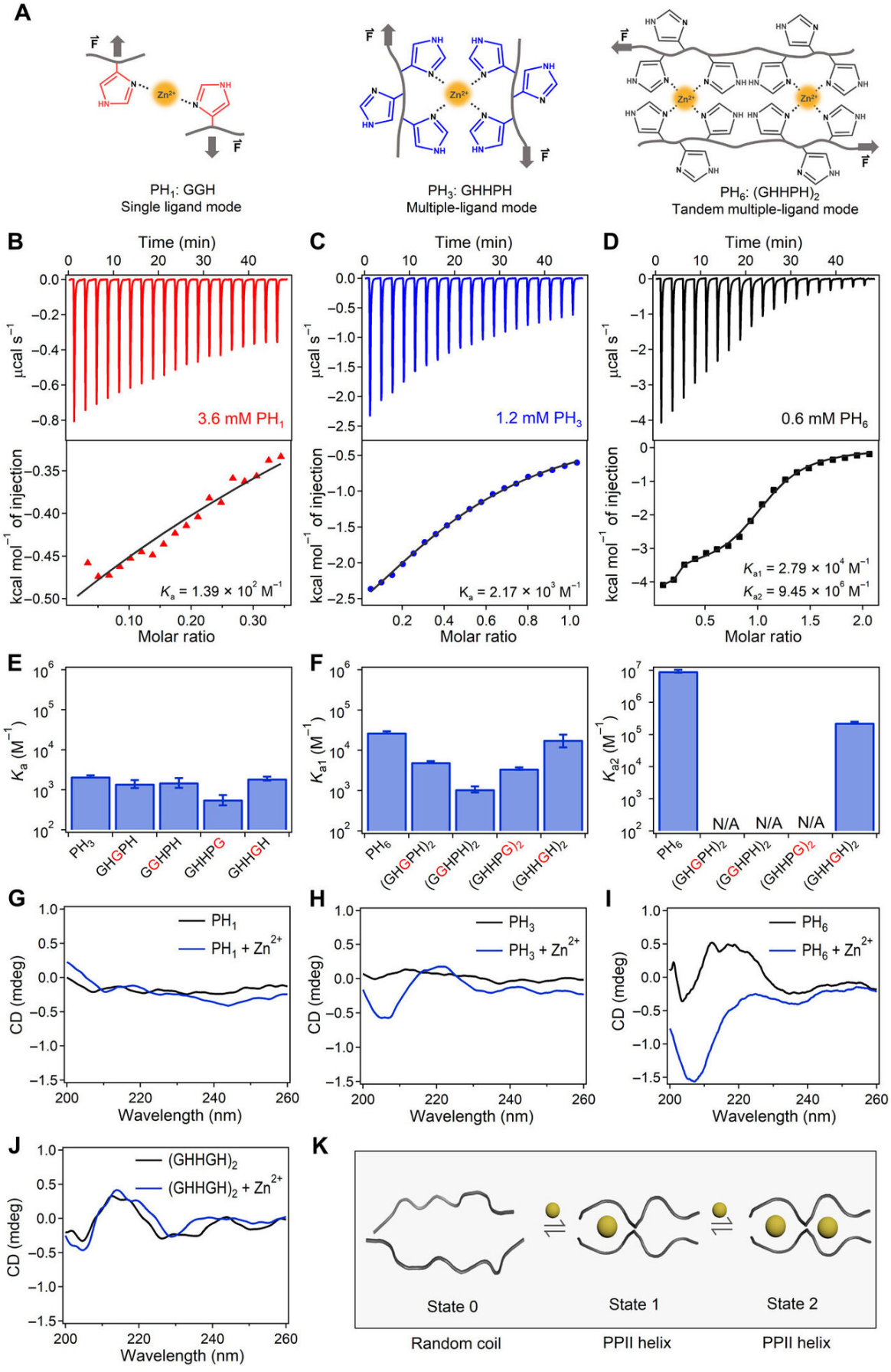
Molecular engineering metal coordination interactions for strong, tough, fast-recovery hydrogels

April 29 2020, by Thamarasee Jeewandara


A
PH1: GGH
Single ligand mode
PH3: GHHPH
Multiple-ligand mode
PH6: (GHHPH)2
Tandem multiple-ligand mode
B
C
D
Time (min)
μcal s−1
3.6 mM PH1
1.2 mM PH3
0.6 mM PH6
kcal mol−1 of injection
Ka = 1.39 × 10^2 M−1
Ka = 2.17 × 10^3 M−1
Ka1 = 2.79 × 10^4 M−1
Ka2 = 9.45 × 10^6 M−1
Molar ratio
E
F
Ka (M−1)
Ka1 (M−1)
Ka2 (M−1)
PH3
GHGPH
GGHPH
GHHPG
GHHGH
PH6
(GHGPH)2
(GGHPH)2
(GHHPG)2
(GHHGH)2
N/A
G
H
I
CD (mdeg)
PH1
PH1 + Zn2+
PH3
PH3 + Zn2+
PH6
PH6 + Zn2+
Wavelength (nm)
J
(GHHGH)2
(GHHGH)2 + Zn2+
K
State 0
State 1
State 2
Random coil
PPII helix
PPII helix

Cooperativity engineering, binding constants, and molecular mechanism of the metal ion coordination interactions at the molecular level for load bearing. (A) The metal ion coordination complexes formed by single ligands (PH1, left) are dynamic and weak. When forming a metal chelation site made of multiple ligands (PH3, middle), the metal ion binding becomes much stronger and less dynamic than that of single ligands. Furthermore, when arranging two metal chelation sites in tandem (PH6, right), the binding affinity, mechanical strength, and association rate can be improved due to cooperativity between the two sites. (B to D) ITC titration data of PH1 (left), PH3 (middle), and PH1 (right) peptides with ZnCl2 in 1 M tris buffer (pH 7.60, containing 300 mM KCl) at 25°C. (E) Zn2+-binding constants (Ka) of PH3 and mutated PH3 peptides. The mutated amino acids are highlighted in red. The error bars represent the fitting errors. (F) Zn2+-binding constants of PH6 and mutated PH6 peptides. Left and right panels correspond to Ka1 and Ka2 for the two binding sites of PH6. Only PH6 and (GHHGH)2 peptides exhibited two binding constants. The rest of the peptides showed single-site binding characteristics. The error bars represent the fitting errors. (G to J) CD spectra of (G) PH1: GGH; (H) PH3: GHHPH; (I) PH6: (GHHPH)2; and (J) (GHHGH)2 peptides in the absence and presence of Zn2+ ions. The relative contents of PPII structures of PH1 and PH3 are 9.6 and 34.2% based on the height of the major CD peak at 205 nm, assuming that the PH6-Zn2+ complex shows 100% PPII helical structure. (K) Schematic illustration of the cooperative Zn2+-binding mechanism of PH6. The conformational change of the first coordination site leads to structural changes of the second one to a conformation more favoring Zn2+ binding. N/A, not applicable. Credit: Science Advances, doi: 10.1126/sciadv.aaz9531

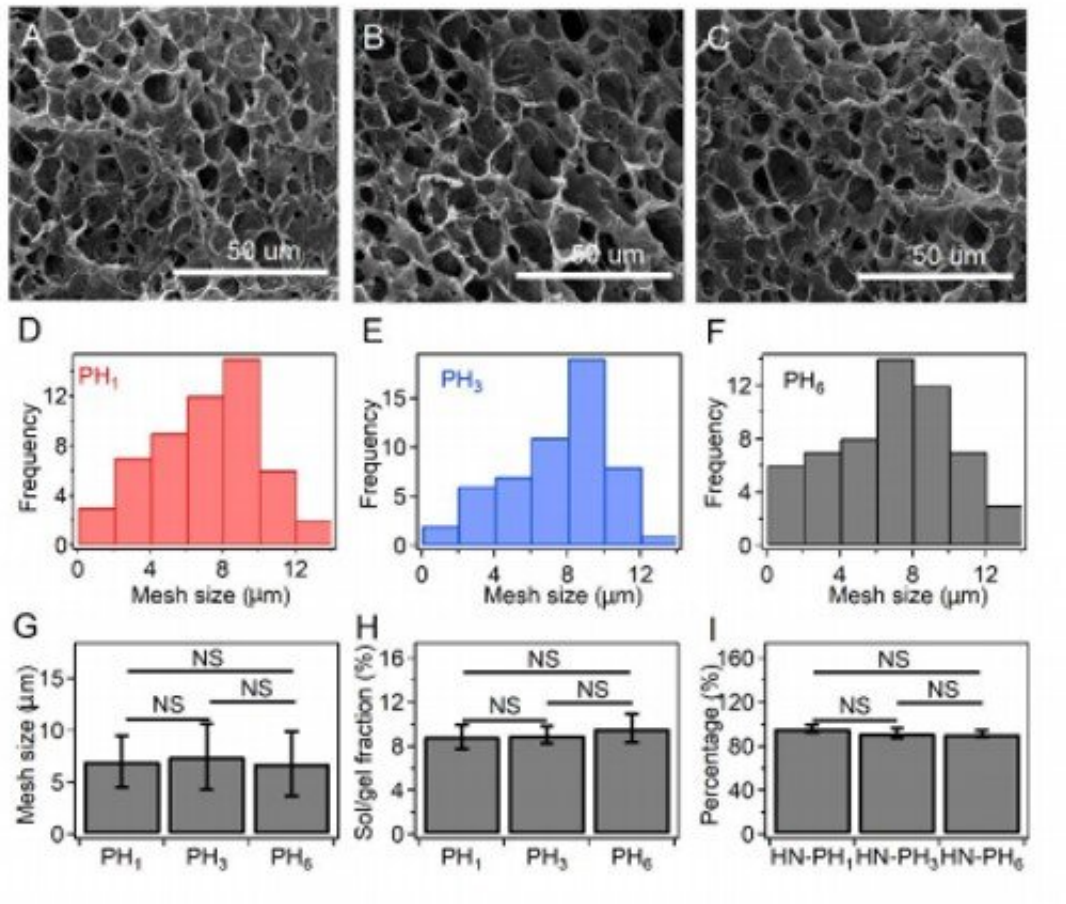
Load bearing tissues such as muscles and cartilages typically show high elasticity, toughness and fast recovery rates. However, combining such mechanical properties in the lab to build synthetic biomaterials is fundamentally challenging. In a new study now published on *Science Advances*, Wenxu Sun and a research team in physics, engineering

mechanics and smart devices in China, developed a strong, tough and fast-recovery hydrogel. The team engineered the material using crosslinkers with cooperative dynamic interactions. They designed a histidine-rich decapeptide (10 amino acid chain) containing two tandem (consecutive) zinc (Zn) binding motifs to facilitate thermodynamic stability, stronger binding strength and faster binding rate of the construct, compared to single binding protein motifs or isolated ligand proteins. The engineered hybrid network hydrogels with the peptide zinc complex exhibited high stability, toughness and fast recovery in seconds. The research team expect the scaffolds to effectively manage load-bearing tissue engineering applications and function as building blocks for soft robotics. The new results provide a general route to tune mechanical and dynamic properties of hydrogels at the molecular level.

When we walk, our muscles, cartilage and tendons are subject to substantial mechanical loads, but biological tissues can recover rapidly to function reliably for many mechanical cycles. Bioengineers have explored soft hydrogels with muscle-like mechanical properties as biomechanical actuators, synthetic cartilage, artificial muscle, ionic skin and in soft robotics. They have devoted many efforts to enhance the mechanical strength and toughness of hydrogels by introducing special energy dissipation mechanisms. Quick recovery is also a unique trait for load-bearing soft tissue, aside from mechanical strength and toughness, but synthetic hydrogels still lack a mechanism for quick recovery. For instance, traditional double network (DN) or hybrid network (HN) hydrogels with short polymer chains as sacrificial networks cannot typically recover soon—often taking a minutes to days.

The strength of a hydrogel depends on the lifetime of its crosslinkers, where slow binding/unbinding kinetics lead to strong hydrogels, while fast exchange rates yield soft ones. To obtain high strength and toughness the crosslinkers must be slow, but to achieve fast recovery, the crosslinkers must be dynamic with high rates of association and

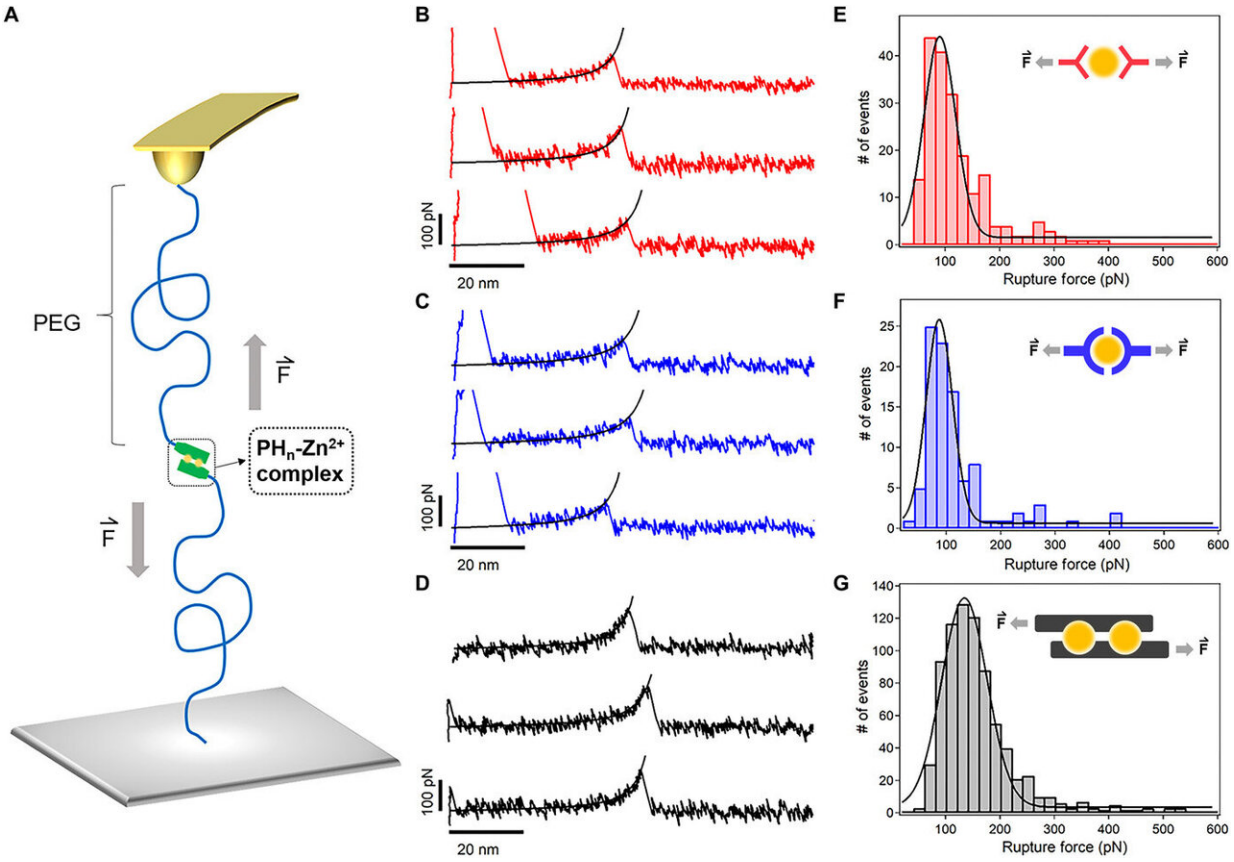
dissociation. To overcome this contradiction, naturally occurring load-bearing materials have used cooperativity of weak interactions. In this work, Sun et al. similarly engineered hybrid network (HN) hydrogels with a specifically designed peptide-metal complex as the physical crosslinker. The team formed efficient metal binding sites in a peptide sequence to engineer hydrogels with the requisite characteristics.



Mesh size, sol/gel fractions, and the actual percent of peptides being incorporated to the hydrogel network. (A-C) SEM images of the HN-PH1 gel (A), the HN-PH3 gel (B) and the HN-PH3 gel (C) before adding Zn2+ ions. (D-F) Mesh size distributions of the HNPH1 gel (D), HN-PH3 gel (E) and HN-PH6 gel (F) estimated from the SEM images using the ImageJ software. (G) Average mesh size of HN-PHn gels in the absence of Zn2+ ions. (H) Sol/gel fractions of different HN-PHn gels prior to adding zinc. (I) The percentage of peptides being

incorporated in the hydrogel network. The initial peptide concentrations were 0.3 M, 0.10 M, and 0.05 M for PH1, PH3, and PH6, respectively. The percentage of the peptides being incorporated in the hydrogels was similar, as estimated by subtracting the faction of eluted peptides from the total amount used. Error bars indicate the mean ± S.D. NS: $p > 0.05$. Credit: Science Advances, doi: 10.1126/sciadv.aaz9531

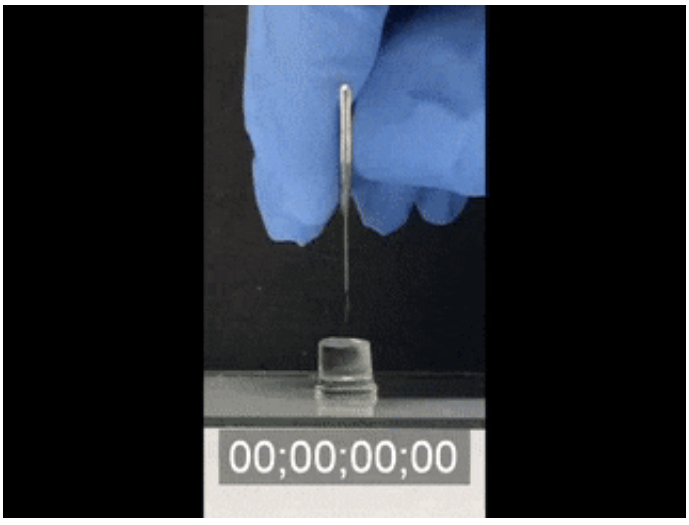
The team first designed three short histidine-rich peptides (HR-peptides) as ligands to bind with zinc ions (Zn^{2+}) and construct HN hydrogels. They denoted the peptide sequences as PH_1, PH_3 and PH_6 based on the number of linked histidines. Sun et al. synthesized the peptides using solid-phase peptide synthesis and purified it with high-performance liquid chromatography. They observed the formation of Zn^{2+} histidine coordination complexes using ultraviolet (UV) and Raman spectroscopy. The specifically designed peptide sequence allowed synergistic and cooperative Zn^{2+} binding affinity, compared to peptides with random histidine residues on their sequences. The scientists studied the molecular mechanism of cooperative zinc ion binding to PH_6 using circular dichroism, the results suggest conformal changes of the first coordination site of PH_6 to be critical for cooperative binding and showed how structural changes favored additional Zn^{2+} binding.



Single-molecule force spectroscopy of the metal ion coordination complexes. (A) Schematic diagram of the AFM-based single-molecule force spectroscopy experiments. The peptide ligands were linked to the cantilever tip and the substrate via a PEG linker (MW, 5 kDa). (B to D) Typical force-extension curves for the rupture of PH1-Zn2+ (red), PH3-Zn2+(blue), and PH6-Zn2+ (black) complexes at a pulling speed of 1000 nm s−1. Worm-like chain fitting of the force-extension curves (black lines) confirmed that the peak at an extension of ~50 nm corresponds to the rupture of a single metal ion chelation bond. (E to G) The rupture force histograms for PH1-Zn2+ (red), PH3-Zn2+(blue), and PH6-Zn2+ (black), respectively. The Gaussian fitting shows the average rupture forces of 90 ± 29, 87 ± 24, and 135 ± 41 pN, respectively. The proposed Zn2+ ion binding modes for the three peptides are shown in the insets. Credit: Science Advances, doi: 10.1126/sciadv.aaz9531

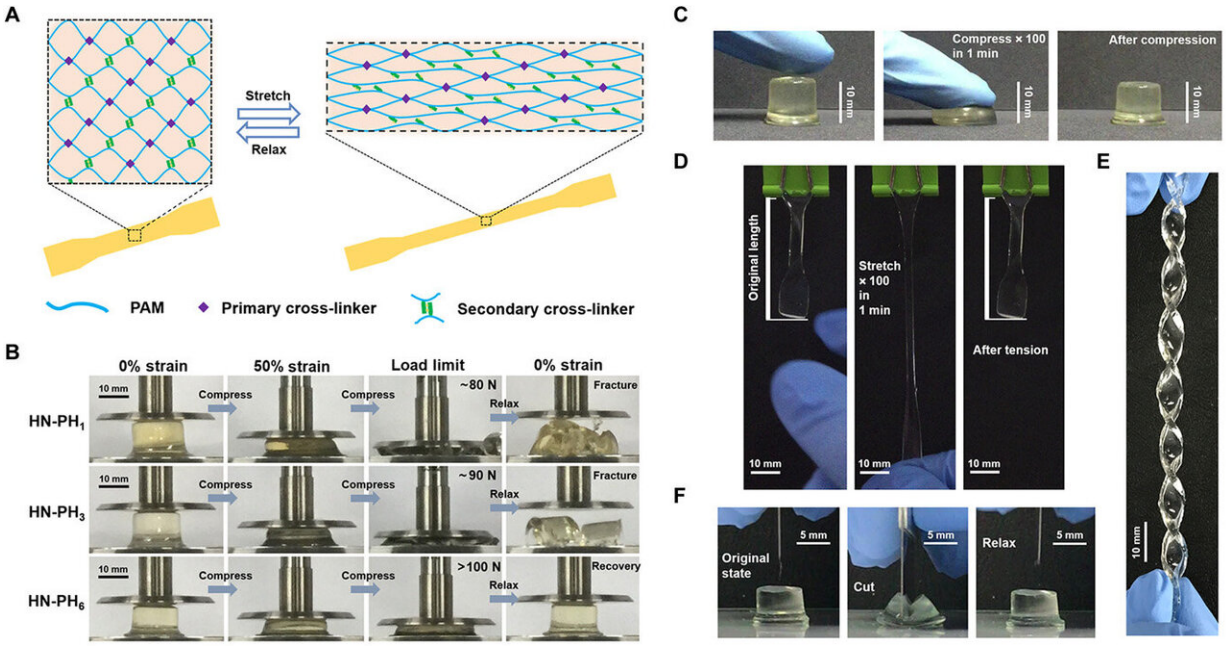
Sun et al. used advanced techniques such as atomic force microscopy (AFM)-based single-molecule force spectroscopy (SMFs) to measure the mechanical stability of the HR-peptide-Zn^{2+} complexes i.e. crosslinkers of the hydrogel at the molecular level. The average rupture forces were much higher for PH_6 compared to other types of hydrogels, confirming the toughness of the hydrogel. The results showed that the mechanical stability of the metal-ligand complexes could be improved considerably based on the binding sites.

The team explored if changes to the intrinsic properties of crosslinkers could alter macroscopic mechanical properties of the hydrogel by preparing a series of hybrid network (HN) hydrogels. They used HR-peptide-Zn^{2+} as sacrificial crosslinkers and covalent bonds as permanent crosslinkers in the constructs and named the resulting hydrogels as HN-PH_1, HN-PH_3, and HN-PH_6, based on the peptide sequence used. The network structures were similar in all three hydrogels but the HN-PH_6 gel was more compressible compared to the others, while functioning effectively under stressful mechanical environments. Interestingly, the scientists could even twist the HN-PH_6 hydrogel into a spiral shape and compress the material with a sharp blade without causing it permanent damage.



Compressing the HN-PH6 hydrogel using a sharp blade does not damage the material. Credit: Science Advances, doi: 10.1126/sciadv.aaz9531

The team conducted tensile mechanical tests on the gels and correlated the results on the bulk level with those at the molecular level, to show remarkably higher break strain, Young's modulus and toughness for the HN-PH_6 gels. Sun et al. then examined the recovery property of the material based on loading-unloading cycles and found HN-PH_6 gels to almost totally recover its macroscopic mechanical properties in minutes. However, if they cut up the HN-PH_6 gels into pieces, the hydrogel could not self-heal since covalent crosslinkers do not reform after fracture. To understand the experimental outcomes, the research team also conducted theoretical analyses and proposed cooperative zinc binding on PH_6 to be an important factor, among other factors to form strong and tough hydrogels with fast recovery rates.



Structure and properties of HN-PHn HN hydrogels cross-linked by the peptide-Zn2+ coordination complexes. (A) Schematic illustration of the network structure of HN-PHn hydrogels. The network comprises covalent bonds as the primary cross-linkers and ligand-metal interactions as the secondary cross-linkers. (B) Optical images of the HN-PH1 (top), HN-PH3 (middle), and HN-PH6 (bottom) hydrogels under a compression-relaxation cycle. The HN-PH1 and HN-PH3 gels were fractured, whereas the HN-PH6 gel was almost fully recovered. (C) Optical images of the HN-PH6 gel under an extreme compressive condition (compressed to >70% strain for 100 times at 1.6 Hz). (D) Optical images of the HN-PH6 gel under an extreme tensile condition (stretched to >150% strain for 100 times at 1.6 Hz). (E) Optical image of the HN-PH6 gel twisted into a spiral shape. (F) Optical images of the HN-PH6 gel compressed with a sharp blade and relaxed. No detectable cut was observed on the gel. PAM, polyacrylamide. Photo credits: Wenxu Sun, Nanjing University. Credit: Science Advances, doi: 10.1126/sciadv.aaz9531

In this way, Wenxu Sun and colleagues developed a novel hydrogel material, bioinspired by histidine residues found in natural load-bearing materials. Combining such outstanding mechanical properties in the lab has remained a challenge due to the inability to effectively harness the unique metal ion binding properties that are encoded in natural proteins. In this work, Sun et al. used bioinspired Zn^{2+}-binding peptide as crosslinkers to form the desired hydrogels at the molecular level, highlighting the importance of cooperative metal coordination during materials synthesis. They intend to examine additional mechanical features, such as adhesion to other tissues, before conducting practical applications in tissue engineering.

More information: Wenxu Sun et al. Molecular engineering of metal coordination interactions for strong, tough, and fast-recovery hydrogels, *Science Advances* (2020). DOI: 10.1126/sciadv.aaz9531

C. Cvetkovic et al. Three-dimensionally printed biological machines powered by skeletal muscle, *Proceedings of the National Academy of Sciences* (2014). DOI: 10.1073/pnas.1401577111

Jeong-Yun Sun et al. Highly stretchable and tough hydrogels, *Nature* (2012). DOI: 10.1038/nature11409