Supplemental Materials

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Materials and General Methods

Fmoc-protected amino acids were purchased from Novabiochem. Trifluoroacetic acid (TFA), anisole, thioanisole, 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) were obtained from Sigma-Aldrich. PL-Rink resin was purchased from Agilent Technologies. 2-(6-Chloro-1H- benzotriazole-1-yl)-1,1,3,3-tetramethylaminium hexafluorophosphate (HCTU) was purchased from Chem Impex International. Fluorescein-NHS was obtained from Fisher Scientific. Lambda Protein Phosphatase was purchased from New England BioLabs(Catalog#: P0753S), specific activity is ~800,000 unit/mg. Alkaline phosphatase (ALP, A1130) was obtained from Biomatik. All peptides synthesized were prepared with an amidated C-terminus.

Peptide Synthesis

All peptides were prepared via standard Fmoc solid-phase peptide synthesis using an ABI 433A peptide synthesizer, with PL-Rink resin and activation by HCTU. The resin-bound peptide was cleaved using a cocktail of TFA/thioanisole/ethanedithiol/anisole (90:5:3:2) for 2h under argon atmosphere. The solution was filtered and the resin was washed using additional TFA. Crude peptide was obtained by concentrating the filtrate and precipitating with cold ether. The crude product was purified by reverse phase HPLC, equipped with a semi-preparative Vydac C18 column. HPLC solvents consisted of solvent A (0.1% TFA in water) and solvent B (0.1% TFA in 9:1 acetonitrile/water) at 40 ^{o}C. A gradient of 0-20% solvent B was first used over 15 min, followed by a gradient of 20-60% solvent B over an additional 80 min. All peptides were lyophilized after purification, then analyzed by using analytical HPLC and LC-MS.

Dephosphorylation assay

Typically, 0.5 wt% PP1 (or PP2) solution in pH 7.4 BTP buffer (50 mM BTP, 150 mM NaCl, 1 mM $MnCl_2$) was treated with lambda protein phosphatase (0.092 U/µL) at 37 ^{o}C. Aliquots (40 µL) were taken at desired times and mixed with 40 µL standard B and 200 µL standard A. The obtained clear solution was analyzed using analytical HPLC to quantify dephosphorylated peptide. A gradient of 0-15% solvent B was first used over 10 min, followed by a gradient of 15-35% solvent B over an additional 40 min.

Circular Dichroism Spectroscopy

Circular Dichroism (CD) spectra were collected on an AVIV model 420 circular dichroism spectrometer. Wavelength and time-dependent spectra for all peptides were obtained in a 0.1mm quartz cell. Wavelength scans were collected by scanning in 1 nm step intervals with a 3s averaging time. All CD samples were prepared to achieve a final buffer composition of 50 mM BTP, 150 mM NaCl, 1mM $MnCl_2$, pH 7.4. A 2x stock of dephosphorylated peptide was prepared in D.I water, chilled on ice, and an equal volume of ice-cold, 2x buffer solution (pH 7.4, 100 mM BTP, 300 mM

NaCl, 2mM $MnCl_2$) was added. Sample were equilibrated at 37 ^oC for 5 min. After which, a desired amount of enzyme was added into the peptide solution and the CD signal was monitored from 193 nm to 260 nm. In temperature dependent experiment, the samples were immediate placed on the cell holder pre-equilibrated at 5 ^oC. Temperature dependent wavelength scans were collected every 5 ^oC up to a maximum temperature of 70 ^oC with a 10 min equilibration time at each temperature. The concentration of the 2x peptide solution in water was determined by measuring the absorbance at 220 nm (ε = 15750 cm^{-1} M^{-1})[1]. Mean residue ellipicity [θ] was calculated using the equation $[\theta] = (\theta_{obs}/10lc)/r$, where θ_{obs} is the observed ellipticity in millidegrees, l is the length of the cell (cm), c is the concentration (M), and r is the number of residues.

Oscillatory Rheology

All rheological experiments were performed on an AR G2 rheometer (TA Instruments) equipped with a steel 25 mm parallel geometry tool. For enzyme-based experiments, phosphorylated peptide solutions were prepared to achieve a final buffer composition of 50 mM BTP, 150 mM NaCl, 1mM $MnCl_2$, pH 7.5. A 2x stock of dephosphorylated peptides was prepared in D.I water, chilled on ice, and an equal volume of ice-cold, 2x buffer solution (pH 7.4, 100 mM BTP, 300 NaCl, 2mM $MnCl_2$) was added. A typical time-dependent experiment was performed as follows: peptide solutions were transferred to the rheometer stage after the addition of enzyme at room temperature; the geometry was lowered to 0.5 mm and the temperature was increased to 37 ^oC over 1 min, after which the storage modulus was monitored. To avoid dehydration, a layer of S6 standard silicon oil was applied around the edge of the sample at the beginning of the experiments. Dynamic frequency and strain sweep experiments were performed to ensure that the time-sweep data were collected in the linear regime of strain and frequency. Briefly, a dynamic frequency sweep experiment was carried out in the frequency range from 0.1 to 100 rad/s at the constant strain (0.2%) after the time-sweep experiment. A dynamic strain sweep was subsequently performed varying the strain from 0.1 to 1000% at constant frequency (6 rad/s).

Transmission Electron Microscopy (TEM)

The sample was prepared by diluting 5 µL of prepared 1.0 wt% hydrogel into 195 µL of water to generate a 40x dilution. A drop of peptide solution was placed on 200-mesh copper grid covered by carbon film (Electron Microscopy Science) and allowed to stand for 1 min, which was then blotted by filter paper. Subsequently a drop of 0.75% uranyl formate was placed on the grid and allowed to stand for 1-2 min, then blotted with a piece of filter paper and left to air dry. Images were taken with a Hitachi 7650 at 80 kv accelerating voltage. Average fibril diameters were measured via ImageJ software by taking 20 independent measurements from distinct fibrils in the field of view.

Cell Viability Assay

Hydrogel samples (50 µL) were prepared in a 96 well plate via the addition of 0.575 U/µL LPP into peptide solution in pH 7.4 HEPES buffer (25 mM HEPES, 150 mM NaCl, 1 mM $MnCl_2$). The

plate was placed into an incubator at 37°C and 5% CO_2 and allowed to equilibrate for 48 h. 100 µL serum-free DMEM media was added to the top of each gel and equilibrated overnight. Human dermal fibroblast cells were trypsinized and counted using a hemocytometer. The resulting suspension was diluted with serum containing DMEM, 100 µL cells (10 000 cells) were placed onto the top of hydrogel and control tissue polystyrene surface. After 24h incubation, the medium was removed and washed gently with serum-free DMEM medium to remove the serum proteins, which can lead to an increase in background fluorescence. Cell viability was evaluated by using a Live/dead assay. Typically, 100 µL serum-free medium containing both 1 µM calcein AM and 2 µM ethidium homodimer was added into each well. The dye was allowed to incubate for 10 min before washing with serum-free medium. Fluorescence microscopy was performed on an EVOS FL Auto, Thermo Fisher Scientific.

Actin and Nuclear Staining of Human Dermal Fibroblasts.

PP1 hydrogel (200 µL) was prepared in an 8 well confocal microscopy chamber as stated above. Human dermal fibroblast cells (400 µL solution containing 20,000 cells) were placed onto the top of hydrogel and control tissue polystyrene surface. After 24 h incubation, cells were fixed using a 4% paraformaldehyde solution (supplemented with 0.5% BSA) in Ca/Mg-free PBS for 30 min. Cells were washed three times with PBS and permeabilized for 5 min using 0.5% TRITON-X-100 (supplemented with 0.5% BSA) in water. The cells were rinsed again and stained with 2 µg/mL Hoechst 33342 for 20 min. Next, a 200 µL solution of Alexa fluor phalloidin in Ca/Mg-free PBS was added and incubated for 1 h. The image was taken on a Zeiss 710 LCM confocal microscope.

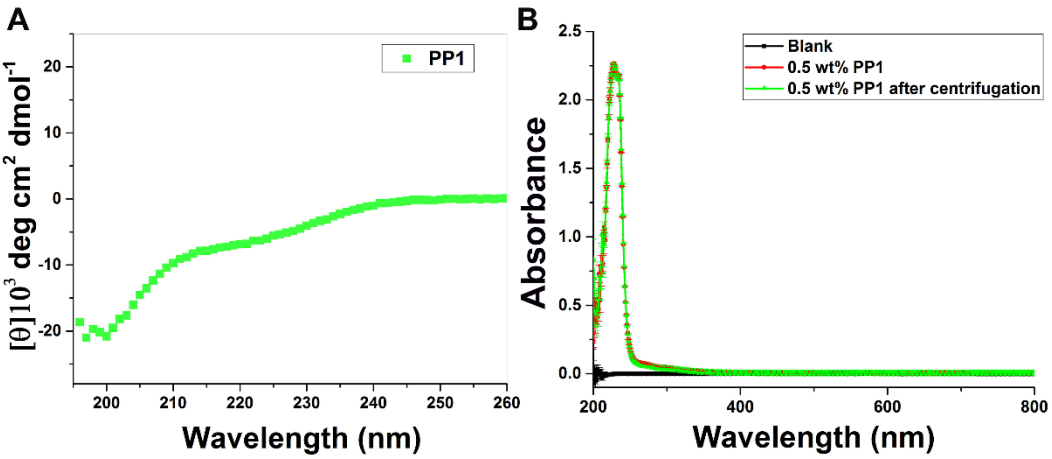

Figure S1. A) CD wavelength spectrum of 0.5 wt% PP1 in BTP buffer (pH 7.4, 50mM BTP, 150 mM NaCl) showing that the peptide is unfolded. B) Absorbance of 0.5 wt% PP1 in buffer before and after centrifugation at 10 000 rpm for 5 min. No absorbance loss is observed after centrifugation nor light scattering at long wavelengths, indicating that PP1 is low molecular weight (monomer) and does not assembled into large molecular weight species, which would have sedimented resulting in loss of absorbance.

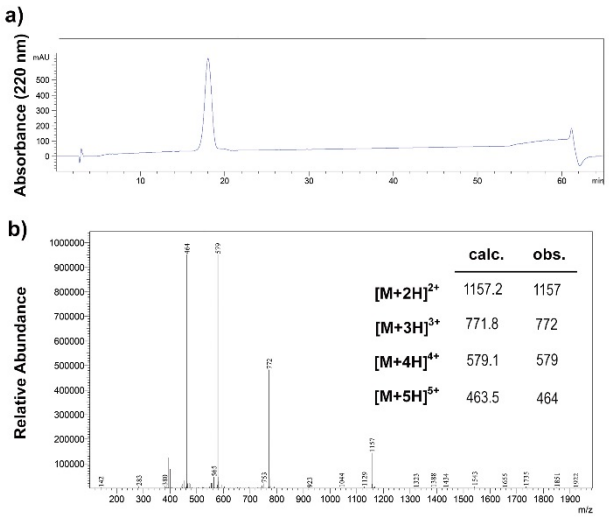

Figure S2. Analytic HPLC and MS spectra of PP1 upon treatment with ALP (25 U/mL) for 48h. PP1 remains unreacted indicating that PP1 is not a substrate of ALP.

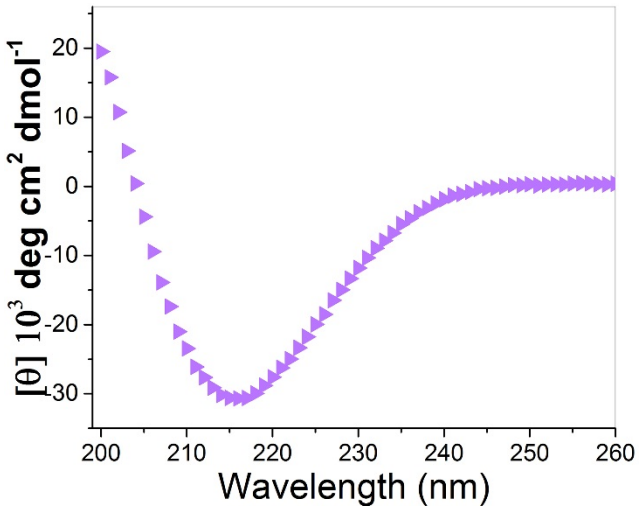

Figure S3. CD spectra of 0.5 wt% dephosphorylated PP1 prepared non-enzymatically via chemical synthesis. Peptide dissolved in pH 7.4 BTP buffer (50 mM BTP, 150 mM NaCl, 1mM $MnCl_2$).

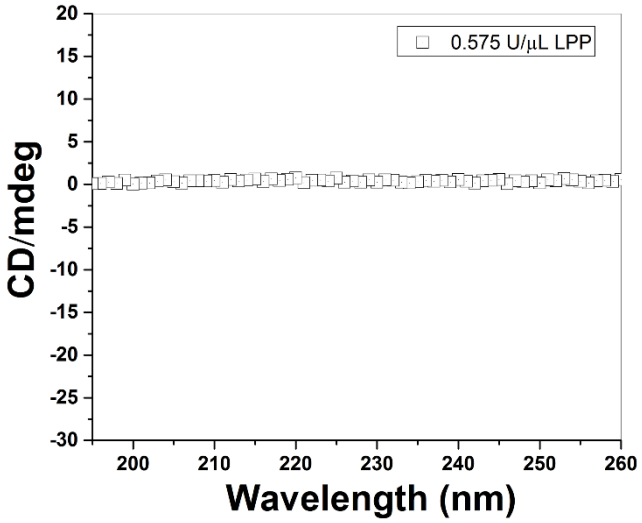

Figure S4. CD spectrum of LPP at the concentration of 0.575 U/μL LPP was diluted in pH 7.4 BTP buffer (50 mM BTP, 150 mM NaCl, 1mM $MnCl_2$).

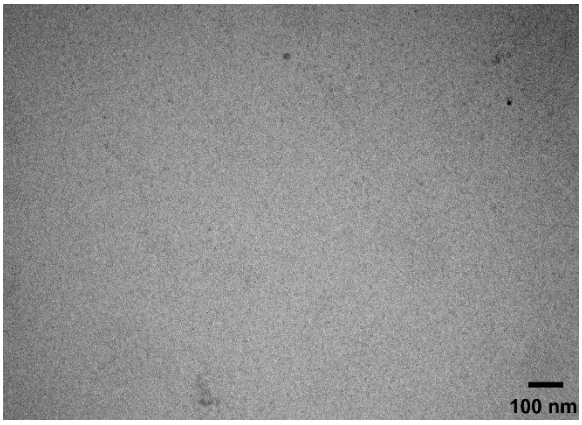

Figure S5. TEM images of phosphorylated PP1 (0.5 wt%) in pH 7.4 BTP buffer (50 mM BTP, 150 mM NaCl, 1mM $MnCl_2$).

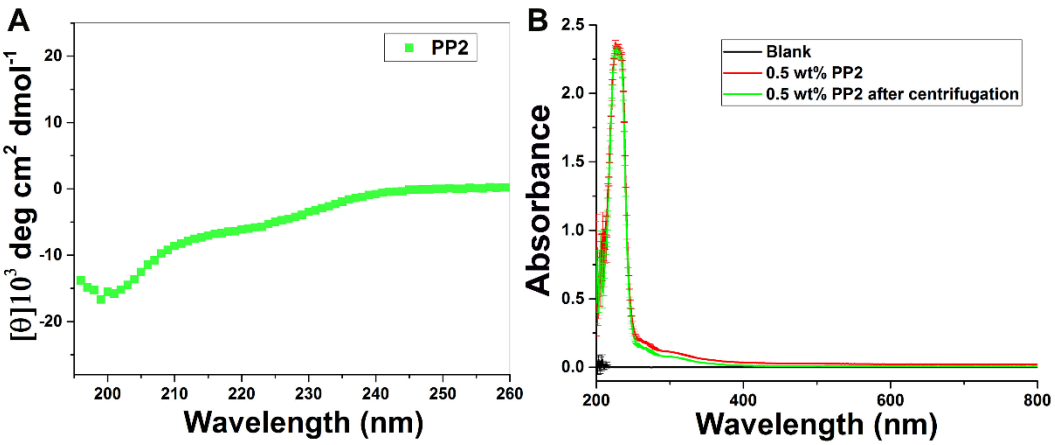

Figure S6. A) CD wavelength spectrum of 0.5 wt% phosphorylated PP2 in BTP buffer (pH 7.4, 50mM BTP, 150 mM NaCl) showing that the peptide is unfolded. B) Absorbance of 0.5 wt% PP2 in buffer before and after centrifugation at 10 000 rpm for 5 min. No absorbance loss is observed after centrifugation nor light scattering at long wavelength, indicating that PP2 is low molecular weight (monomer) and does not assembled into large molecular weight species.

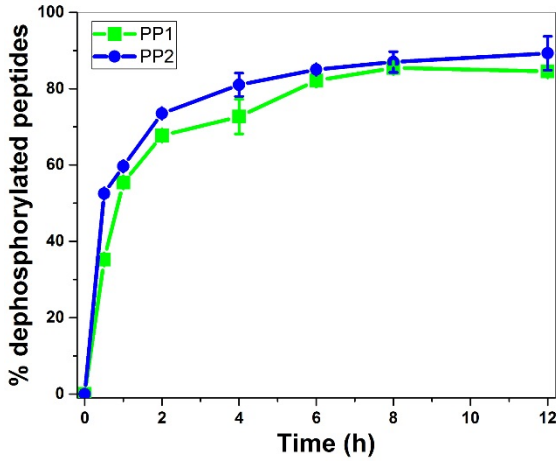

Figure S7. Time-dependent dephosphorylation of PP1 and PP2 (0.5 wt%) in the presence of 0.092 U/µL lambda protein phosphatase (LPP).

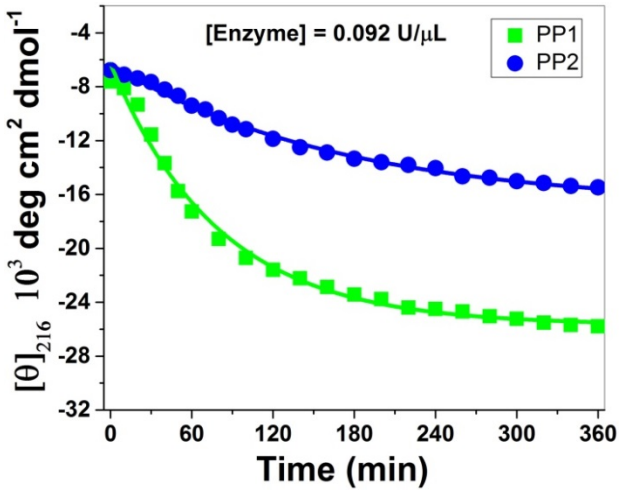

Figure S8. Mean residue ellipticity at 216 nm for 0.5 wt% PP1 and PP2 was monitored as a function of time in the presence of 0.092 U/μL LPP. Peptide solutions were prepared in pH 7.4 BTP buffer (50mM BTP, 150 mM NaCl) containing 1mM $MnCl_2$ at 37°C.

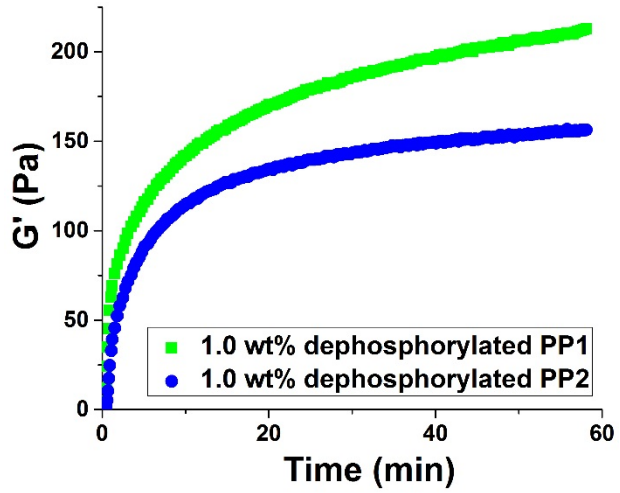

Figure S9. Time-dependent rheology shows hydrogel formation of 1.0 wt% dephosphorylated PP1 and PP2 in pH 7.4 BTP buffer (50mM BTP, 150 mM NaCl) containing 1mM $MnCl_2$ at 37°C.

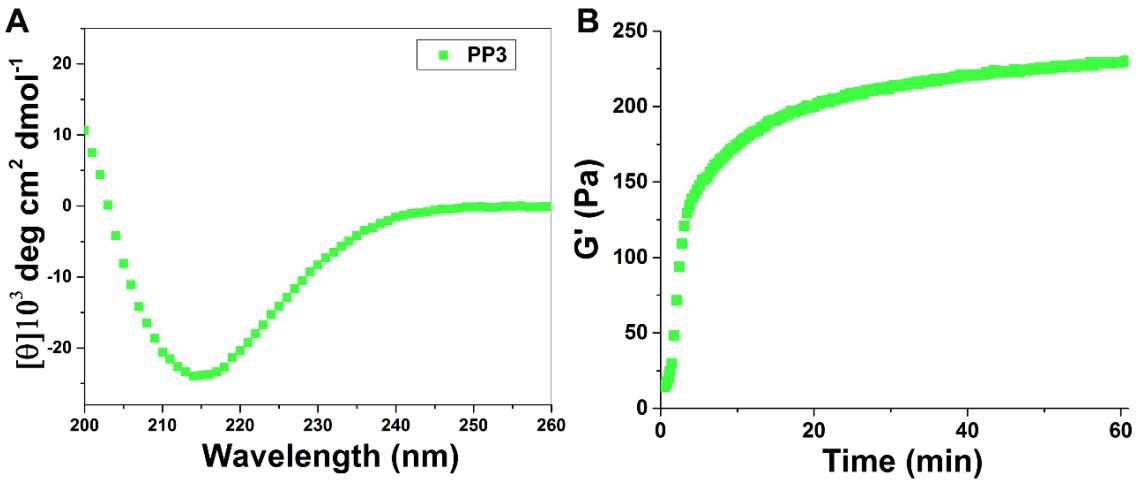

Figure S10. A) CD spectrum of 0.5 wt% phosphorylated PP3 in buffer (pH 7.4, 50mM BTP, 150 mM NaCl), indicating the formation of β-sheet structure. B) Time-dependent rheology shows hydrogel formation of 0.5 wt% phosphorylated PP3 in pH 7.4 BTP buffer (50mM BTP, 150 mM NaCl) at 37ºC.

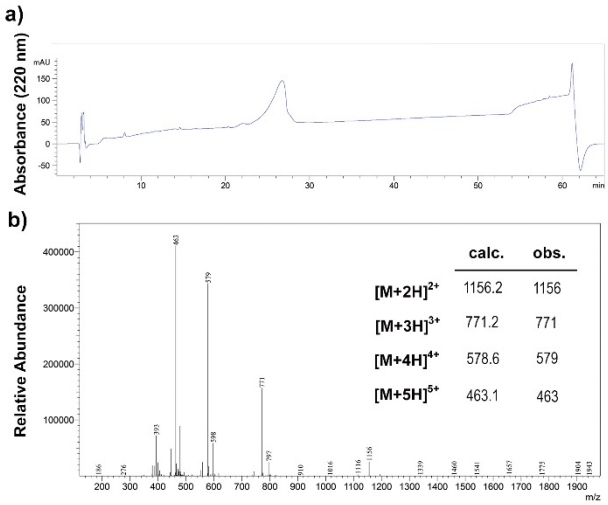

Figure S11. Analytic HPLC of LC-MS Spectra of assembled phosphorylated PP3 after treatment with LPP (0.575 U/μL) for 6 months. No change in mass is observed.

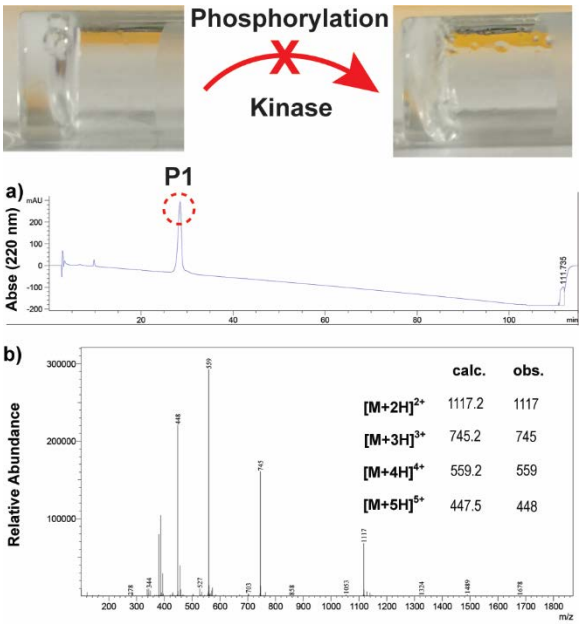

Figure S12. Optical images of hydrogel of dephosphorylated PP1 (labeled P1 in Figure) treated with or without protein kinase A (PKA, 25, 000 U) for 3 days. Analytic HPLC of LC-MS Spectra of assembled dephosphorylated PP1 after treatment with PKA indicate that the self-assembled dephosphorylated PP1 is unable to be phosphorylated by the enzyme and as a consequence, gel disassembly does not occur.

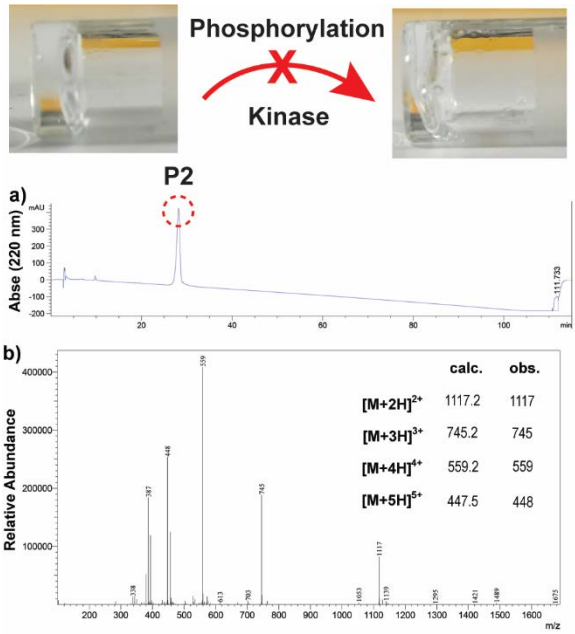

Figure S13. Optical images of hydrogel of dephosphorylated PP2 (labeled P2 in Figure) treated with or without protein kinase A (PKA, 25, 000 U) for 3 days. Analytic HPLC of LC-MS Spectra of assembled dephosphorylated PP1 after treatment with PKA indicate that the self-assembled dephosphorylated PP2 is unable to be phosphorylated by the enzyme and as a consequence, gel disassembly does not occur.

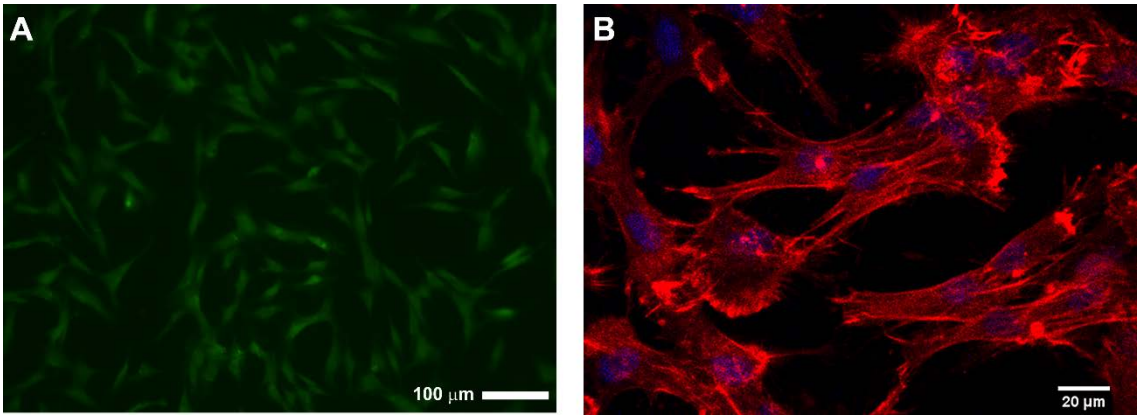

Figure S14. (A) Live/dead assay and (B) F-actin staining of human dermal fibroblasts after being seeded onto 1.0 wt% dephosphorylated PP2 hydrogel for 24h.

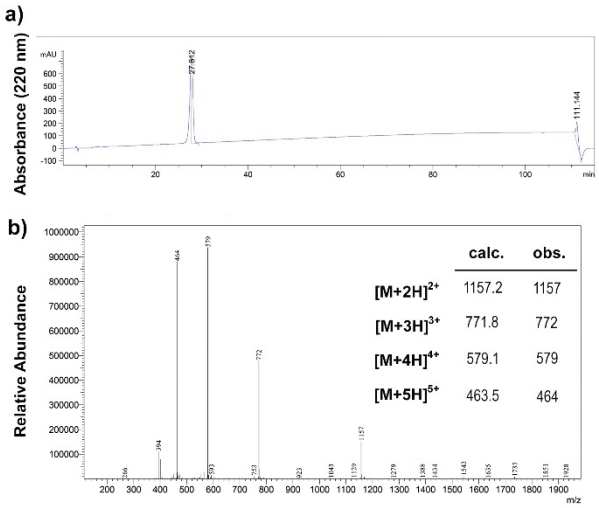

Figure S15. a) Analytical HPLC (0%-100% B over 100 min) and b) ESI (+) mass spectrum of purified PP1.

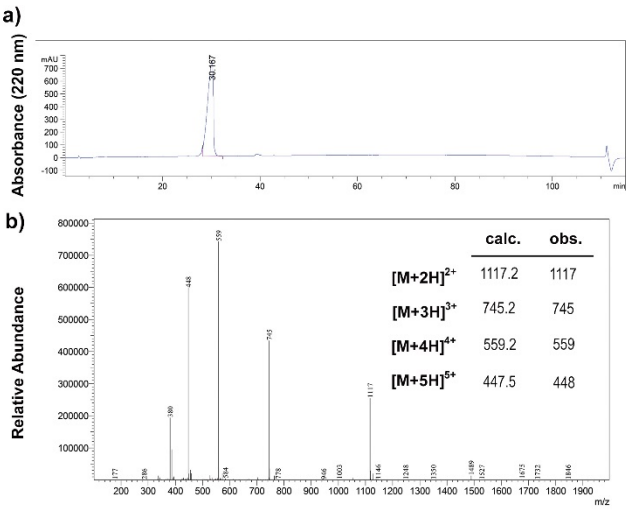

Figure S16. a) Analytical HPLC (0%-100% B over 100 min) and b) ESI (+) mass spectrum of purified dephosphorylated PP1.

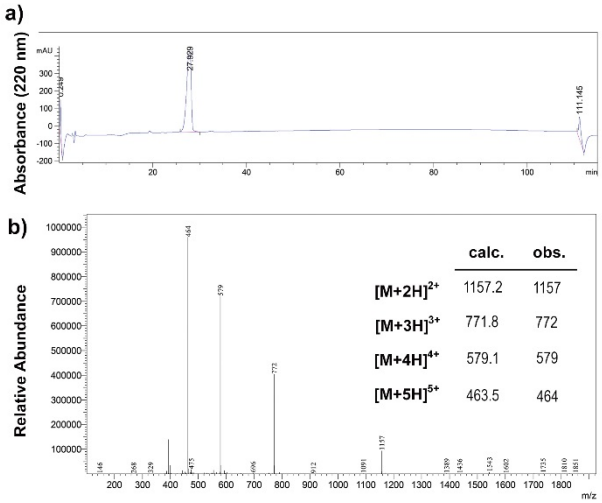

Figure S17. a) Analytical HPLC (0%-100% B over 100 min) and b) ESI (+) mass spectrum of purified PP2.

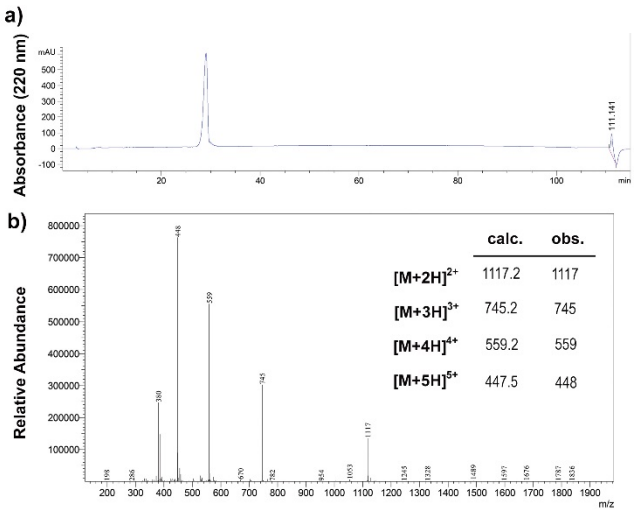

Figure S18. a) Analytical HPLC (0%-100% B over 100 min) and b) ESI (+) mass spectrum of purified dephosphorylated PP2.

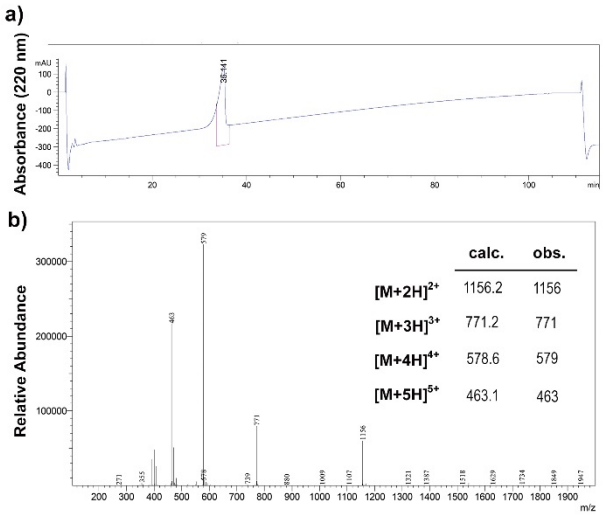

Figure S19. a) Analytical HPLC (0%-100% B over 100 min) and b) ESI (+) mass spectrum of purified PP3.

Supplemental References

[1] J. P. Schneider, D. J. Pochan, B. Ozbas, K. Rajagopal, L. Pakstis, J. Kretsinger, *J. Am. Chem. Soc.* **2002**, *124*, 15030-15037.