Supporting Information for:

Thermosensitive Metalloprotein-based Hybrid Hydrogels for Reversible and Highly Selective Removal of Lead(II) from Water

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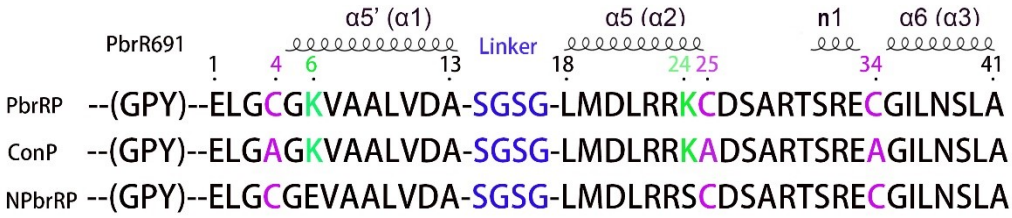

Figure S1. The amino acid sequence of the PbrRP, ConP, and NPbrR. The residues highlighted in green were designed for the conjugation with the acryloyl group by NAS.

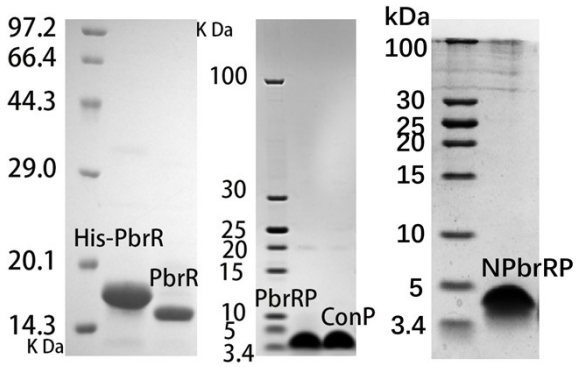

Figure S2. SDS-PAGE characterization of the purified PbrR, PbrRP, ConP, and

NPbrR. All of these proteins were of high purity.

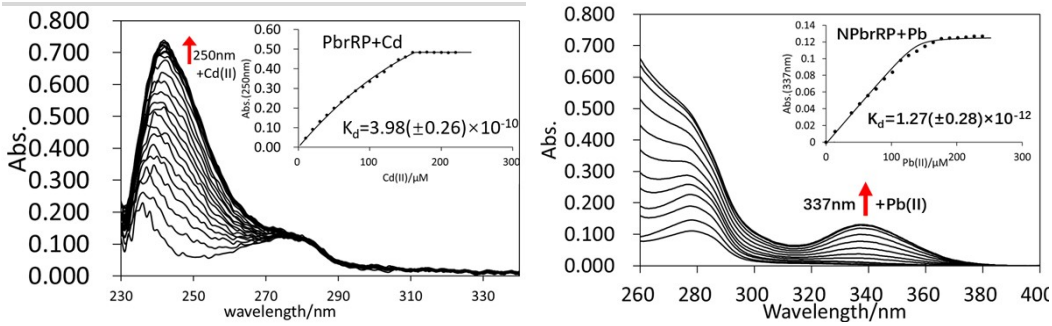

Figure S3. Absorbance spectra curves for the Cd(II) titration of PbrRP and the Pb(II) titration of NPbrRP by UV-vis spectra.

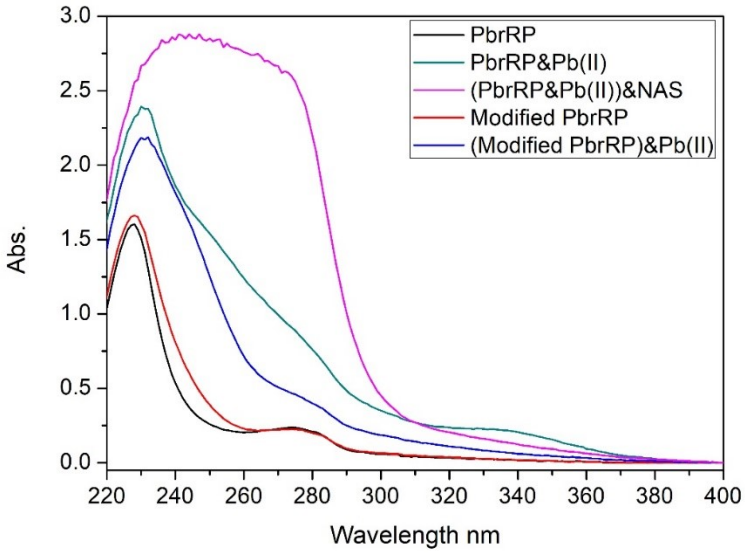

Figure S4. UV-vis spectra anaylysis of the modification of PbrRP in the presence of equivalent of Pb(II).

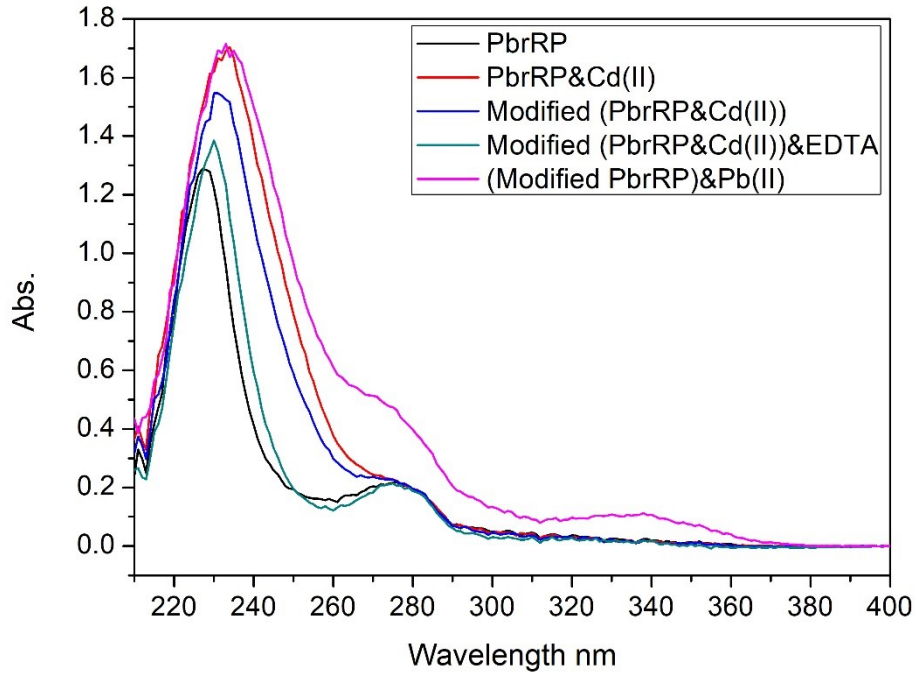

Figure S5. UV-vis spectra anaylysis of the modification of PbrRP in the presence of equivalent of Cd(II).

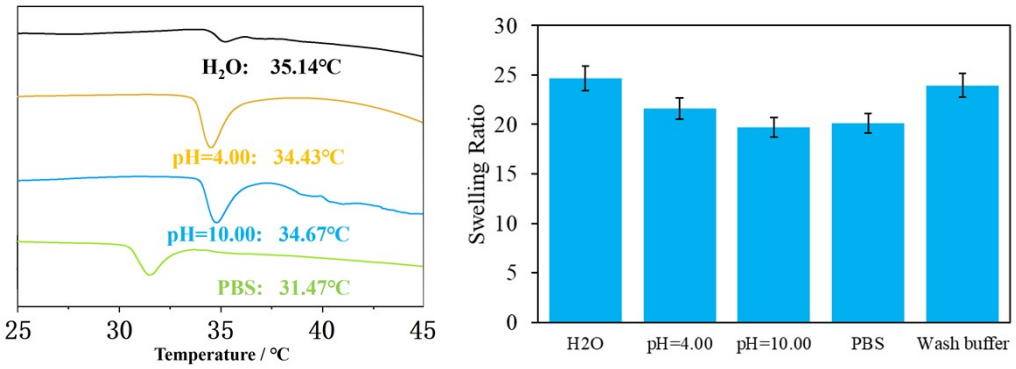

Figure S6. Solvent effects on LCST and the swelling ratio of hydrogels. Changes in the solvent do not have much effect on LCST and swelling ratio, and do not affect the practical application of the hydrogel.

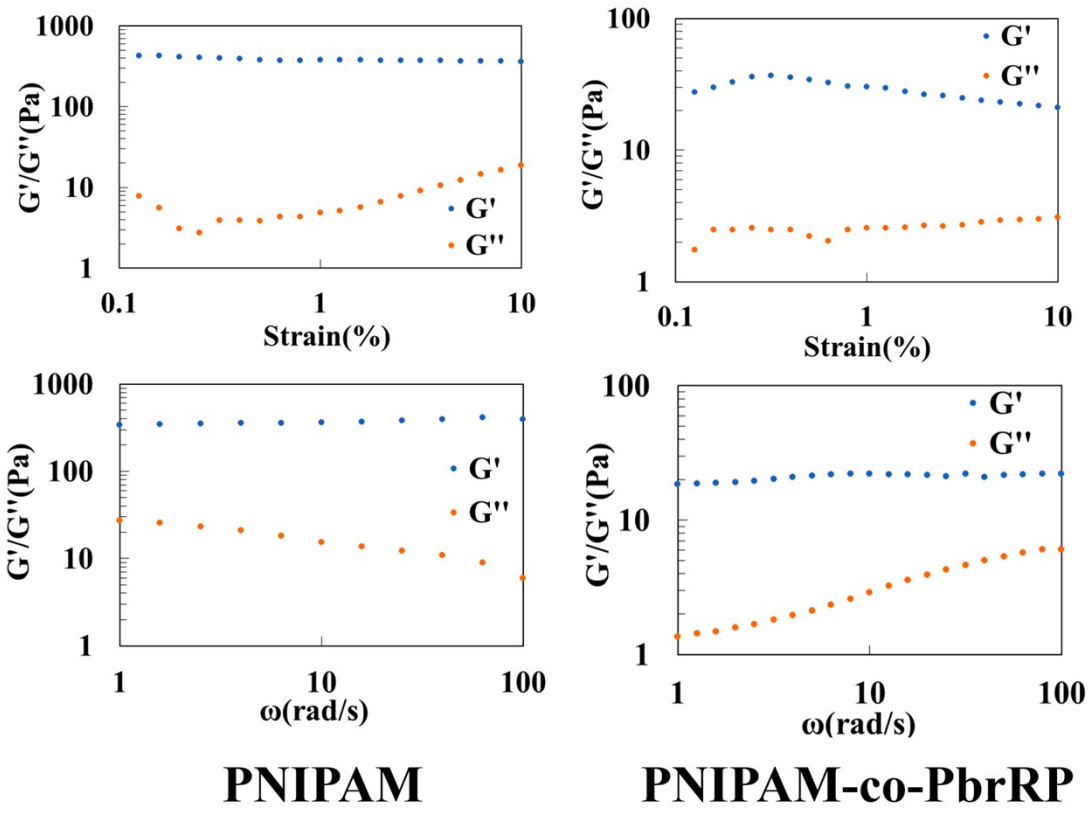

Figure S7. Rheological experiments of PNIPAM and PNIPAM-co-PbrRP. The storage modulus G'remained almost unchanged when the strain was increased from 0.1 to 10%, showing the hydrogel resilience to mechanical deformation. The G'value remained almost constant when the angular frequency was varied from 1 to 100 rad/s, which strongly suggested a stable, covalently cross-linked hydrogel.

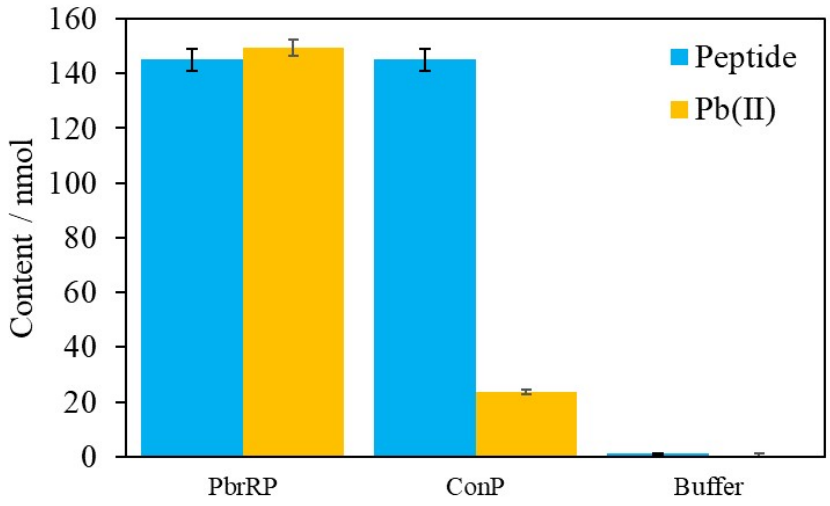

Figure S8. With the Cys-to-Ala PbrRP mutant, ConP still retained some binding ability to Pb(II).

An excess of Pb(II) was added to the purified PbrRP and ConP protein solutions. The

free Pb(II) was later removed by concentrating and diluting the sample through an ultrafiltration tube. The Pb(II) bound to the protein remained in the solution and was measured by ICP-MS.

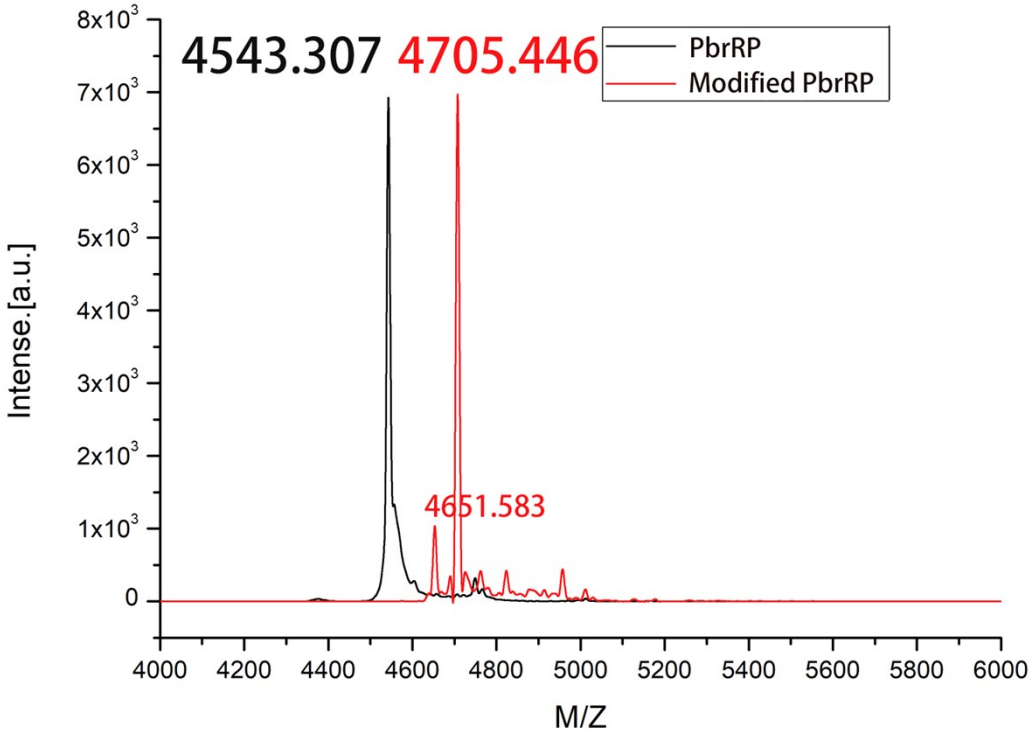

Figure S9. Characterization of the PbrRP and Modified PbrRP by MALDI-TOF MS. The peaks at 4543.307 and 4705.446 represented PbrRP and modified PbrRP, respectively. The results indicated that three acryloyl groups were successfully introduced into the PbrRP. The matrix was sinapic acid and a positive mode was used.

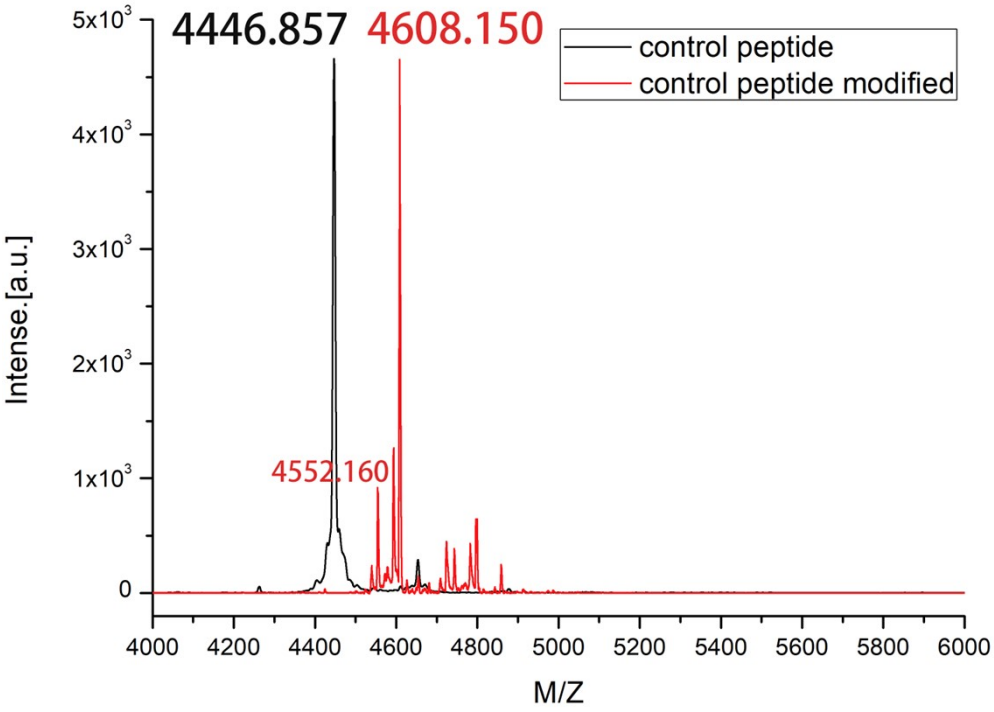

Figure S10. Characterization of the ConP and Modified ConP by MALDI-TOF MS. The peaks at 4446.857 and 4608.150 represented ConP and modified ConP, respectively, indicating that three acryloyl groups were successfully introduced into ConP.

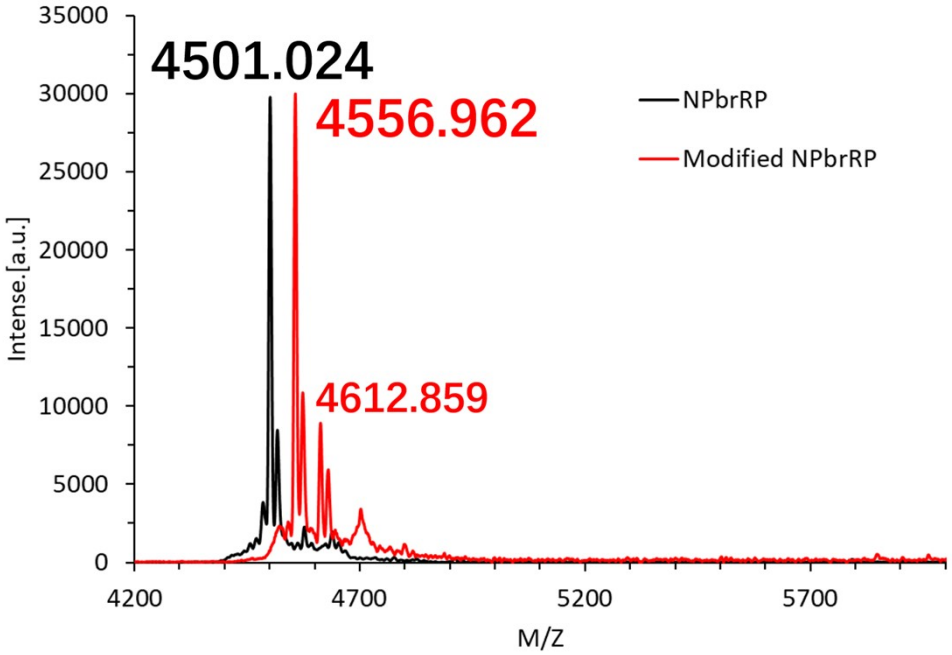

Figure S11. Characterization of the NPbrRP and Modified NPbrRP by MALDI-TOF MS. The peaks at 4501.024 and 4556.962 represented NPbrRP and modified NPbrRP, respectively, indicating that one acryloyl group was successfully introduced into ConP.