Supporting Information

Chemical Signal Regulated Injectable Coacervate Hydrogels

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1. Materials and methods

Materials and instruments

3-(trimethylsilyl)-1-propanesulfonic acid sodium salt (**DSS**), pyrrolidine (**P**), glycine (**Gly**), sodium-3-mercaptopropane-sulfonate (**SH-Na**), 2-acrylamido-2-methylpropane sulfonic acid sodium salt (**AMPS**), 4-((((2-carboxyethyl)-thio)-carbonothioyl)-thio)-4-cyanopentanoic acid (**CETCPA**), rhodamine B, acrylamide (**AAm**), potassium persulfate (**KPS**), *N,N*-methylene-bis(acrylamide) (**MBAA**), Ham's F-12 nutrient mix (cell culture media (**CM**), containing 4.73 mM primary amine, 2.41 mM secondary amine, 0.2 mM thiol and 150 mM inorganic salt, no phenol red, for gel degradation tests), sodium phosphate monobasic, sodium phosphate dibasic, 1,4-dioxane, methanol, chloroform and diethyl ether were purchased from Sigma Aldrich. D_2O (99.96% D) and MeOD (99.8% D) were obtained from Eurisotop. Culture media (DMEM, high glucose, for cell cytotoxicity tests) and NCS (newborn calf serum) were from Gibco. NIH/3T3 cell (mouse fibroblast cells, CRL-1658) culture line was obtained from American Type Culture Collection.

4-vinyl pyridine (**VP**) and *N,N*-dimethylacrylamide (**DMA**) were purchased from Sigma Aldrich and passed through basic alumina to remove inhibitor before use.

Methyl-2-(acetoxymethyl)-acrylate (**ME**) was synthesized according to literature.[1] Chain transfer agent **BDMAT** was synthesized according to literature.[2] Diethyl(α-acetoxymethyl) vinylphosphonate (**DVP**) was synthesized according to literature.[3,4]

The LED reactor (for polymerization) was constructed from a 5-meter strip of 300 RGB 5050 SMD LEDs procured from Ryslux (ebay) with λ_{max} of the blue lights measured to be 441 nm (Ocean Optics USB 4000 fiber coupled spectrometer). These LEDs were wound around a glass beaker of diameter 10 cm.

The tubes (20 mL volume, 27 mm diameter) for rheological measurements were obtained from VWR and were cut to 30 mm height before use.

20G, 21G and 26G needles were purchased from VWR.

NMR spectroscopy

^{1}H-NMR spectra were recorded on an Agilent-400 MR DD2 operating at 400 MHz (at 25°C).

Polymerization conversion (ρ) was calculated by monitoring the reduction of monitoring reduction in the ^{1}H-NMR integrals of the monomer unsaturated protons (∫ M: 5.50 – 6.70 ppm for **VP**, 5.60 – 6.80 ppm for **DMA**, 5.55 – 5.90 ppm for **AMPS**) and the aromatic protons in case of **VP** (7.5 ppm) relative to the internal standard **DSS** (0 ppm). In the case of a copolymerization with both **VP** and **DMA**, the conversion of both monomers was calculated with Equation S1.

$$\rho = \frac{\int M\,(t0) - \int M\,(t)}{\int M\,(t0)} \qquad \text{Equation S1}$$

For a polymerization containing z monomers, $M_{n,conv}$ was calculated according to Equation S2. Here $[M_x]_0$ is the initial concentration of monomer x, $[CTA]_0$ is the initial chain transfer agent (CTA) concentration and M_{Mx} and M_{CTA} are the monomer x and CTA molecular weights, respectively.

$$M_{n,conv} = \sum_{x=1}^{z} \rho_x \times \frac{[M_x]_0}{[CTA]_0} \times M_{Mx} + M_{CTA} \qquad \text{Equation S2}$$

Gel permeation chromatography

The GPC measurements were carried out using a Shimadzu GPC with DMF LiBr (25 mM) as eluent for vinyl pyridine-based polymer (**PVP1** and **PVP2**) characterization or a Shimadzu GPC with aqueous pH 8.0 buffer as eluent for

polyanionic polymer (**PAMPS$_{236}$**) characterization. The DMF system was equipped with a Shimadzu CTO-20AC Column oven, a Shimadzu RID-10A refractive index detector, a Shimadzu SPD-20A UV-Vis detector, PLgel guard column (MIXED, 5 µm), 50 mm x 7.5 mm, and 1x Agilent PLGel (MIXED-C, 5 µm), 300 mm x 7.5 mm, providing an effective molar mass range of 200 to 2 x 10^6 g/mol. DMF LiBr (25 mM) was used as an eluent with a flow rate of 1.0 mL/min at 50°C. The GPC columns were calibrated with low dispersity PMMA standards (Sigma Aldrich) ranging from 800 to 2.2 x 10^6 g/mol, and molar masses are reported as PMMA equivalents. The aqueous system was equipped with a Shimadzu CTO-20AC Column oven, a Shimadzu RID-20A refractive index detector, PL aquagel-OH guard column (8 µm), 50 x 7.5 mm, and 2 x Agilent PL-AquaGel-OH columns (Mixed H, 8 µm), each 300 mm x 7.5 mm^2, providing an effective molar mass range of 100 to 10^7 g/mol. Aqueous buffer was prepared containing 80% (0.20 M $NaNO_3$, 0.01 M NaH_2PO_4 in DI water) and 20% methanol adjusted to pH 8 and filtered through a 0.45 µm PTFE filter. The filtered aqueous buffer was used as an eluent with a flow rate of 1.0 mL/min at 40°C. The GPC columns were calibrated with low dispersity PEO standards (Sigma Aldrich) ranging from 238 to 969000 g/mol, and molar masses are reported as PEO equivalents. A 3rd-order polynomial was used to fit the log Mp vs. time calibration curve for both systems, which was near linear across the molar mass ranges.

Dynamic light scattering

The DLS measurements were performed on a Malvern Zetasizer Nano ZS with a 633 nm laser at a back-scattering angle of 173°. The samples were measured in Brand semi-micro PMMA cuvettes (10 mm path length) sealed with parafilm to prevent solvent loss during experiments (at 25°C).

UV absorption

The UV absorption measurements were performed on a SHIMADZU 1800

spectrophotometer. The samples were measured at 25°C.

Rheometer

The rheological measurements were performed on a rheometer (AR-G2, TA instruments) equipped with a steel plate-and-plate geometry of 20 mm in diameter and equipped with water trap. The sample was placed in a tube (which was glued onto the test platform) of 27 mm in diameter (see Figure S10A). All experiments were performed at 25°C. After adding the reagents into the solutions or gels, we moved the tube to a shaker for 3 min shaking. Finally, we return it to the rheometer for further rheology measurements. Note that the reactant mixing is largely convective. This is clear for the gelation step, because the initial solution is shaken. As the gel breakdown occurs fairly rapidly (the 3 min shaking is already enough to give full breakdown), thus the diffusion timescales of small reagents likely have negligible impact on the observed behavior.

For the tube-free control experiment, the gel sample was directly placed on the test platform for frequency-sweep rheological measurements.

For the time sweep oscillatory tests, the strain (γ) and frequency (ω) were set as 5% and 10 rad/s, respectively. Frequency sweep experiments were performed from 100 rad/s to 0.1 rad/s at fixed strain (γ = 5%), and the tests were carried out until storage modulus (G') reached to equilibrium state. Strain sweep measurements were performed from 0.1% to 1000% at fixed frequency (ω = 10 rad/s). Fixed-frequency measurements with repeated strain jumps from 5% to 500% and back were measured at ω = 10 rad/s (each test lasted 5 min).

Note: During the experiments, the upper plate of the rheometer just touches the upper surface of the gel material. There is no stress from either edges or top face of the plate. An additional control experiment was performed (Figure

S10B), showing there is negligible difference for the measurements of hydrogel samples with or without the tube.

2. Polymer synthesis and characterization

ABA triblock copolymer (PVP1) synthesis

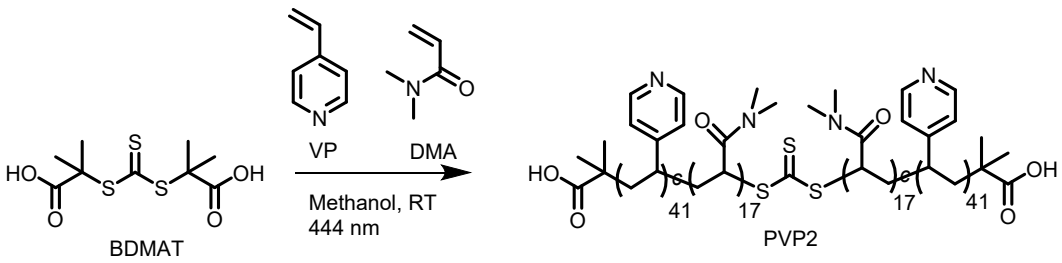


Figure S1. Synthesis route of **PVP2** with **BDMAT** as chain transfer agent.

To synthesize the copolymer **PVP2**, **VP** (2.696 mL), **DMA** (2.576 mL), **BDMAT** (70.6 mg) and **DSS** (54.58 mg) were separately dissolved in MeOD (4.728 mL), and subsequently mixed in a 50 mL flask. Then, the flask was sealed and deoxygenated by bubbling with nitrogen. Finally, the reaction solution was irradiated with a LED lamp (444 nm) at 25°C, and NMR was applied to follow the reaction conversion. For purification, the reaction solution was diluted by 10 mL chloroform and dripped into 1000 mL diethyl ether for excess monomer removal. The precipitate in diethyl ether was collected by filtration. After that, the crude product was re-dissolved in chloroform and precipitated again in diethyl ether. Finally, the product (3 g) was collected and dried in a vacuum drier for 24 h.

The NMR spectra of **PVP2** can be found in Figure S4.

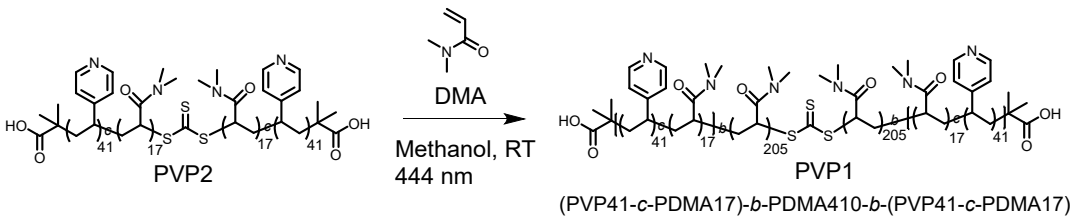


Figure S2. Synthesis route of the ABA triblock copolymer **PVP1** with **PVP2** as macro-chain-transfer agent.

For the synthesis of triblock copolymer **PVP1**, macro-chain-transfer agent **PVP2** (2.839 g) and **DMA** (10.82 mL) were separately dissolved in MeOD (24.18 mL), and subsequently mixed in a 50 mL flask. Then, the flask was sealed and deoxygenated by bubbling with nitrogen. Finally, the reaction solution was irradiated with a LED lamp (444 nm) at 25°C, and NMR was applied to follow the reaction conversion. For purification, the reaction solution was diluted by 10 mL chloroform and dripped into 1500 mL diethyl ether for excess monomer removal. The precipitate in diethyl ether was collected by filtration. After that, the crude product was re-dissolved in chloroform and precipitated again in diethyl ether. Finally, the product (8 g) was collected and dried in a vacuum drier for 24 h.

The NMR spectra of **PVP1** can be found in Figure S4 and the GPC data for chain extension can be found in Figure S7.

Polyanion (PAMPS$_{236}$) synthesis

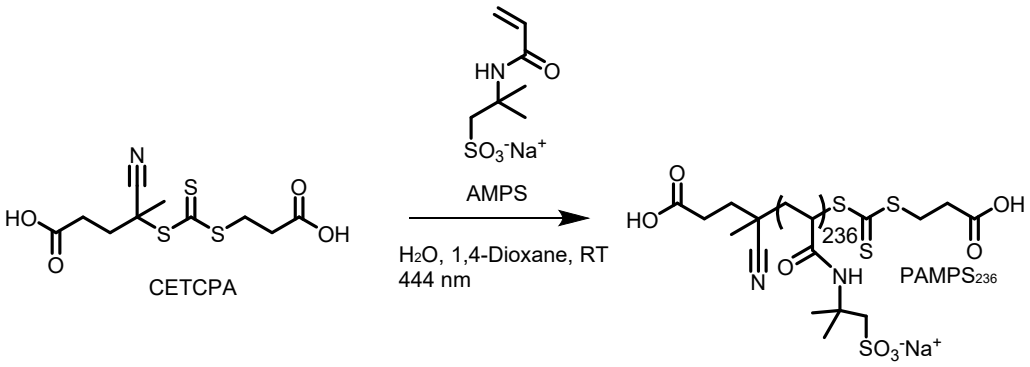


Figure S3. Synthesis of the anionic polymer **PAMPS$_{236}$** with **CETCPA** as chain transfer agent.

For the synthesis of polymer **PAMPS$_{236}$**, **AMPS** (2.2923 g), **DSS** (8.73 mg) and **CETCPA** (12.3 mg) were separately added in a 50 mL flask, then the reactants were dissolved in the mixture of D_2O (4.5 mL) and 1,4-dioxane (0.5 mL). After

that, the flask was sealed and deoxygenated by bubbling with nitrogen. Finally, the reaction solution was irradiated with a LED lamp (444 nm) at 25°C, and NMR was applied to follow the reaction conversion. For purification, the reaction solution was dialyzed in pure water for 24 h and freeze-dried to get the anionic polymer **$PAMPS_{236}$** (2 g).

The NMR spectra of **$PAMPS_{236}$** can be found in Figure S6.

Table S1. ABA Triblock copolymer synthesis and characterization data

Polymer	CTA	[CTA]0: [DMA]0: [VP]0	Polymerization time (h)	NMR conversion (%)	Mn,conv (kDa)
PVP2	BDMAT	1:100:100	31.0	80.43 (VP), 32.98 (DMA)	12.3
PVP1	PVP2	1:455	18.0	90	52.9

Table S2. Polyanion synthesis and characterization data

Polymer	CTA	[CTA]0: [AMPS]0	Polymerization time (h)	NMR conversion (%)	Mn,conv (kDa)
$PAMPS_{236}$	CETCPA	1:250	2.0	94	54.4

Table S3. Molecular weight characterization data for RAFT synthesized polymers

Polymer	$M_{n,conv}$ (kDa)	$M_{n,GPC}$ (kDa)	$Đ$	GPC system
PVP2	12.3	11.6	1.35	DMF
PVP1	52.9	106.8	1.31	DMF
$PAMPS_{236}$	54.4	39.3	1.30	Aqueous

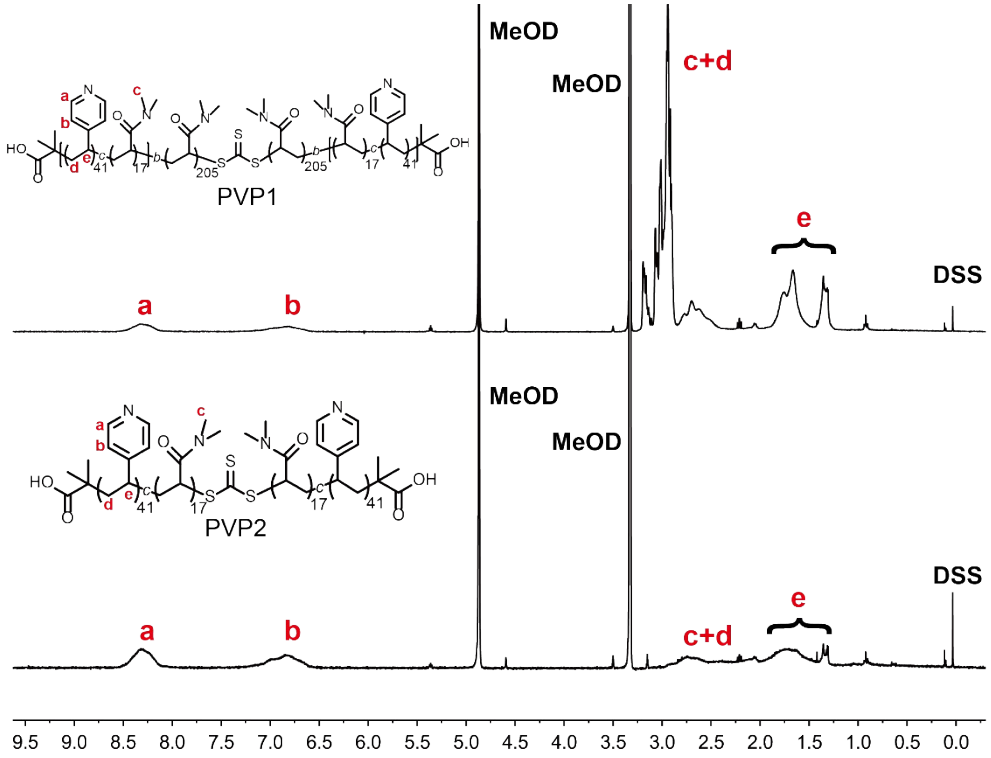


Figure S4. ^{1}H-NMR stacked spectra of **PVP2** (bottom) and **PVP1** (top) in MeOD.

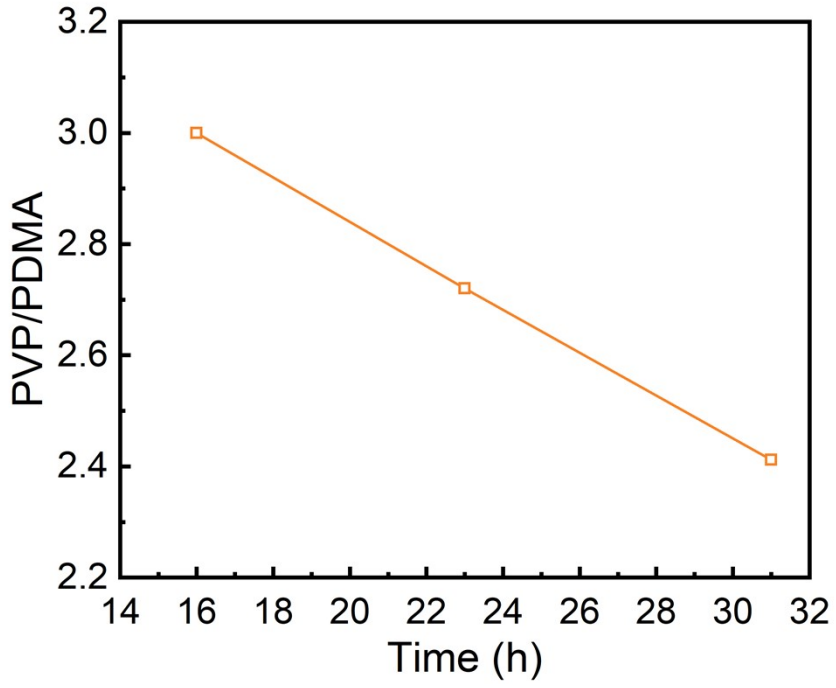


Figure S5. The chain length ratio of PVP and PDMA in the end block of **PVP1** as a function of reaction time.

Figure S5 shows the PVP/PDMA ratio decreases with reaction time, indicating

that the reactivity ratio is not unity. This will result in a compositional gradient. Based on this data, we expect that the at the ends of the A blocks will have a relatively higher **VP** content which decreases towards to central B block.

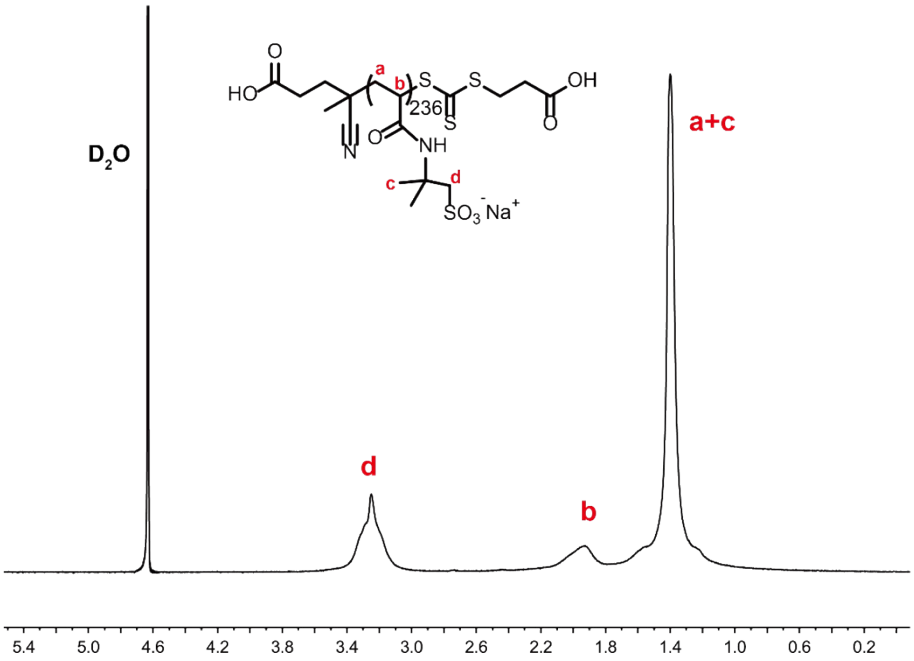


Figure S6. ^{1}H-NMR spectra of **PAMPS$_{236}$** in D_2O.

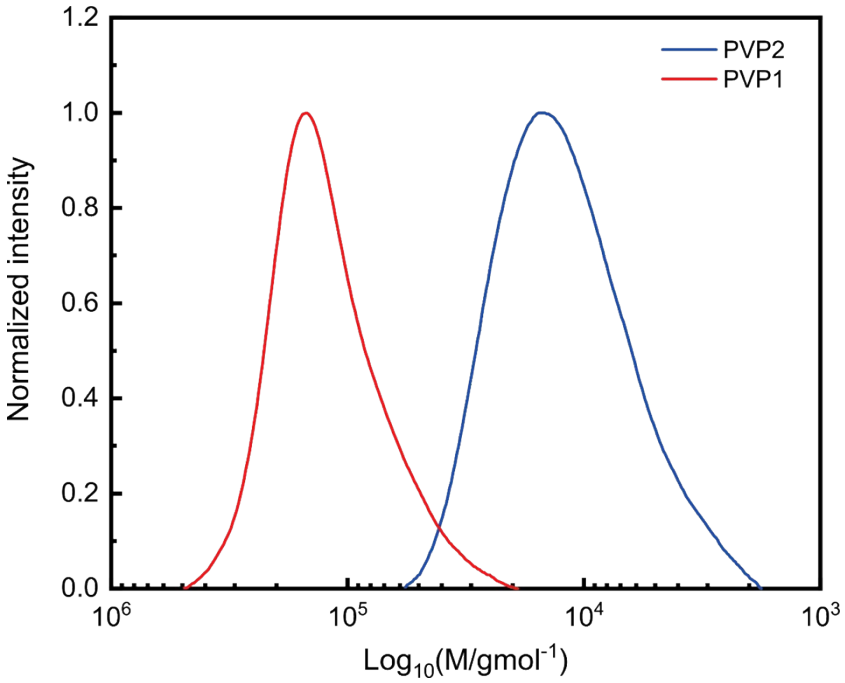


Figure S7. GPC data for chain extension from **PVP2** (blue line) to **PVP1** (red line).

3. Sample preparation

pK_a tests

The aqueous solution of PVP1 (0.035 wt%) were prepared at pH 1.79. The solution pH was cautiously increased by adding 1-2 μL aliquots of aqueous NaOH solution with constant stirring. Following that, the pH and UV absorption of solution were recorded at room temperature. To obtain the pK_a of PVP1, the absorbance at 253 nm was plotted against the solution pH. These data were fitted with an equation from literature by nonlinear curve fitting procedure in Origin software Version 8.[5] The pKa values were taken from the best fit model with ten iterative fitting.

^{1}H-NMR tests

All samples were prepared in 100 mM pH 7.4 phosphate buffer (in D_2O) at 25°C (considering there were non-deuterated H_2O molecules from phosphate buffer stock solution, we suppressed the water peak during our NMR tests), the concentration of **PVP1** was kept at 0.5 wt%. Typically, 5 mg **PVP1**, 332 μL phosphate buffer stock solution (300 mM) and 663 μL D_2O were combined in an NMR tube, followed by addition of **ME** (1.0 eq.) and **P** (1.0 eq.) with the reaction monitored by ^{1}H-NMR. Selected NMR spectra can be found in Figure S8.

DLS tests

All samples were prepared in 100 mM phosphate buffer at 25°C, the concentration of **PVP1** was kept at 0.1 wt%. Typically, for **$PAMPS_{236}$**-free samples,1 mg **PVP1**, 333 μL phosphate buffer stock solution (300 mM, pH 7.4) and 667 μL H_2O were combined in a DLS vial, followed by addition of **ME** (1.0 eq.) and **P** (1.0 eq.) and analyzed by DLS over time. For samples with polyanions, we added the **$PAMPS_{236}$** (1.0 eq.) into 0.1 wt% **PVP1** solution before treated with any chemical signals (1.0 eq. **$PAMPS_{236}$** means that the molar mass for **AMPS** and **VP** are equal). Additional discussions on DLS data

were shown in Figure S11A, S11B, S12A and S12B.

Rheological tests

All samples were in 100 mM pH 7.4 phosphate buffer prepared at 25°C. A 5 wt% coacervate gel can be prepared as below: 50 mg **PVP1** (0.0776 mmol pyridine groups) was added into the vial, then 358 μL H_2O, 317 μL phosphate buffer stock solution (300 mM, pH 7.4) and 275 μL **PAMPS$_{236}$** stock solution (65 mg/mL, 1.195 mM) were added. For the samples which were dyed by rhodamine B (1 μM in the system), 2 μL stock solution of rhodamine B (0.475 mM) was added into the solution. Finally, the vial was placed on a shaker for overnight shaking.

To initiate the gelation, 12.27 mg (11.33 μL, 0.0776 mmol) methyl-2-(acetoxymethyl)-acrylate (1.0 eq. **ME**) was added into the as-prepared solution, then the vial was shook for 3 min and waited for 2 hours for complete reaction.

To disassemble the gel, 5.52 mg (6.37 μL, 0.0776 mmol) pyrrolidine (1.0 eq. **P**) was added on the gel surface, then the vial was shook for 3 min, the solution can be observed. And we illustrated that shaking cannot trigger the sol-gel transitions (see Figure S13).

Diagram of material states

All samples were prepared in 100 mM pH 7.4 phosphate buffer at 25°C. The polymer solutions (1 wt%, 2 wt%, 5 wt% and 10 wt% **PVP1**, with 1.0 eq. **PAMPS$_{236}$**) can be prepared as above. For 15 wt% and 20 wt% samples, as we would like to investigate the formation of hydrophobic micelle gel, there were no **PAMPS$_{236}$** and **ME** inside the **PVP1** solutions.

To induce gel formation, 1.0 eq. **ME** was added into the as-prepared solutions with different polymer mass fractions (1 wt%, 2 wt%, 5 wt% and 10 wt% **PVP1**, with 1.0 eq. **PAMPS$_{236}$**), then the samples was shook for mixing well and

stabilized overnight for further rheological tests. For 15 wt% and 20 wt% **PVP1** samples, they were directly used for rheological tests when the **PVP1** was completely dissolved.

Swollen PAAm gels

Two steps were required to prepare the swollen **PAAm** gels. First step was to synthesis a **PAAm** gel: 250 mg **AAm**, 120 μL **MBAA** stock solution (1.056 mg/mL), 300 μL **KPS** stock solution (4.5 mg/mL) and 580 μL H_2O were combined in a sealed vial, then the solution was placed in the 75°C oven for gelation. After 4 hours, a transparent **PAAm** gel was formed and can be used for next step.

Next step was to exchange the solvent for as-prepared **PAAm** gel: The as-prepared **PAAm** gel was immersed in 20 mL solvents (water or 150 mM pH 7.4 phosphate buffer or cell culture media). The solvent was then replaced with fresh solvent every 6 hours. After 18 hours, the swollen **PAAm** gel can be used after removal of excess solvent on its surface.

Self-healing tests

To observe the macroscopic self-healing behavior of coacervate gels, we prepared two coacervate gels (5 wt% **PVP1** and 1.0 eq. $\mathbf{PAMPS_{236}}$, initiated their gelation with 1.0 eq. **ME**) separately (one was stained by rhodamine B, another one is unstained). Then we pressed two gels together and kept them in a sealed beaker for 5 min. After that, the two separate gels became to one whole gel. When the self-healed gel was kept for 2 days, the dye diffused into everywhere of the whole gel.

Injection tests

In air: The as-prepared gels (5 wt% **PVP1** and 1.0 eq. $\mathbf{PAMPS_{236}}$, activated by 1.0 eq. **ME**) were put into a needle syringe by a tweezer and a needle plunge, then the gel was extruded through either 20G, 21G or 26G needles. We took a

video for recording the injection process with a 26G needle (see Movie S1).

Into a **PAAm** gel: The coacervate gel was put into a needle syringe by a tweezer and a needle plunge, then the gel was injected through a 26G needle into the water or 150 mM pH 7.4 phosphate buffer or cell culture media swollen **PAAm** gels. We also took a video for recording the whole injection process (into a water swollen **PAAm** gel) (see Movie S2).

Degradation tests (in liquid environments)

We injected the coacervate gels into six separate culture dishes. We divided them into two groups: one was kept at room temperature (25°C), while another was kept at physiological temperature (37°C). Each group contained three different culture environments: water, **PB** (150 mM phosphate buffer, pH = 7.4) and **CM** (cell culture media) for comparison.

Specifically, a 5 wt% coacervate gel (with 76.5 mg **PVP1** and 1.0 eq. **PAMPS$_{236}$**, 1.0 eq. **ME** initiated) was prepared and put into a syringe. Then, we injected it onto a culture dish, the mass of the extruded gel should be recorded. Next, a certain volume of media (water or **PB** or **CM**) was added for disassembling the coacervate gel (the calculation of media volume can be found below). Finally, we took the photos to follow the degradation of coacervate gels over time: at 0 h, 0.5 h, 1 h, 1.5 h, 2 h, 3 h, 4 h, 7 h, 12 h, 24 h, 48 h and 72 h.

Calculation of media volume: The initial mass of the coacervate gel was 1530 mg which was made from 76.5 mg **PVP1** (0.1188 mmol **VP**). We injected 52.48 mg gel (the molar mass of **VP** for injected gel was 52.48 / 1530 x 0.1188 = 0.004075 mmol) onto the culture dish. To create an amine-rich environment, we kept the molar mass of amines 4 times as much as pyridine groups (To simplify calculation, the concentration of amines in cell culture media was counted as 4 mM). Thus, the volume of **CM** was 0.004075 x 4 / 4 = 0.004075 L = 4.075 mL. The volumes of **PB** and water were calculated as above. In Table

S4, we listed the calculated media volume in our experiments.

Table S4. Volume of media for degradation tests (in liquid environment)

	In water (25°C)	In PB (25°C)	In CM (25°C)	In water (37°C)	In PB (37°C)	In CM (37°C)
Volume of media / mL	4.20	4.08	4.08	4.15	4.03	4.25
Mass of injected coacervate gels / mg	54.1	52.5	52.5	53.4	51.9	54.7

Degradation tests (in swollen PAAm gels)

We injected the coacervate gels (initiated by 1.0 eq. **ME**) into six separate **PAAm** gels (swollen in different media). And we divided them into two groups: one was kept at room temperature (25°C), while the other was kept at physiological temperature (37°C). Each group contained three different swollen **PAAm** gels: water swollen, **PB**-swollen and **CM**-swollen **PAAm** gels for comparison.

First, a 5 wt% coacervate gel (with 76.5 mg **PVP1**, 1.0 eq. **ME** activated) was prepared and put into a syringe. Then, we injected it into the swollen **PAAm** gels for recording its degradation and the photos were taken over time: at 0 h, 0.5 h, 1 h, 2 h, 4 h, 7 h, 12 h, 24 h, 36 h and 48 h.

Note: As it was difficult to control the final mass of swollen **PAAm** gels, we tried to control the mass of injected coacervate gels. We firstly defined that the mass increment of **PAAm** gel was: $\Delta m = m_{swollen} - m_{initial}$. Specifically, we injected 15 mg coacervate gel into the swollen **PAAm** gel with minimum mass increment. For other swollen **PAAm** gels with higher mass increments, the mass of injected gel was scaled up (compare with the value of minimum mass increment) based on the mass increment of swollen **PAAm** gels. Since the mass increments of the swollen **PAAm** gels were much higher than injected

pyridine-based polymer, we believed that the swollen **PAAm** gels possessed an amino acid-rich environment for coacervate gels degradation. In Table S5, we listed the mass for the injected coacervate gels in our experiments.

Table S5. Mass of injected coacervate gel (into swollen **PAAm** gels) for degradation tests

	In water swollen PAAm gel (25°C)	**In PB-swollen PAAm gel (25°C)**	**In CM-swollen PAAm gel (25°C)**	**In water swollen PAAm gel (37°C)**	**In PB-swollen PAAm gel (37°C)**	**In CM-swollen PAAm gel (37°C)**
Mass increment for swollen PAAm gels / g	1.72	1.68	1.62	1.64	1.75	1.59
Mass of injected coacervate gels / mg	16.2	15.8	15.3	15.5	16.5	15.0

Cell cytotoxicity tests

The coacervate hydrogel was prepared as described above (with 5 wt% **PVP1** and 1.0 eq. **PAMPS$_{236}$**) and its gelation was activated by 1.0 eq. **ME**. A commercial CCK-8 assay (Adamas) was used in accordance with the manufacturer's instructions. NIH-3T3 mouse fibroblast cells were seeded in a 96 well plate at a density of 5 x 10^3 cells per well. After 24 hours, the culture medium (DMEM, 10% NCS, 1 % Anti Anti) was replaced by fresh media.

For **ME**-activated gel treatments between 20 and 10000 μg/mL (gel concentration in the well), the media was pre-incubated with the gel for 2 h at 37°C in culture medium, causing gel dissolution and then diluted to the required concentration. For **PAMPS$_{236}$** treatments between 7 and 350 μg/mL (**PAMPS$_{236}$** concentration in the well), the media was pre-incubated with the **PAMPS$_{236}$** for

2 h at 37°C in culture medium, then diluted to the required concentration. For **PVP1** treatments between 5 and 500 μg/mL (**PVP1** concentration in the well), the media was pre-incubated with the **PVP1** for 2 h at 37°C in culture medium, then diluted to the required concentration. For **PVP1$^+$** (obtained by adding 1 eq. **ME** into **PVP1** solution between 5 and 500 μg/mL) treatments, the media was pre-incubated with the **PVP1$^+$** for 2 h at 37°C in culture medium, then diluted to the required concentration. For **ME** treatments between 0.1 and 25 μg/mL (**ME** concentration in the well), the media was pre-incubated with the **ME** for 2 h at 37°C in culture medium, then diluted to the required concentration.

A 5 wt% **DVP**-activated coacervate gel can be prepared as below: 11.5 mg **PVP1** (0.0178 mmol pyridine groups, 1.0 eq.) was added into the vial, then 87 μL H_2O, 73 μL phosphate buffer stock solution (300 mM, pH 7.4) and 59 μL **PAMPS$_{236}$** stock solution (70 mg/mL, 1.0 eq.) were added. Finally, 8.4 mg (7.56 μL, 0.0356 mmol) diethyl(α-acetoxymethyl) vinylphosphonate (2.0 eq. **DVP**) was added into the as-prepared solution to initiate the gelation. For **DVP**-activated gel treatments between 1000 and 10000 μg/mL (gel concentration in the well), the media was pre-incubated with the gel for 2 h at 37°C in culture medium, causing gel dissolution and then diluted to the required concentration.

The plate was then incubated at 37°C for 48 h in a humidified, 5% CO_2 atmosphere. Then 10 μL CCK-8 solution was added to each well. This included wells containing no cells with and without gel treatments for background reference. The plate was then further incubated for 4 h with the absorbance at 450 nm measured using a 96-well plate reader.

The viability is then reported as per Equation S3. Note: each treatment was run in triplicate, with average values reported and error bars as standard deviation.

$$\text{Cell viability}\ (\%) = 100 \times \frac{\text{Abs}\ (450,\text{treat}) - \text{Abs}\ (450,\text{no cells})}{\text{Abs}\ (450,\text{no treat}) - \text{Abs}\ (450,\text{no cells})}$$

Equation S3

Abs (450, treat) is the absorbance at 450 nm for cells incubated with coacervate gels, Abs (450, no treat) is the absorbance at 450 nm for cells incubated with culture medium (DMEM, 10% NCS, 1 % Anti Anti), Abs (450, no cells) is the absorbance at 450 nm for vials incubated without addition of CCK-8 solution.

For **DVP** samples, the media was pre-incubated with the **DVP** for 2 h at 37°C in culture medium, then diluted to the required concentration. A commercial MTS assay (Promega CellTitre 96® AQ_{ueous} One Solution) was applied to evaluate the cell viability: After adding 20 μL MTS solution into the well, the plate was incubated for 4 h with the absorbance at 490 nm measured using a 96-well plate reader. And the viability can be calculated based on Equation S3 (with 490 nm absorbance).

4. NMR study of reaction

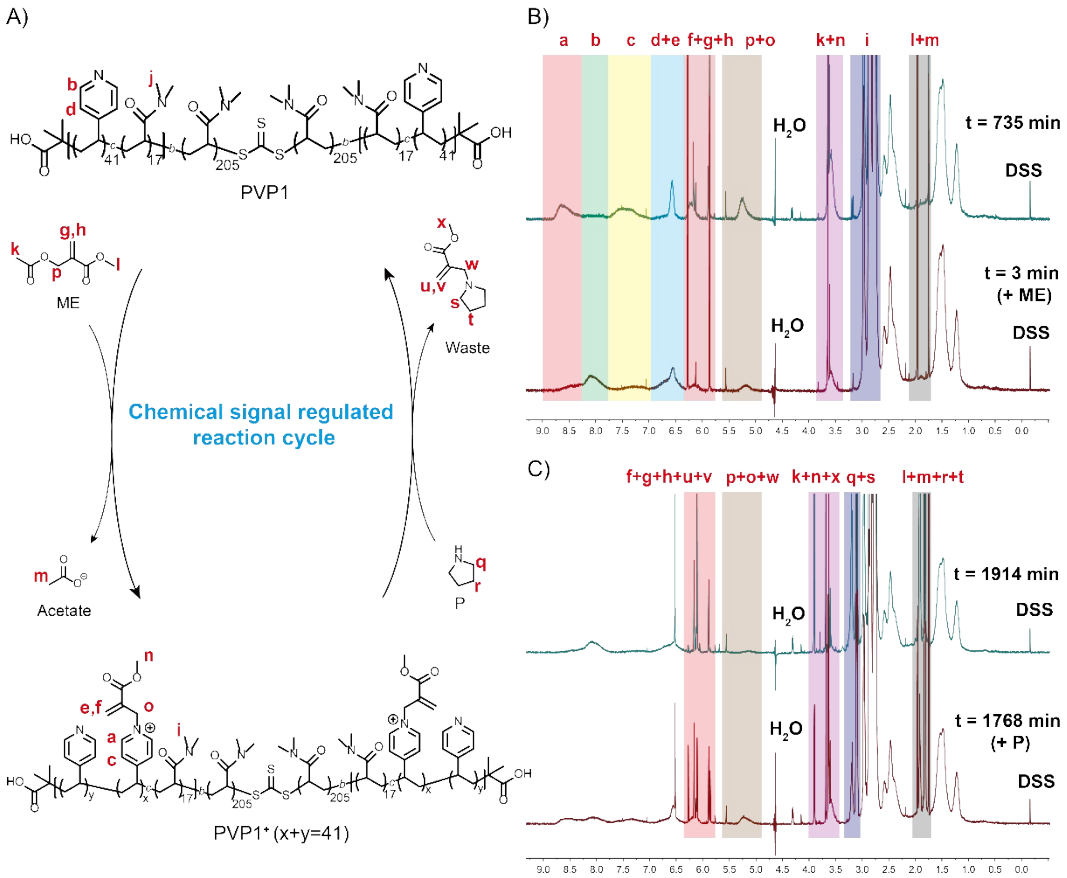


Figure S8. A) Scheme of chemical reaction network (CRN) for reversible cationization of pyridine groups. B) Example ^{1}H-NMR (D_2O with 100 mM, pH 7.4 phosphate buffer, water peak was suppressed) from **ME** (1.0 eq.) activated

PVP1 ionization experiment at t = 3 min and 735 min. Peaks labelled in colors were integrated to quantitate the extent of conversion to cationic **PVP1⁺**. C) Example ^{1}H-NMR (D_2O with 100 mM pH 7.4 phosphate buffer, water peak was suppressed) from **P** (1.0 eq.) triggered **PVP1** de-ionization experiment at t = 1768 min and 1914 min. The mass fraction of **PVP1** for NMR measurements was kept at 0.5 wt%.

5. pK_a study of PVP1

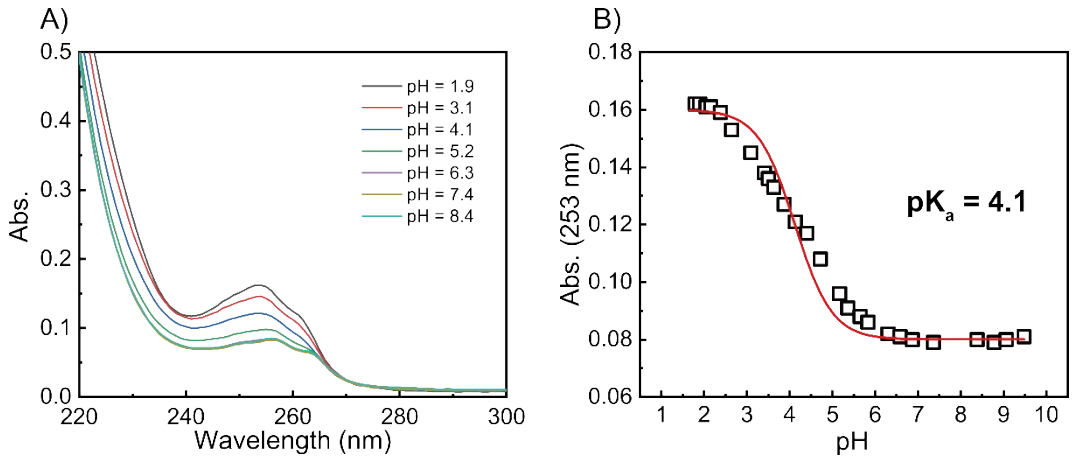


Figure S9. A) Absorption spectra of **PVP1** (0.035 wt%) at different pH values. B) Dependence of the absorbance at 253 nm on pH.

6. Devices for rheological study of coacervate gels

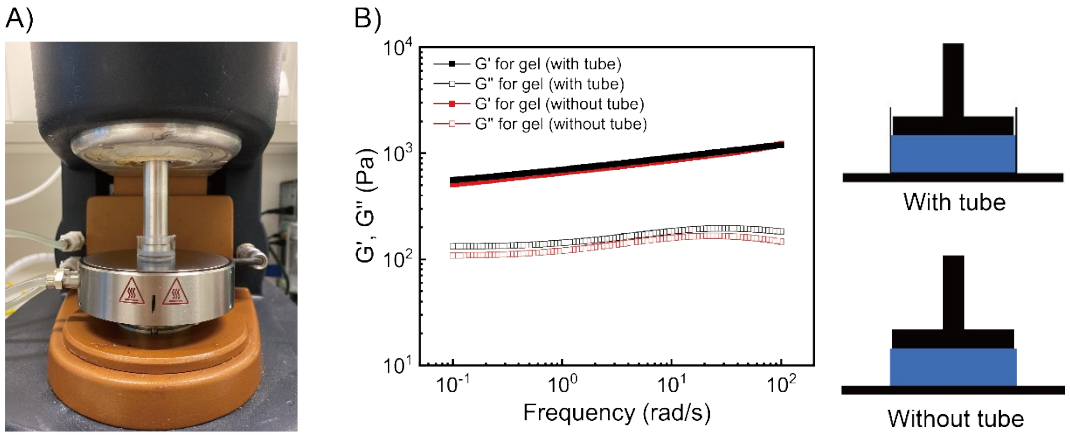


Figure S10. A) Rheometer and home-made tubes with lowered geometry. B) Frequency rheological measurements for detecting the impact of home-made

tubes on mechanical performance of gel. The samples were prepared with 5 wt% **PVP1** and 1.0 eq. **PAMPS$_{236}$** (overall polymer content: 6.8 wt%), in 100 mM pH 7.4 phosphate buffer.

7. Rheological study for sol-gel transition of hydrophobic micelle gels

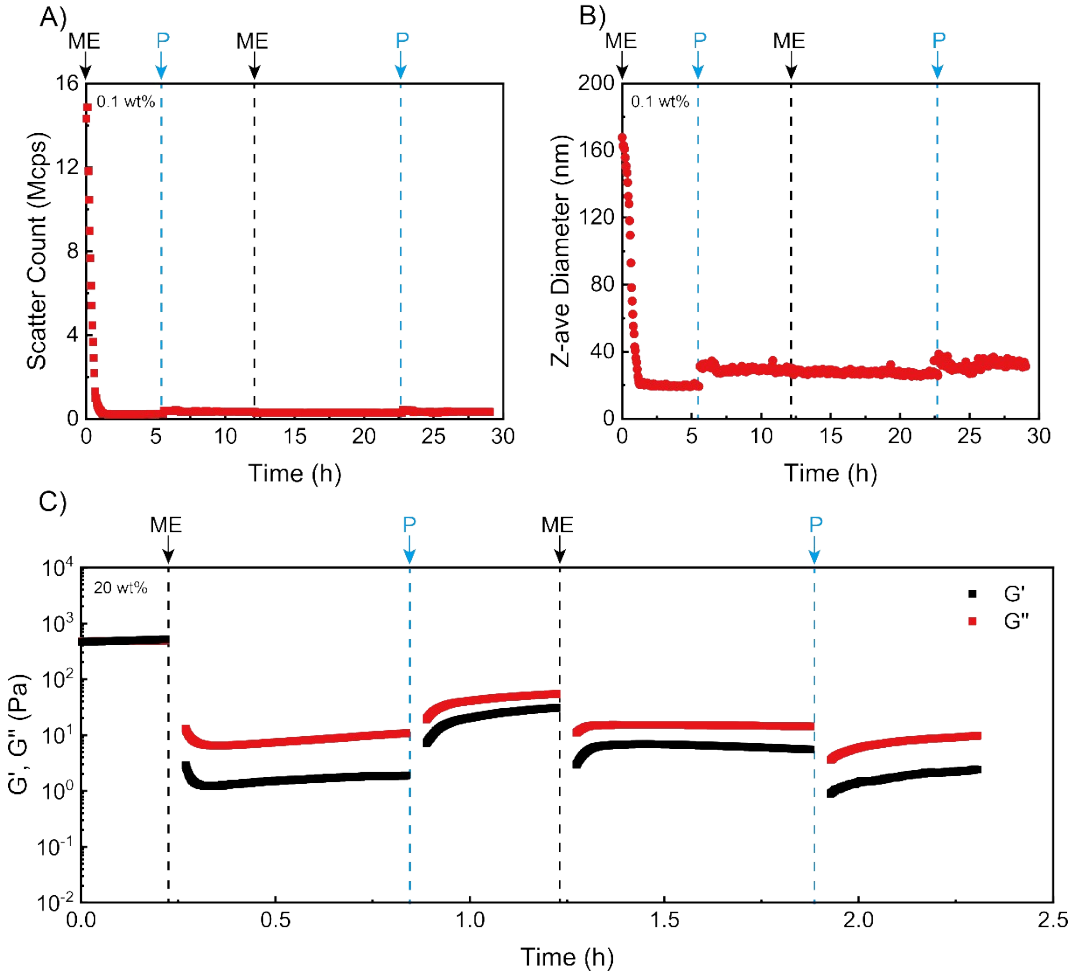


Figure S11. Dynamic light scattering for 2-cycles 1.0 eq. **ME** and **P** sequential additions following A) scatter count and B) Z-average diameter. The samples were prepared in 100 mM pH 7.4 phosphate buffer with 0.1 wt% **PVP1**. C) Rheological test monitoring the **ME**-driven (1.0 eq.) disassembly and **P**-induced (1.0 eq.) recovery process of the hydrophobic micelle gel (2 cycles). The samples were prepared in 100 mM pH 7.4 phosphate buffer with 20 wt% **PVP1**.

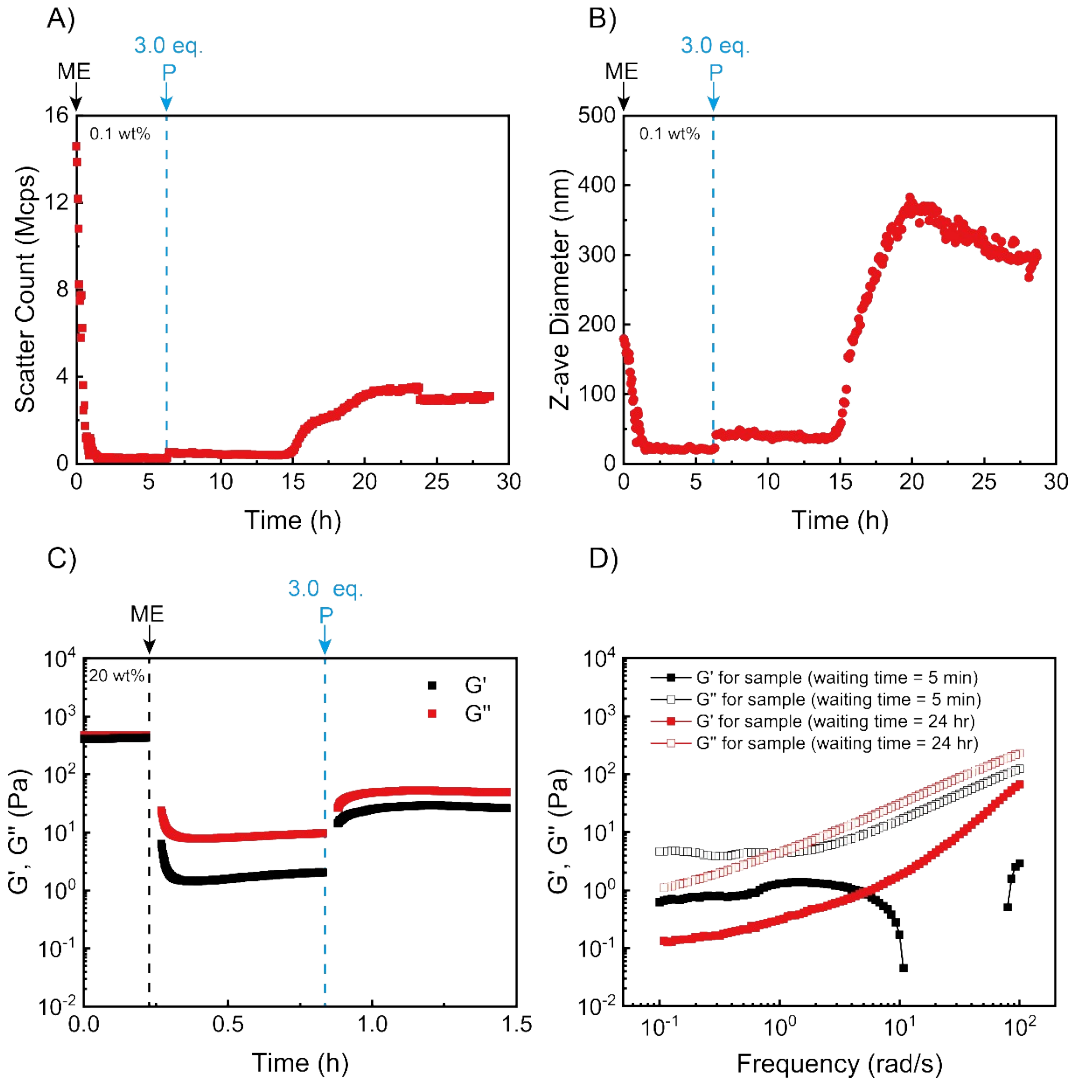


Figure S12. Dynamic light scattering for 1-cycle 1.0 eq. **ME** and 3.0 eq. **P** sequential additions following A) scatter count and B) Z-average diameter. The samples were prepared in 100 mM pH 7.4 phosphate buffer with 0.1 wt% **PVP1**. C) Rheological test monitoring the **ME** (1.0 eq.) driven disassembly and excess **P** (3.0 eq.) induced recovery process of the hydrophobic micelle gel. D) Frequency sweep rheological measurement of the **PVP1** solution which was stabilized for 5 mins and 24 hours with sequential additions of 1.0 eq. **ME** + 3.0 eq. **P**. The samples for rheological measurements were prepared in 100 mM pH 7.4 phosphate buffer with 20 wt% **PVP1**.

We prepared a **PVP1** solution (0.1 wt% **PVP1**, in the absence of **PAMPS$_{236}$**) in 100 mM pH 7.4 phosphate buffer. At the beginning, **PVP1** formed the

hydrophobic micelles by itself with a similar scatter count and size (15 Mcps, 170 nm) as the system with **PAMPS$_{236}$** (Figure S11A and S11B). When the **ME** (1.0 eq.) was added, the neutral hydrophobic pyridine groups were ionized, resulting in micelles disassociation. Then, 1.0 eq. **P** was introduced into solution for regenerating the neutral pyridine groups. However, the hydrophobic micelles did not reappear. We also repeated one more cycle to demonstrate the irreversibility of hydrophobic micelles (Figure S11A and S11B). Even we treated them with excess **P** (3.0 eq.), the scatter count can only increase to 25% as before in further 8 hours, and the particles size cannot recover to the original value (Figure S12A and S12B).

One may wonder whether the hydrophobic micelle gels were reversible. To answer this question, we firstly prepared the hydrophobic micelle gel (20 wt% **PVP1**), then we treated the hydrophobic micelle gel with 1.0 eq. **ME** to disassociate it to liquid state. However, the resultant solution seems not reversible with 1.0 eq. **P** addition (Figure S11C). In fact, even we treated the solution with excess **P** (3.0 eq.), it still cannot recover to starting gel state (Figure S12C). Moreover, we recorded the rheological data for the solution (1.0 eq. **ME** + 3.0 eq. **P** treated) at 5 min and 24 h to see whether the gel could re-appear with different stabilization time (Figure S12C and S12D). We found that there was a slightly increase for G’ and G’’ over time, but the rheological curve still characterized a liquid state.

8. Rheological study for detecting the impact of shaking on sol-gel transition of coacervate gels

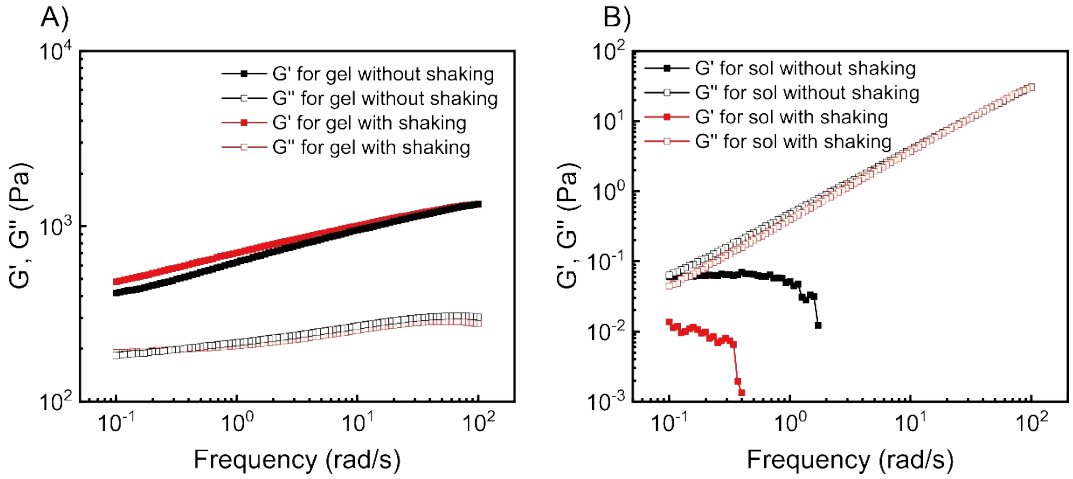


Figure S13. Frequency rheological measurements for detecting the impact of shaking on mechanical performance of A) gel and B) solution. The samples were prepared with 5 wt% **PVP1** and 1.0 eq. **PAMPS$_{236}$** (overall polymer content: 6.8 wt%), in 100 mM pH 7.4 phosphate buffer.

One may wonder whether shaking or the volume change of system (due to addition of chemical signals) could induce the sol-gel (or gel-sol) transition. To answer this question, comparative experiments were conducted. We first prepared the gel (or solution) and recorded their original rheological data, then we added 30 μL H_2O into the vial, shook the samples for 3 min and recorded the rheological data again (see Figure S13). We found that for gel (or solution), shaking or samples volume change (3% increment) of system only showed negligible impacts on mechanical strength and cannot trigger the sol-gel transitions.

9. Rheological study for sol-gel transition of coacervate gels

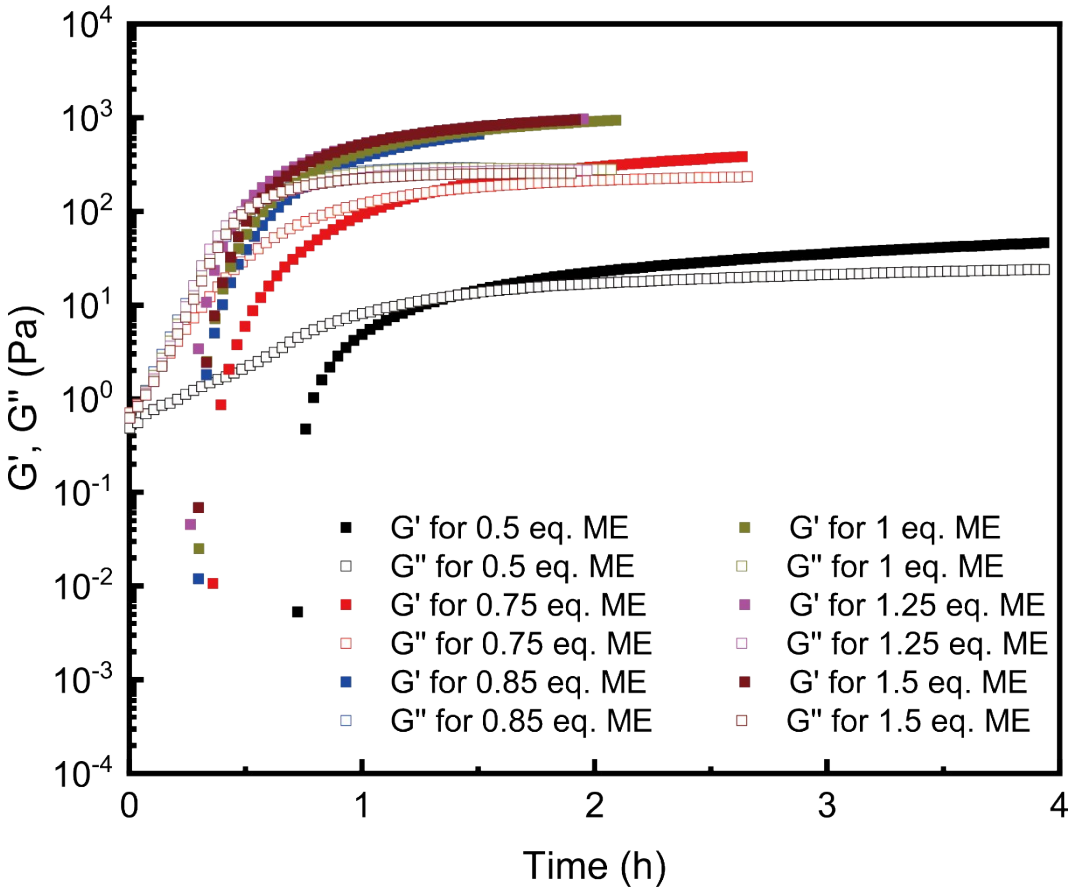


Figure S14. Time-dependent rheological measurements of coacervate gels activated by 0.75 eq., 0.85 eq., 1.0 eq., 1.25 eq. and 1.5 eq. **ME**. All samples: 5 wt% **PVP1** and 1.0 eq. **PAMPS$_{236}$** (overall polymer content: 6.8 wt%, in 100 mM pH 7.4 phosphate buffer).

10. Rheological study for diagram of material states

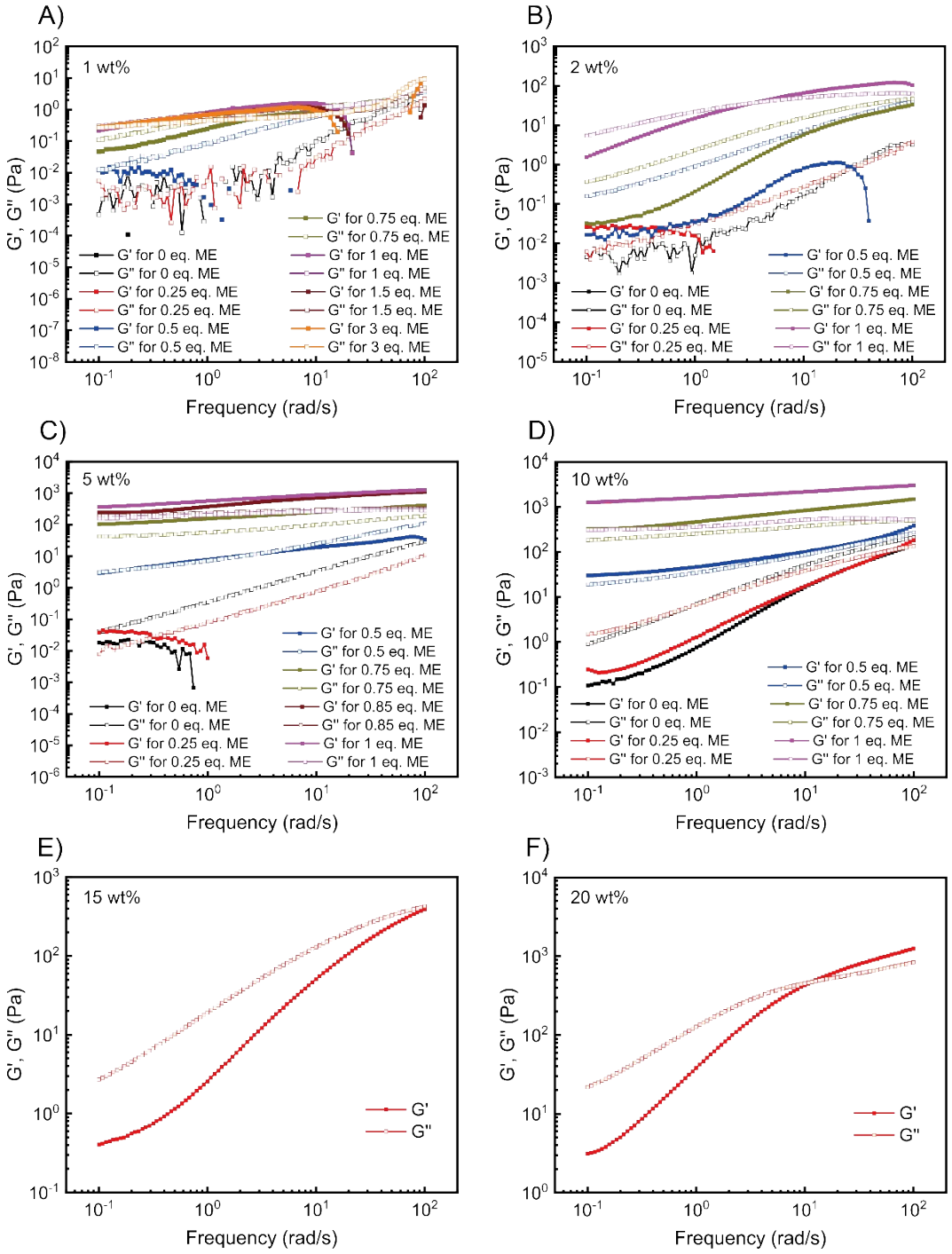
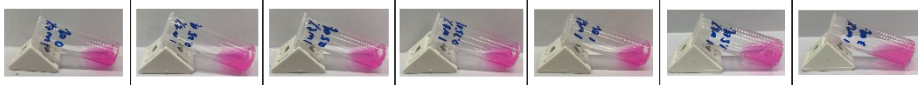
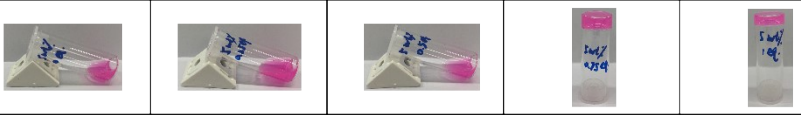
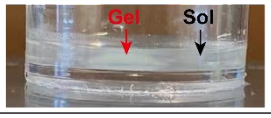
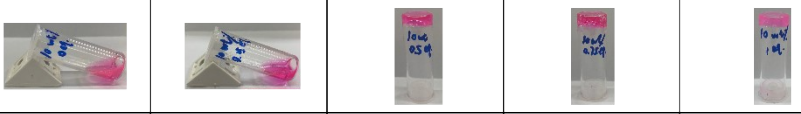


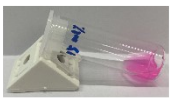
Figure S15. Frequency sweep rheological measurements of the samples with A) 1 wt%, B) 2 wt%, C) 5 wt%, D) 10 wt%, E) 15 wt% and F) 20 wt% **PVP1** which were triggered by different **ME** equivalents. For 1 wt%, 2 wt%, 5 wt% and 10 wt% samples (overall polymer content: 1.4 wt%, 2.7 wt%, 6.8 wt% and 13.6 wt%): **PVP1** and 1.0 eq. **PAMPS$_{236}$**, in 100 mM pH 7.4 phosphate buffer; for 15 wt% and 20 wt% samples: **PVP1**, in 100 mM pH 7.4 phosphate buffer.

ME equivalent	0 eq.	0.25 eq.	0.5 eq.	0.75 eq.	1 eq.	1.5 eq.	3 eq.
1 wt%							
Materials state	Sol	Sol	Sol	Sol	Sol	Sol	Sol

ME equivalent	0 eq.	0.25 eq.	0.5 eq.	0.75 eq.	1 eq.
2 wt%					
Materials state	Sol	Sol	Sol	Sol	Gel

ME equivalent	0 eq.	0.25 eq.	0.5 eq.	0.75 eq.	1 eq.
5 wt%					
Materials state	Sol	Sol	Sol-gel mixture (Intermediate)	Gel	Gel
Note	 5 wt% Polymer with 0.5 eq. ME				

ME equivalent	0 eq.	0.25 eq.	0.5 eq.	0.75 eq.	1 eq.
10 wt%					
Materials state	Sol	Sol	Gel	Gel	Gel

ME equivalent	0 eq.
15 wt%	
Materials state	Sol

ME equivalent	0 eq.
20 wt%	
Materials state	Gel

Figure S16. Photos of the samples with 1 wt%, 2 wt%, 5 wt%, 10 wt%, 15 wt% and 20 wt% **PVP1** which were initiated by different **ME** equivalents. The samples (in red) were dyed by rhodamine B. For 1 wt%, 2 wt%, 5 wt% and 10 wt% samples (overall polymer content: 1.4 wt%, 2.7 wt%, 6.8 wt% and 13.6 wt%): **PVP1** and 1.0 eq. **PAMPS$_{236}$**, in 100 mM pH 7.4 phosphate buffer; for 15 wt% and 20 wt% samples: **PVP1**, in 100 mM pH 7.4 phosphate buffer.

Theoretically, if the storage moduli (G') of the sample was higher than its loss moduli (G'') over the entire range of frequencies, we considered the sample as

a gel. For the sample with a crossover point of G' and G'' in rheological measurements, we further determined its material state by visual observation. Typically, if the material can stay at the bottom of an inversed vial for 1 min, we considered it as a gel, or it should be considered as a solution. If G' of a sample was lower than G'' over the entire range of frequencies, we considered the sample as a solution.

11. Rheological study for degradation of coacervate gels

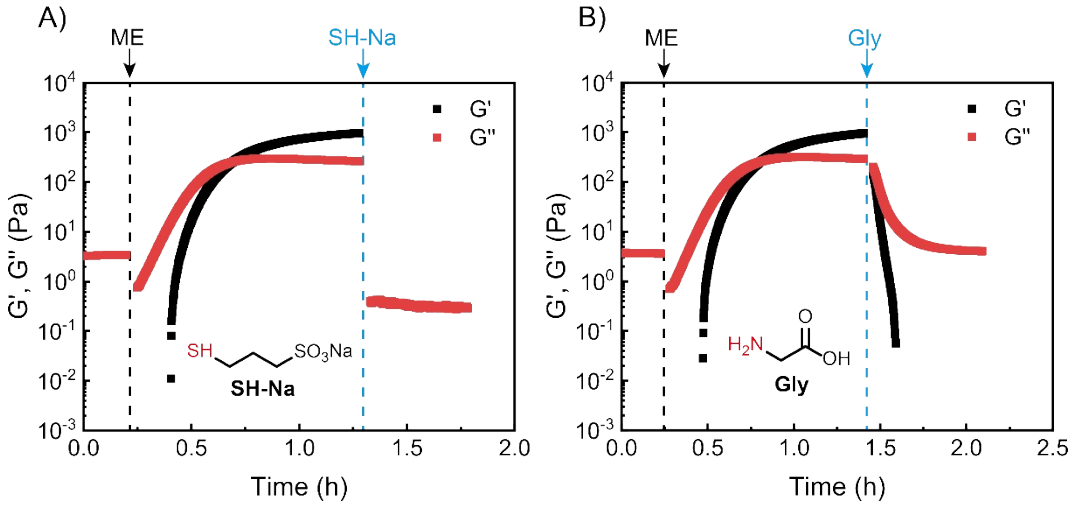


Figure S17. Time sweep rheological test of the **ME**-activated gelation process and A) thiol **SH-Na** or B) primary amine **Gly** induced disassembly process of the coacervate gels.

Herein, we illustrated that our gels can be disassociated by the thiol and the primary amine. We found that our gels possessed the nucleophile-degradability (for a primary amine – **Gly**, a secondary amine – **P** and a thiol – **SH-Na**, see Figure S17).

12. Degradation tests (in the liquid environments)

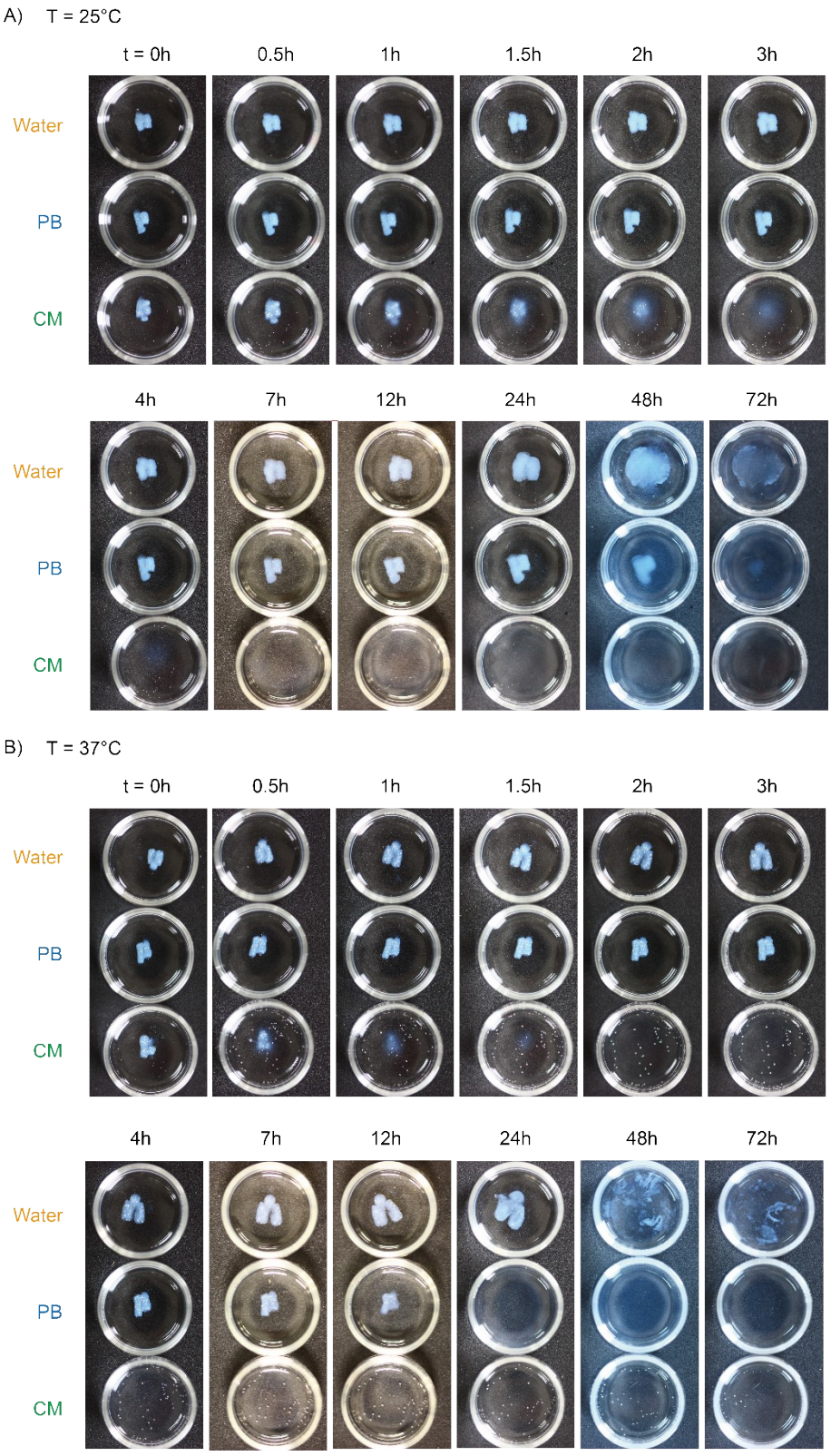


Figure S18. Degradation of coacervate gels in water or 150 mM pH 7.4 phosphate buffer (**PB**) or cell culture media (**CM**) at A) 25°C and B) 37°C over

time. All samples: 5 wt% **PVP1** and 1.0 eq. **PAMPS$_{236}$** (overall polymer content: 6.8 wt%), in 100 mM pH 7.4 phosphate buffer (initiated by 1.0 eq. **ME**).

13. Cell cytotoxicity tests

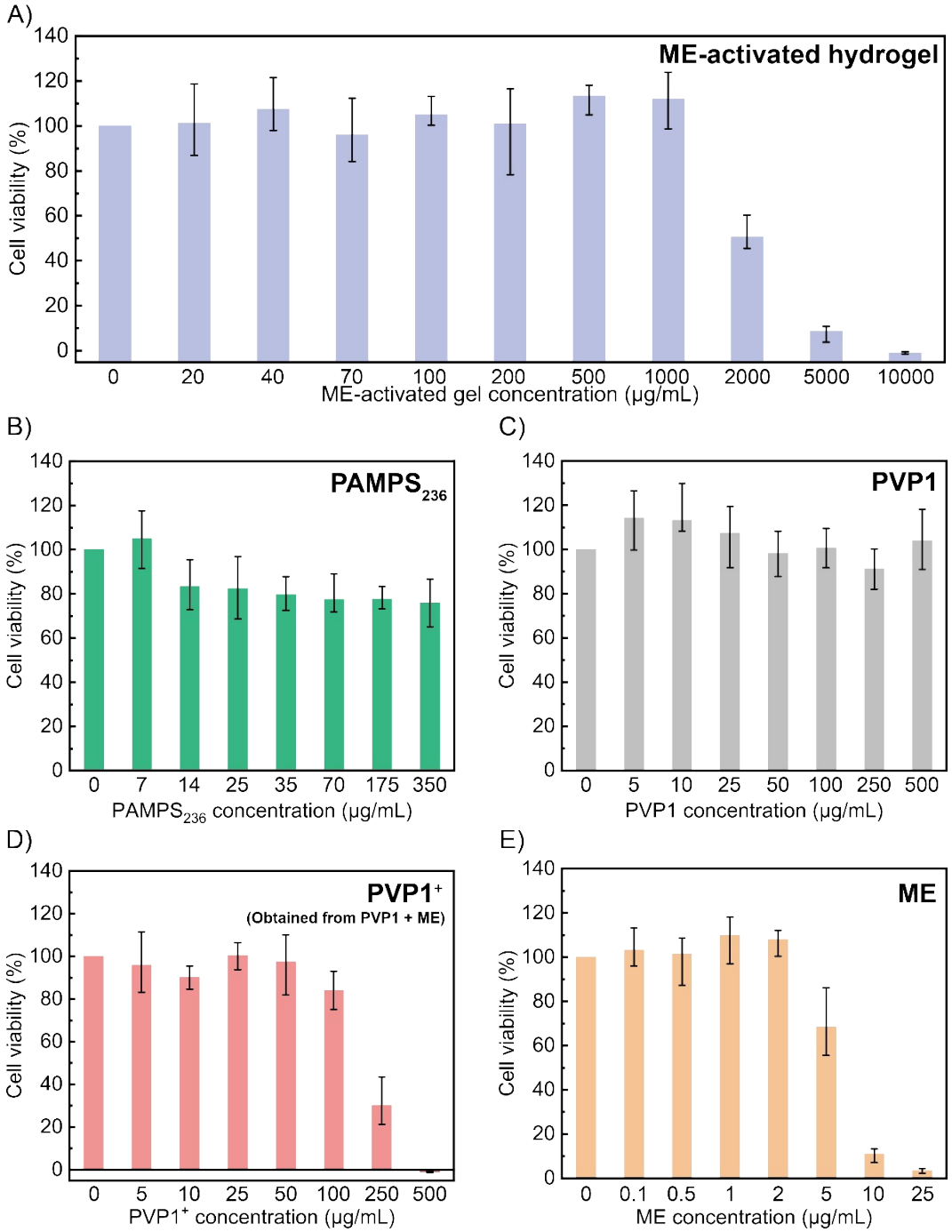


Figure S19. Biocompatibility of A) **ME**-activated coacervate hydrogels, B) **PAMPS$_{236}$**, C) **PVP1**, D) **PVP1$^+$** and E) **ME**. The NIH-3T3 mouse fibroblast cells were incubated in the presence of coacervate gels, **PAMPS$_{236}$**, **PVP1**, **PVP1$^+$**

and **ME** (in different concentrations) for 48 hours. The cell viability of the blank sample was defined as 100%. Error bars are calculated from three independent measurements.

A cell cytotoxicity measurement was performed to evaluate the biocompatibility of the coacervate gels. NIH-3T3 mouse fibroblast cells were cocultured with varying concentration of **ME**-activated coacervate gels, **PAMPS$_{236}$**, **PVP1**, **PVP1^{+}** and **ME** in cell culture media at 37°C (in 5% CO_2 atmosphere). We defined the cell viability of the blank sample (without coacervate gels) as 100%. After 48-hour incubation, we noticed that the **ME**-activated gel shows no toxicity in low concentration (gel concentration ≤ 1000 μg/mL) but substantial toxicity in higher concentration (gel concentration > 1000 μg/mL) (Figure S19A). The **PAMPS$_{236}$**, **PVP1** and **DVP** exhibits no toxicity (viability above 80% up to 350 μg/mL **PAMPS$_{236}$**, above 95% up to 500 μg/mL **PVP1** and above 80% up to 236 μg/mL **DVP**, respectively; Figure S19B-C and S20A), while **PVP1^{+}** and **ME** decreased cell viability below 65% at 250 and 10 μg/mL, respectively (Figure S19D-E). Moreover, the **DVP**-activated coacervate hydrogels display no significant toxicity at high gel concentration (viability above 85% up to 10 mg/mL gel concentration, Figure S20B).

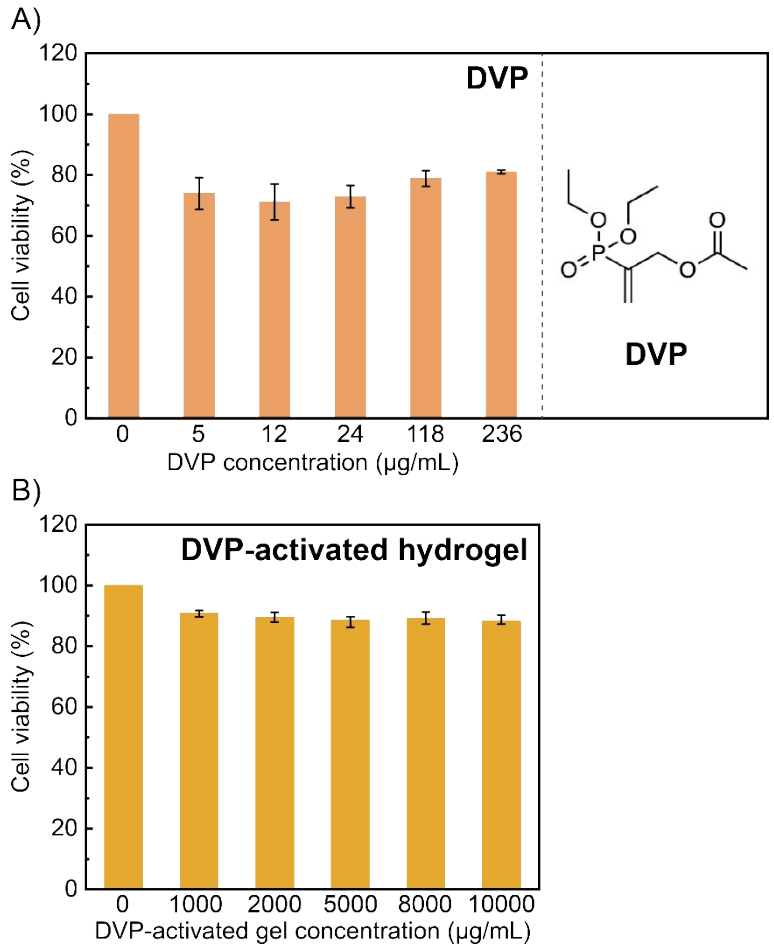


Figure S20. Biocompatibility of **DVP** and **DVP**-activated coacervate hydrogels. The NIH-3T3 mouse fibroblast cells were incubated in the presence of A) **DVP** and B) **DVP**-activated coacervate hydrogels (in different concentrations) for 48 hours. The cell viability of the blank sample was defined as 100%. Error bars are calculated from three independent measurements.

14. Rheological study for the swollen PAAm gels

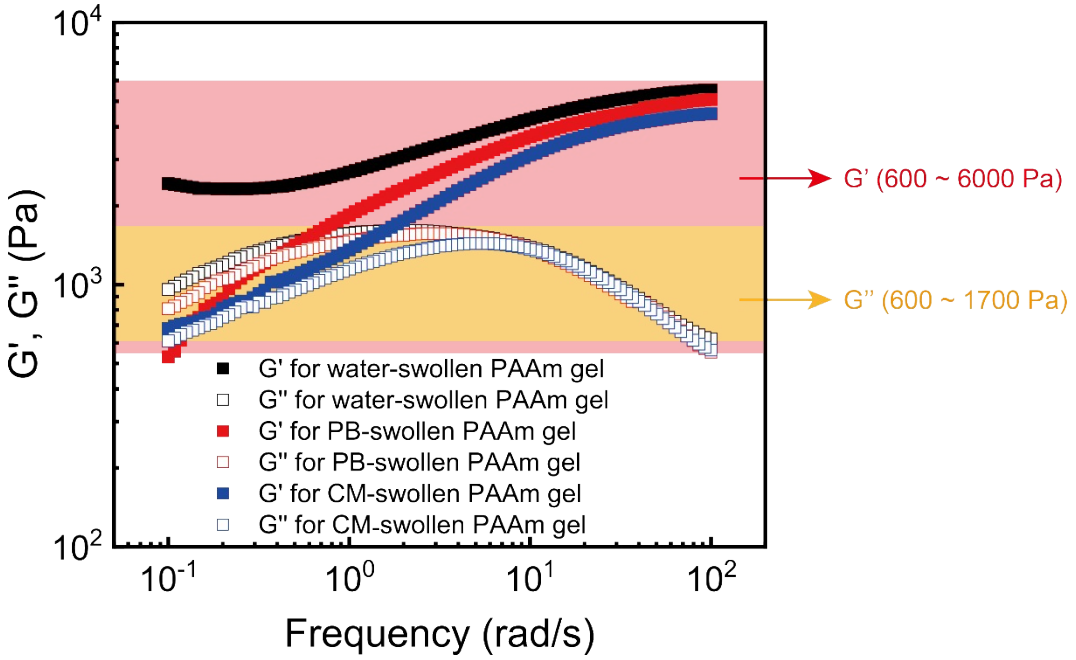


Figure S21. Frequency sweep rheological measurements of water, or 150 mM pH 7.4 phosphate buffer (**PB**) or cell culture media (**CM**) swollen **PAAm** gels.

According to literature, the mechanical strengths for human tissue are 500 Pa < G', G'' < 10000 Pa,[6] our swollen **PAAm** gels possess similar rheological value (G' 600 ~ 6000 Pa, G'' 600 ~ 1700 Pa, see Figure S21). Moreover, even the **PAAm** gels were swollen in different media, the difference of their mechanical performance was negligible.

15. Degradation tests (in the swollen PAAm gels)

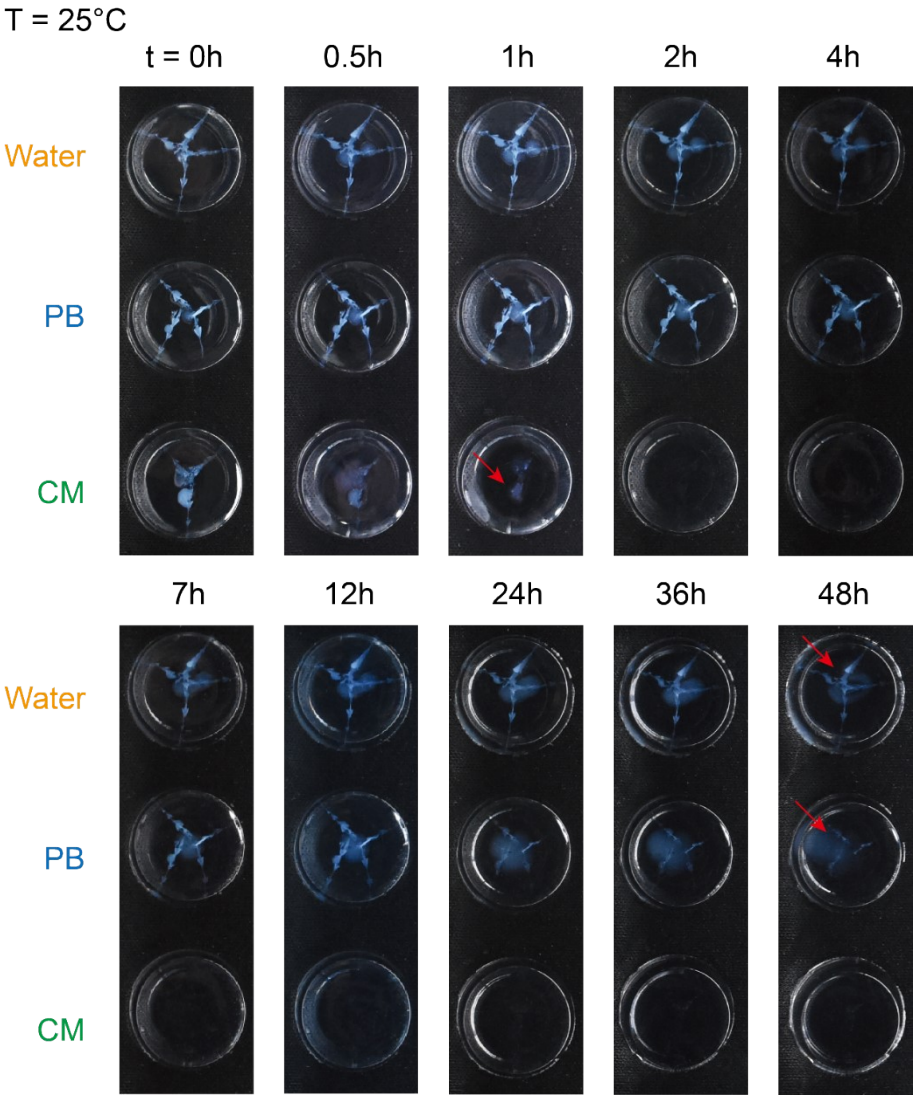


Figure S22. Degradation of coacervate gels in water or 150 mM pH 7.4 phosphate buffer (**PB**) or culture media (**CM**) swollen **PAAm** gels at 25°C over time. Red arrows are inserted to point out the gel residue. All samples: 5 wt% **PVP1** and 1.0 eq. **PAMPS$_{236}$** (overall polymer content: 6.8 wt%), in 100 mM pH 7.4 phosphate buffer (initiated by 1.0 eq. **ME**).

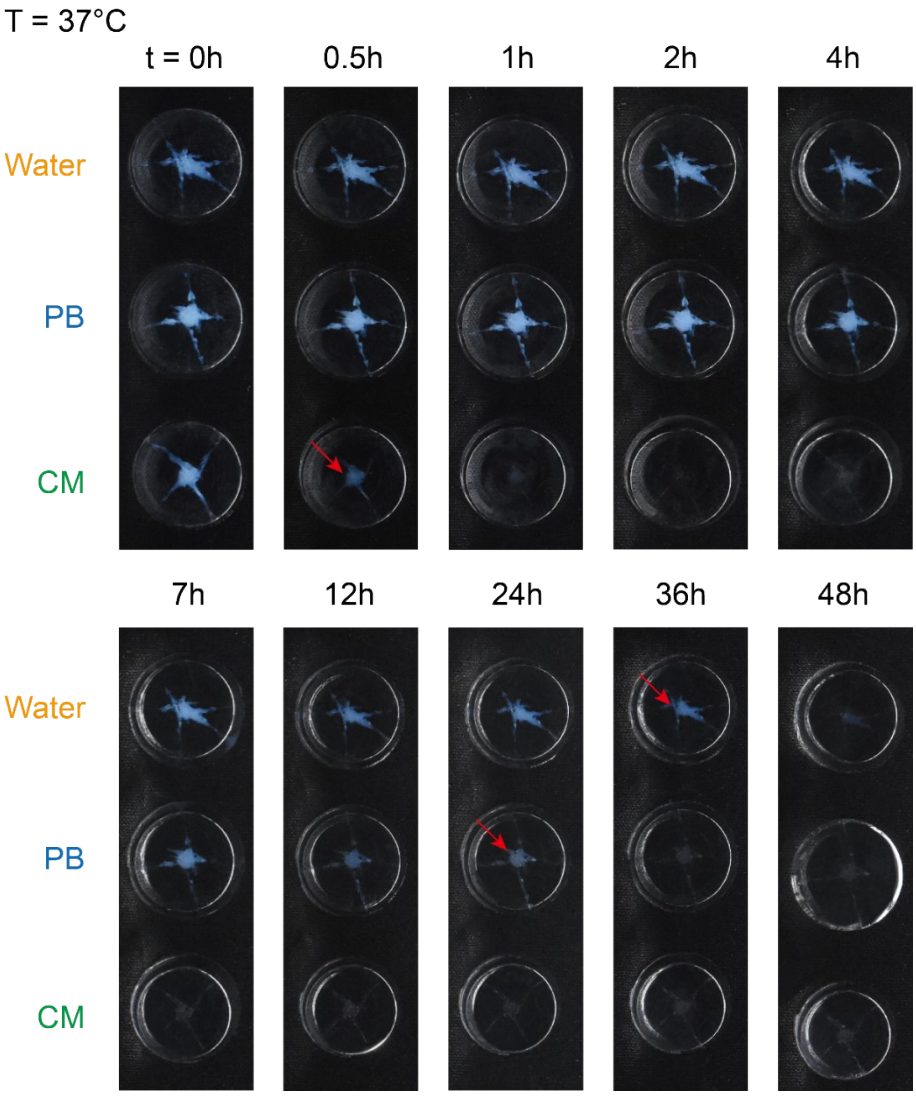


Figure S23. Degradation of coacervate gels in water or 150 mM pH 7.4 phosphate buffer (**PB**) or culture media (**CM**) swollen **PAAm** gels at 37°C over time. Red arrows are inserted to point out the gel residue. All samples: 5 wt% **PVP1** and 1.0 eq. **PAMPS$_{236}$** (overall polymer content: 6.8 wt%), in 100 mM pH 7.4 phosphate buffer (initiated by 1.0 eq. **ME**).

Temperature / °C	25			37		
Medium	Water	PB	CM	Water	PB	CM
Time / h	**Ratio / %**					
0	15.4	14.4	11.9	20.0	20.4	16.1
0.5	17.2	16.8	11.2	18.5	17.9	4.7
1	24.0	20.4	2.3	19.1	18.3	0.0
2	13.8	14.1	0.0	19.7	18.5	0.0
4	7.4	7.7	0.0	17.4	14.9	0.0
7	9.3	8.5	0.0	15.5	10.6	0.0
12	9.7	10.8	0.0	10.3	5.4	0.0
24	8.1	3.3	0.0	11.7	6.1	0.0
36	7.4	1.3	0.0	7.8	0.0	0.0
48	4.2	0.6	0.0	0.0	0.0	0.1

Figure S24. Degradation ratios of coacervate gels (in the swollen **PAAm** gels). The ratios (blue pixels/total pixels) which are below 0.5% are highlighted in orange.

Note that at 37°C, after degradation of the coacervate hydrogel (blue areas) some voids (grey areas) appeared which were caused by needle injection. To more objectively analyze the degradation process, we analyzed the photos using ImageJ software. We determined the number of pixels above a set threshold in the blue channel (value: 80 – 255) compared to total number of pixels (background, value: 0 – 255) (Figure S24). We define complete degradation for when the ratio (blue pixels/total pixels) is below 0.5%, and the corresponding time (complete degradation) is the degradation time of coacervate hydrogel.

Therefore, as shown in Figure S22, S23 and S24, at 25°C, the coacervate gels are visible in the water-swollen and **PB**-swollen **PAAm** gel even after 48 hours (blue pixel ratio retention 4.2% and 0.6%, respectively). While in the **CM**-swollen **PAAm** gel, the coacervate gels only needs 2 hours to dissolve. At 37°C, the coacervate gels requires 48 hours to completely dissolve in the water-swollen **PAAm** gel but 36 hours to dissolve in the **PB**-swollen **PAAm** gel. For the coacervate gels in the **CM**-swollen **PAAm** gel, the degradation time is about 1 hour.

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