

In situ elucidating phase transition and differentiated hydrogen-bonding nanoconfinement in single nano-hydrogel

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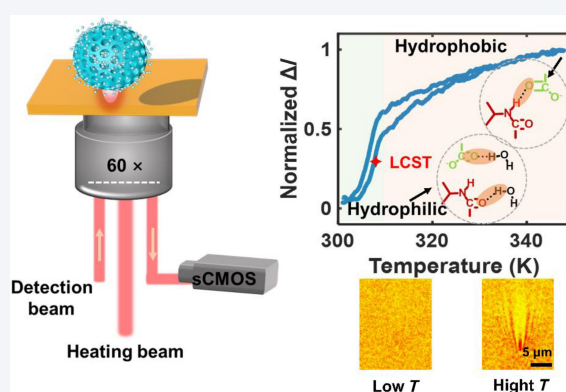
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Cite this article: Nano Research, 2026, 19, 94908979. <https://doi.org/10.26599/NR.2026.94908979>

ABSTRACT: Stimuli-responsive smart polymer hydrogels have garnered significant interest across multidisciplinary fields, with conventional time-resolved bulk characterization techniques extensively applied to probe their ensemble behavior. However, a critical microscopic profiling of their fundamental dynamics down to single nanoparticle level remains elusive, particularly for resolving transient volume phase transition and metastable intermediates triggered by thermal and hydrogen-bonding regulation. To bridge this gap, we demonstrated an *in situ* single-nanoparticle strategy integrating localized thermoregulation with surface plasmon resonance imaging by introducing programmable optical tweezer system. This platform enables real-time tracking of volume phase transitions within individual nano-hydrogels under localized thermoregulation. Crucially, we identify transient intermediate states and thermodynamics of this transition event. The intrinsic volume phase transition temperature of single nano-hydrogel invariant to thermal modulation rate is quantified, governed by the equilibrium of hydrogen-bond nanoconfinement within polymer structure. By tuning the crosslinking density of three-dimensional network polymers within a single nano-hydrogel, heterogeneous volume phase transition behavior through pH-controlled hydrogen bonding nanoconfinement is also decoded. This *operando* single-entity plasmonic imaging methodology, synergized with spatiotemporally controlled thermal fields, provides a platform for investigating stimulus-adaptive behavior of thermally responsive nanomaterials at the single nanoparticle level.

KEYWORDS: nano-hydrogels, plasmonic imaging, thermal volumetric phase transition, hydrogen-bond nanoconfinement, spatiotemporal manipulation



1 Introduction

Intelligent hydrogel materials have shown great application potential in interdisciplinary fields of artificial intelligence actuators, drug delivery, and optical devices due to their excellent stimulus response characteristics and flexibility. It provides key parameters for the design of intelligent carriers that can achieve efficient on-demand drug release in complex biological environments. In the field of tissue engineering, it is helpful to construct intelligent scaffolds that can dynamically regulate the cell microenvironment through photothermal effect. In addition, the thermoelectric active ions are encapsulated in the hydrogel-based battery, and the

temperature and environmental temperature gradient are used to trigger thermal volume phase transition (VPT) to accelerate the thermal diffusion of ions, so as to realize the continuous output of electric energy, meet the demand of flexible electronic power supply and reduce the dependence on traditional batteries [1–6]. As the core characteristic of intelligent hydrogel materials, VPT determines its performance boundary in the interdisciplinary field [3, 7–10]. However, traditional research focuses on the collective response of bulk hydrogels. This approach conceals inherent thermodynamics of VPT governed by the hydrogen-bonding nanoconfinement effect at single-particle scale, particularly intermediate state information of the thermally-regulated transient VPT process. Therefore, in-depth research and accurate measurement of the VPT process of hydrogels is crucial to promote the development of hydrogel-based nanodevices. At present, an *in situ* measurement method with both spatial resolution and time resolution is urgently needed to realize real-time monitoring of VPT process under spatiotemporally

Received: March 25, 2026; Revised: June 29, 2026

Accepted: June 29, 2026

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coupled thermal perturbations (through the programmed design of external stimuli such as laser and chemical environment, the spatial precise control of the phase transition behavior of a single nanohydrogel is realized).

At present, time-resolved overall characterization technique mainly obtains the macroscopic behavior characteristics of hydrogel by measuring the average performance of bulk hydrogel [11–13]. However, this behavior cannot distinguish the contribution from particle-particle interactions versus the intrinsic behavior of single particles. Meanwhile, conventional microscopy techniques face challenges in achieving dynamic tracking of the phase transition process of hydrogels while precisely controlling the thermal field. Although significant progress has been made in synthesizing nanogels with controllable size, morphology, and properties, due to the high sensitivity of nanogels to their environment, their properties differ significantly from those of gels with comparable length scales [11, 14–17]. Therefore, investigating how the dynamic equilibrium of hydrogen-bond networks at the nanoscale affects VPT threshold and whether local thermal field induces intermediate state formation during transient volume phase transitions has become a crucial scientific problem to be addressed. Realizing real-time monitoring of spatiotemporally coupled thermal perturbations and VPT represents the core technical approach for elucidating the non-continuous phase transition kinetics of hydrogels under dynamic thermal drive.

In this work, we propose a photothermal-plasmonic imaging strategy. A programmable optical tweezer system is utilized to construct a local thermal field gradient, which is then coupled with surface plasmon resonance imaging method to establish a high-resolution and real-time research platform. This platform identifies hydrated intermediate states by capturing plasmonic signal transitions and quantifies the contribution of hydrogen-bond reconstruction to phase transitions, enabling precise tracking of individual nanohydrogel VPT processes. This method can record the phase transition process of individual nanohydrogels under local thermal regulation in real-time, accurately analyze the transient intermediate states and phase transition thermodynamics. It is demonstrated that the intrinsic VPT temperature of individual nanohydrogels is independent of the thermal regulation rate, which is essentially determined by the equilibrium of hydrogen-bond nanoconfinement within the polymer structure. In addition, by adjusting the crosslinking density of nanohydrogels, the heterogeneous VPT behavior caused by hydrogen-bond nanoconfinement under pH control is revealed. This *in situ* single-entity plasmonic imaging method with spatiotemporally controllable thermal field collaboration paves a new path for studying the stimulus-adaptive behavior of thermally responsive nanomaterials in nanoconfined spaces.

2 Experimental

2.1 Materials and characterization

P(NIPAM₉₅-co-AA₅) nano-hydrogel was synthesized following the method reported by Ref. [18]. A 0.01 g amount of acrylic acid (AA), 0.33 g of N-isopropylacrylamide (NIPAM), and 0.03 g of N,N'-methylenebisacrylamide (BIS), all purchased from Adamas-beta, were added to a three-port flask containing 50 mL of DI water, and the temperature was raised to 70 °C. N₂ was continuously introduced into the reaction and fully stirred until the solution was

transparent. After a certain temperature, 0.2 mM ammonium persulphate (APS) (General-reagent) solution was added, and the reaction was carried out at 70 °C for 4 h to obtain nano-hydrogel. After cooling, the nano-hydrogels were repeatedly dialyzed with deionized (DI) water. These two monomers were randomly distributed in the network, and the polymer chains can expand in the hydrogel network. The synthesized nano-hydrogel particles were characterized by scanning electron microscope (SEM) (Zeiss Gemini 300), atomic force microscopy (AFM) (Bruker Dimension), Raman (Renishaw inVia) and dynamic light scattering (DLS) (Malvern).

2.2 Sample preparation

The prepared nano-hydrogel solution was diluted to 1/100 of the original volume, and a PDMS cell was placed on the glass covering the Au film. Then 2 mL DI water was added dropwise to the PDMS reaction cell, and 20 µL nano-hydrogel was suspended in the PDMS reaction cell, waiting for 10 min, and finally the DI water was sucked away.

2.3 Optical system

The whole experiment was carried out on an inverted microscope (Ti-U, Nikon). The top optical path used an optical tweezers system (Aresis; Tweez 250si) to focus a 60× oil-immersion lens (N.A. = 1.49) on the Au film to locally heat a single nano-hydrogel (1064 nm near-infrared laser). A filter was used to prevent most of the 1064 nm (Nd:YAG) from entering the camera, and the bottom optical path was used as a detection beam for plasmonic imaging. The surface plasmon resonance wave of Au film was excited by a 680 nm (super luminescent light emitting diode, Q-Photonics) collimated beam, and the reflected light was collected by a sCMOS (Hamamatsu, ORCA-Flash 4.0) camera to obtain a plasmonic image with the frame rate of 50 fps.

3 Results and discussion

3.1 Plasmonic imaging of transient VPT of single nano-hydrogel

Individual P(NIPAM₉₅-co-AA₅) is deposited on the Au film electrode by drop-casting and characterized by SEM (Figs. S1 and S2 in the Electronic Supplementary Material (ESM)). The combination of plasmonic imaging system and optical tweezers (heating system) is introduced to study the thermal VPT of single nano-hydrogel on the substrate, as shown in Fig. 1(a). The gold film loaded with hydrogel nanoparticles is placed on an inverted microscope equipped with a 60× oil immersion objective (N.A. = 1.49). A collimated, low-power p-polarized super luminescent diode (SLED) incident light beam ($\lambda = 680$ nm) is directed at an appropriate angle onto the Au film surface to excite surface plasmon polaritons (SPPs) at the interface. The interaction between hydrogel nanoparticles and SPPs generates a wave pattern with a parabolic tail that represents the point spread function of plasmonic imaging method. The plasmonic image is displayed on an sCMOS camera by collecting the reflected light beam. Insets show SEM and plasmonic images of a specific nanohydrogel. Another focused near-infrared light beam ($\lambda = 1064$ nm) is integrated onto Au film based on an inverted optical microscope, adjusting the temperature of single nanoparticle on the substrate surface through localized heating of Au film. This precisely localized heating approach

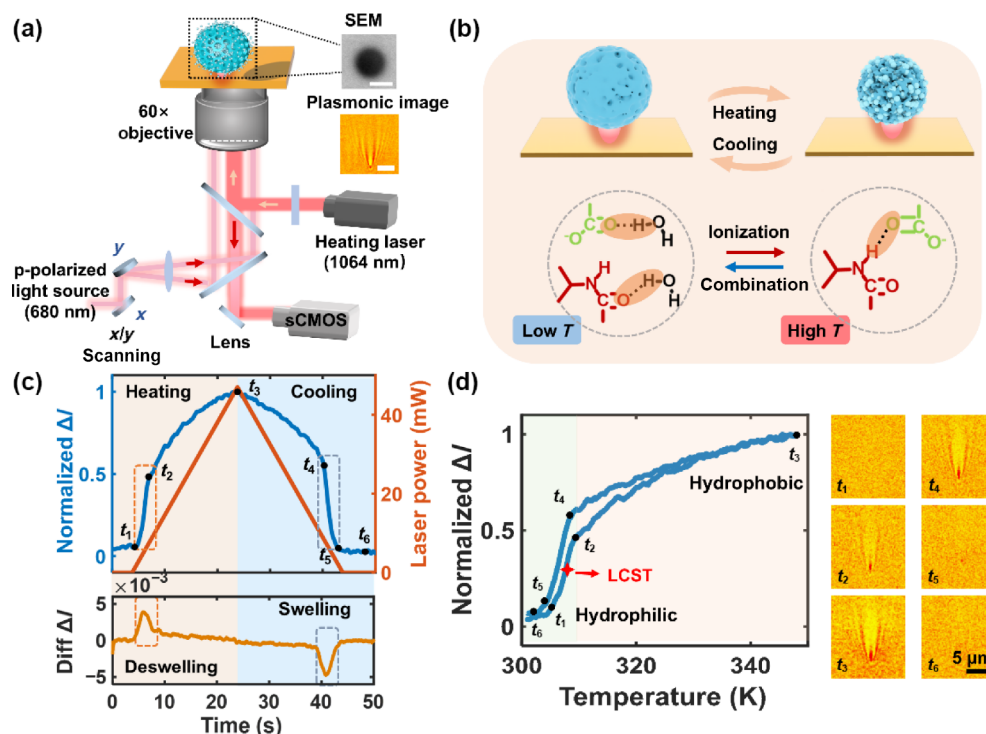


Figure 1 Monitoring the transient thermal VPT behavior of single nano-hydrogel by plasmonic imaging. (a) Schematic illustration of monitoring the thermal-induced VPT of single nano-hydrogel with surface plasmon resonance imaging strategy. The scattering intensity of single nano-hydrogel serves as a sensitive probe for two critical parameters, which include the dielectric constant modulation during phase transitions, and the nanoscale separation distance between the hydrogel and metallic surface. This dual-parameter detection mechanism allows precise tracking of both thermal fluctuations and temperature-dependent conformational changes in individual nano-hydrogels. The SEM image and corresponding plasmonic image of a single nano-hydrogel are shown in the insets, and the scale bar is 500 nm and 5 μm , respectively. (b) Schematic diagram of formation and dissociation of hydrogen bond network of single nano-hydrogel during thermal modulation. (c) Time-lapsed plasmonic intensity variation of single nano-hydrogel (blue curve) and corresponding transition rate of VPT event (orange curve) regulated by thermal control. The first derivative of plasmonic intensity curve can be defined as the transition rate in a representative volume phase transition cycle. (d) Dynamics for transient volume phase transition event of single nano-hydrogel during thermal cycling. The snapshots of plasmonic images for a single nano-hydrogel at different time nodes in a representative volumetric phase transition cycle. The red arrow points out the LCST location of this nano-hydrogel. Scale bar: 5 μm .

enables temperature modulation within an extremely confined spatial domain. When combined with plasmonic imaging method, it allows for simultaneous thermal field control and high-spatial-resolution observation of phase transition behaviors in individual nanohydrogels under varying temperatures.

To monitor the VPT of single nano-hydrogel, a detection beam continuously irradiates the Au film surface, providing a stable detection light source for real-time capture of the nanohydrogel's optical response. The heating beam, with the aid of a programmable optical tweezers system, achieves precise power regulation based on a preset program. The camera continuously records time-lapsed plasmonic images during the heating process at a rate of 50 frames per second (fps). The heating beam power is modulated in a triangular waveform (linearly increasing from 0 to 47 mW within 20 s and then decreasing back to 0, red curve in Fig. 1(c)), enabling local temperature control of individual nanohydrogel during the heating-cooling process. The blue curve in Fig. 1(c) shows the representative plasmonic intensity-time profile of the nanohydrogel at the heating spot: in the initial stage of linear heating, the optical signal remained stable as the temperature had not reached the VPT threshold; a sudden signal increase occurred at time t_1 (t_1 – t_2), marking the onset of VPT Stage I; with further increase in power, the signal transitioned smoothly from t_2 to t_3 , corresponding to the characteristics of VPT Stage II. During the cooling phase, the optical signal first attenuated slowly from t_3 to t_4 , then dropped sharply from t_4 to t_5 and recovered to the initial

state, confirming the reversibility of the VPT process. First-derivative processing of the intensity curve (orange curve in Fig. 1(c)) improved signal sensitivity. The prepared P(NIPAM₉₅-co-AA₅) nanohydrogels exhibit temperature-responsive switching properties and tunable deformability, showing significant VPT behavior and high swelling/expansion ratios in integrated microsystems.

By applying the temperature conversion formula in Fig. S3 in the ESM, the original laser power-dependent plasmonic intensity profile of the nanohydrogel in Fig. 1(d) is successfully transformed into a temperature-intensity curve, thereby enabling precise determination of the VPT characteristics for individual nanohydrogels. The lower critical solution temperature (LCST) is defined as the maximum temperature of the first-order derivative of the optical intensity curve. The volume phase transition temperature (VPTT) (T_D and T_S) in Fig. 1(d) during the deswelling and swelling processes are 308.0 and 306.4 K, respectively, slightly above the LCST ($\approx$ 305 K) in polymer aqueous solutions [19]. First, the VPT during the cooling stage occurs at a lower temperature, which is a sign of hysteresis, consistent with the traditional thermal VPT curve measured from bulk samples [11, 12]. Second, the VPT process exhibits its most pronounced changes near the LCST, while demonstrating progressively attenuated responses at temperatures further removed from this transition threshold. Third, the optical signal returns to its initial state when the temperature again back to below the LCST, so this temperature-sensitive characteristic is

reversible. Bright-field (BF) microscopy failed to resolve the two-step VPT process when monitoring identical heating protocols (Fig. S4 in the ESM), whereas plasmonic imaging method successfully captured the rapid phase transition dynamics of nanogels through its superior sensitivity and spatial resolution. The transitional behaviors during the phase transformation evolution can be distinguished, namely the intermediate states, referring to the intermediate steps of the phase transformation that can be stably identified. The time-lapsed plasmonic images of Fig. 1(d) can be observed that the hydrophobic state has a higher optical contrast than the hydrophilic state. According to the literature, this optical contrast depends on the dielectric constant and volume [20, 21]. Since the dielectric constant decreases during the heating process [22–24], and an increase in optical contrast is observed under different laser powers, it can be concluded that volume expansion plays a dominant role in this special material. Above the LCST, the hydrogen-bond network undergoes rapid reconstruction, causing polymer chains to collapse and the nanohydrogels to expel water rapidly, shrinking into a compact spherical conformation (Fig. 1(b)). The height map reconstructed in Fig. S5 in the ESM illustrates the volume contraction phenomenon of the P(NIPAM₉₅-co-AA₅) nanohydrogel during the swelling process.

In summary, we directly observed and quantitatively analyzed the whole process of thermally induced VPT of nano-hydrogels at the single particle level for the first time by plasmonic imaging technology. Notably, in addition to confirming the reversibility and thermal hysteresis, we directly observed its clear two-stage kinetic characteristics (rapid contraction and slow) through high spatial-temporal resolution imaging, presenting a typical non-continuous phase transition evolution path. These results clearly show that the phase transition behavior of P(NIPAM₉₅-co-AA₅) hydrogel in nano-confined environment is significantly different from that in free solution, and its VPTT will shift due to interfacial interaction.

3.2 Thermodynamics analysis at the single nanohydrogel level

Compared to hard colloidal particles, the distinctive soft nature of nanohydrogels gives rise to rich and tunable dynamic behaviors in multiphase systems. Investigating the VPT kinetics of individual nanohydrogels effectively circumvents interference from complex factors in bulk systems, such as uneven heat transfer and interparticle interactions. By monitoring the time-resolved evolution of plasmonic signals, we can track in real time the rates and sequence of key events during phase transitions, including volumetric changes and hydrogen-bond reorganization, thereby achieving high spatiotemporal resolution characterization of VPT kinetics.

The kinetic behavior of a single nano-nanogel in the process of deswelling and swelling is studied by plasmonic imaging method combined with laser-induced pulse heating technology. Figures 2(a) and 2(b) show the dependence of the deswelling and swelling rates of P(NIPAM₉₅-co-AA₅) nano-hydrogel on the temperature scan rate. The shoulder peak area decreases with the decrease of the temperature scan rate. A possible reason is that heating causes the destruction of hydrogen bonds nanoconfinement inside the nano-hydrogel, accompanied by the release of free water and the contraction of polymer chains. Slower heating and cooling rates provide sufficient time for the hydrogel to contract and expand. It reveals that the VPTT of the nano-hydrogel is an intrinsic transition temperature that is independent of temperature scan rate. To

quantify the kinetics of the VPT process of nano-hydrogels, a periodic square wave signal is applied to the nano-hydrogel for kinetic monitoring. Upon exceeding the VPTT with the applied laser temperature, the nano-hydrogel contraction elicits an abrupt increase in the optical signal. Conversely, abrupt laser deactivation triggers a gradual cooling of the system to ambient temperature, prompting the nano-hydrogel to re-swell. The optical signal demonstrates incomplete recovery to the initial baseline at 367.0 K, a phenomenon corroborated by plasmonic imaging (Fig. 2(h)). Notably, during repeated laser on-off cycles, the re-swelling process of the nano-hydrogel exhibits pronounced volume fluctuations with excellent spatial uniformity (Figs. 2(c) and 2(d)). A single-exponential function fitting of the optical intensity curve is performed to derive the time constants for deswelling (τ_d) and swelling (τ_s) processes, as illustrated in Fig. 2(e). The relaxation behavior it represents is the time-averaged result of the coordinated effect of hydration changes and network structure reconstruction. The inset depicts plasmonic images captured at the instant of laser shutdown and post-thermal equilibrium. The deswelling kinetic constant τ_d exhibits similar transition kinetics, allowing for a rapid thermal trigger response as low as several tens of milliseconds. The swelling dynamic constant τ_s increased from 0.09 to 0.86 s and showed a linear correlation, while the increase in laser temperature provides a longer time for the recovery of the nano-hydrogel (Fig. 2(f)). Under all experimental conditions, deswelling exhibited faster transition kinetics than the re-swelling process. The possible reason is that the temperature of the nano-hydrogel rapidly increases within a small volume centered around the heated laser in a short period of time. The three-dimensional network structure of the nano-hydrogel acts as a temporary storage of water, resulting in a rapid VPT. During the cooling process, it is necessary to balance the system's temperature with that of the surrounding environment, resulting in a relatively slow relaxation process, which causes significant differences in the re-swelling kinetics of the nano-hydrogel. For different pulse heating times, the re-swelling kinetic constant τ_s is similar. The nano-hydrogel completely recovers to its original volume at a shorter pulse heating time (0.2 s), as shown in Fig. 2(g).

In summary, several key characteristics of the phase transition kinetics of P(NIPAM₉₅-co-AA₅) nanohydrogels are quantitatively revealed at the single particle level by pulse heating and plasmonic imaging techniques. Firstly, the VPTT is an intrinsic property, which is independent of the temperature scanning rate. Secondly, the phase transition process exhibits strong kinetic asymmetry, the rate of deswelling is much faster than that of reswelling. Finally, the nanohydrogels exhibited excellent reversibility and responsiveness under fast pulse stimulation. These findings clearly point out that heat transfer is a key rate-limiting step in controlling the switching speed of interfacial nanogels.

3.3 Regulation of hydrogen-bond nanoconfinement of single nanohydrogel

Related research indicates that the state of water affects the VPT behavior of nano-hydrogels, categorizing the water release process into bound water and free water. Inspired by this, we employed a method of naturally placing the same nano-hydrogel and rehydrating it multiple times to verify the mechanism of VPT Stage I, based on the principle of adjusting a certain proportion of reversible hydrogen-bonds to ensure strain recovery after stress release and tunable hydrogen-bond nanoconfinement (a

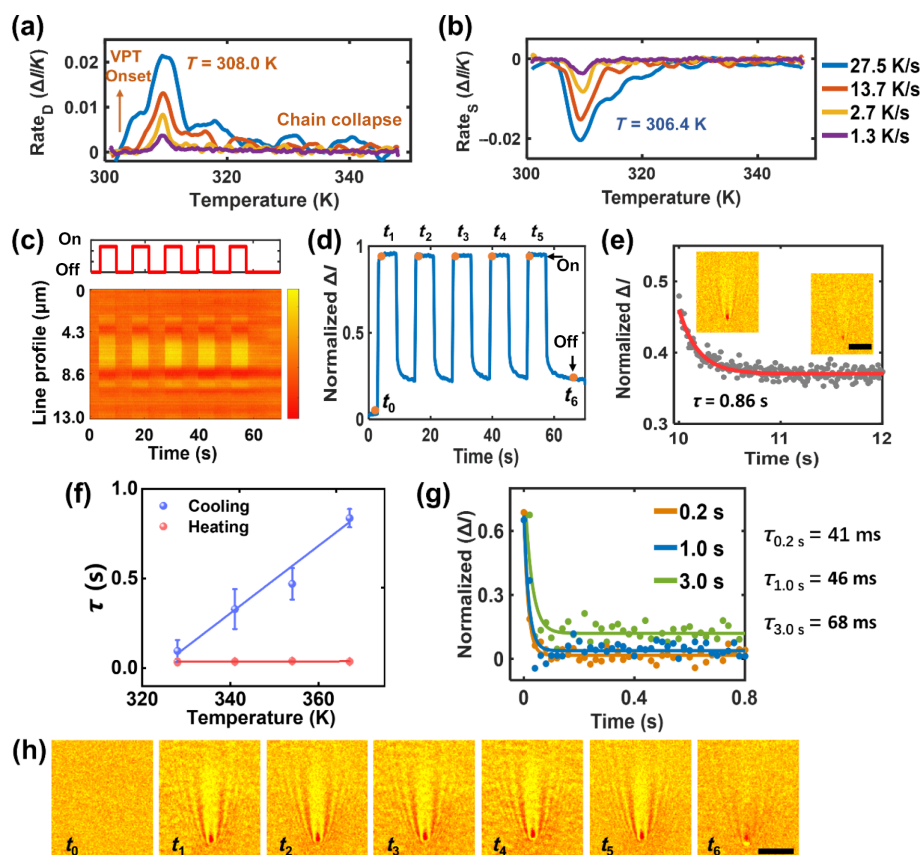


Figure 2 Evaluating and tuning VPT thermodynamics of single P(NIPAM₉₅-co-AA₅) nano-hydrogel by thermal-controlled plasmonic imaging strategy. (a) and (b) The transition rate of deswelling and swelling process of single P(NIPAM₉₅-co-AA₅) nano-hydrogel with different scan rate of thermal regulation. (c) 2D line profile across the horizontal direction of plasmonic pattern of single nano-hydrogel under periodic square-wave pulse thermal regulation. (d) Time-lapsed plasmonic intensity curve of the nano-hydrogel in C under periodic square-wave pulse thermal regulation. (e) Swelling kinetics of the nano-hydrogel. The insets reveal representative plasmonic images at initial and equilibrium state of the nano-hydrogel. Scale bar: 5 μ m. (f) Thermal-dependence of transition time constant during swelling and deswelling process of single P(NIPAM₉₅-co-AA₅) nano-hydrogel. (g) Swelling kinetics of the nano-hydrogel under different duration time of the fixed heating laser pulse. The temperature of heating laser pulse is 367.0 K. (h) Snapshots of plasmonic images of the nano-hydrogel at various intermediate states in d. Scale bar: 5 μ m.

comprehensive system of dynamic hydrogen bonding interactions between polymer networks and water molecules in nanoscale space).

Real-time monitoring of individual P(NIPAM₉₅-co-AA₅) nanohydrogels revealed distinct optical responses under different hydration states (Figs. 3(a)–3(c)). Results show that after 2 h of exposure to the environment, the initial plasmonic intensity of the nanohydrogels decreases significantly due to the loss of free water molecules in the hydrogel network (Fig. 3(b)), with the plasmonic images failing to recover to their original state. Notably, upon re-exposure to a high-humidity environment, the nanohydrogels undergo rapid rehydration, and both the optical intensity and plasmonic imaging properties fully recover to the initial level (Fig. 3(c)). This phenomenon clearly demonstrates that the dynamic exchange of water molecules can effectively drive the reorganization of hydrogen-bond nanoconfinements within the nanohydrogels. Furthermore, the complete recovery of optical properties indicates that the three-dimensional polymer network maintains its structural integrity during rehydration, thereby preserving the mechanical stability of the nanohydrogel system. For the nano-hydrogels studied herein, the swelling equilibrium ratio is a key criterion, which is calculated using the ratio of optical intensities in the first stages of swelling and deswelling (Fig. 3(d)). The competition between hydrogen-bonds among hydrated

molecules in nano-hydrogels plays a crucial role in the monotonic variation of relative humidity. We verified that water molecules promote the rapid regeneration of hydrogen-bond clusters between the polymer matrix and aqueous phase through multiple hydrations (Figs. 3(e) and 3(f)). For clarity, the transition of hydrogen-bonds during the dynamic hydration process between water molecules and polymers is illustrated in Fig. 3(g). At low temperatures, intermolecular hydrogen-bonds form between water molecules and the polymer, endowing the polymer with good water solubility. When water molecules are reduced, hydrogen-bond clusters are formed between polymer molecules, prompting the polymer chains to arrange closely.

Nano-hydrogel can utilize pH to regulate hydrogen-bonds to achieve reversible dynamic response behavior. Inspired by this, we designed a strategy that regulates multiple reversible hydrogen-bonds while maintaining large deformation to explore the pH-induced transition of hydrogen-bond nanoconfinement. Nanohydrogels treated with 0.5 M H₂SO₄ solution exhibit a significantly enhanced rate of abrupt optical signal change during VPT Stage I. Moreover, the optical signal curves show excellent reproducibility after multiple cycles of DI water and H₂SO₄ treatment, confirming the reversibility of the response (Figs. 4(a)–4(c) and Fig. S8 in the ESM). The VPTT of the nano-hydrogel during the deswelling and swelling processes is 306.1 and 305.3 K,

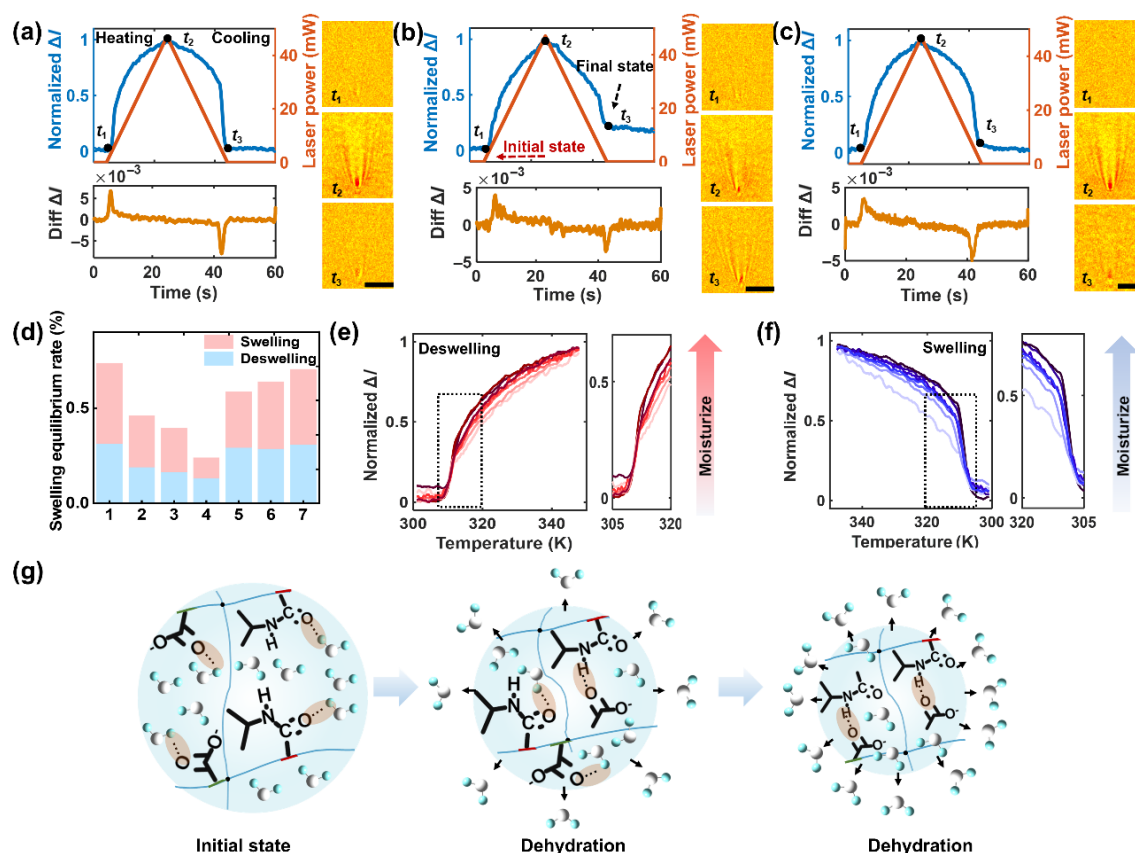


Figure 3 Regulating hydrogen bond nanoconfinement of single P(NIPAM-co-AA₃) nano-hydrogel during VPT dynamics. (a)–(c) Time-lapsed plasmonic intensity variation of single nano-hydrogel (blue curve) at the initial VPT period, the hydrophobic VPT period after natural drying two hours in the air and the final VPT period by complementing moisture, respectively. The corresponding transition rate variations of VPT event (orange curve) regulated by thermal control are plotted and snapshots of single-nano-hydrogel plasmonic images at distinct intermediate states. Scale bar: 5 μm. (d) Swelling equilibrium rate of single P(NIPAM-co-AA₃) nano-hydrogel under various conditions. The initial VPT period refers to Condition 1, and the subsequent hydrophobic VPT periods measured after 0.5, 1.0, 2.0 hours of natural air-drying respectively correspond to Conditions 2–4. The last three conditions characterize the VPT evolution by progressive complementing moisture, reflecting the first-stage, second-stage, and final equilibrium states of hydration. Swelling equilibrium rate is defined as the ratio of optical intensity during the first phase of swelling and deswelling event. (e) and (f) Thermal-dependent of plasmonic intensity curves during deswelling and swelling process of single P(NIPAM-co-AA₃) nano-hydrogel under Conditions 1–7. Right inset figures magnifying region from 305.0 to 320.0 K. (g) Schematic illustrations of transition evolution of hydrogen bonds nanoconfinement effect during the dynamic dehydration and hydration event of water molecules within a nano-hydrogel.

respectively, which are slightly lower than the temperature of the polymer after immersion in the DI water. We systematically analyzed the VPT characteristics of nanohydrogels in environments with different sulfuric acid concentrations by regulating hydrogen-bond nano-constraints via pH control. Results showed that in 0.5 M H₂SO₄, the nanohydrogels exhibited the most significant volume jump signal and the lowest VPTT (Figs. 4(d) and 4(e)), while the rate gradually decreased with further increase in concentration (Fig. 4(f)). The AFM characterization shows that the nano-hydrogels at different acid concentrations present a truncated spherical morphology (Figs. S11 and S14 in the ESM).

The volume expansion of a single nanogel is 1.5 times that of its initial state in 0.5 M H₂SO₄. It is generally believed that when nano-hydrogel is placed in an acid bath, the following interactions occur: (i) Hydrogen-bonds form between the protonated carboxyl groups and the copolymer monomer units, reducing the water solubility of the polymer; (ii) the acid bath environment promotes the absorption of water by the nano-hydrogel due to the increased osmotic pressure difference between the internal and external ions, leading to the formation of a three-dimensional network polymer of physical cross-linking, significantly increasing the expansion volume of the nano-hydrogel and reducing the mechanical strength

of the nano-hydrogel; (iii) mobile ions (H⁺ and SO₄²⁻) provide good ionic conductivity, and it is expected that the absorbed water molecules will significantly reduce the physical crosslinking strength of the nano-hydrogel, thereby plasticizing the entire nanogel [6, 25, 26]. These interactions have dynamic adaptability during the copolymerization process, leading to the development of different hydrogen-bond networks. When immersed in H₂SO₄ solution of a specific concentration, the polymer network experiences an osmotic pressure gradient across its interface, which drives water molecules into the nano-hydrogel matrix, inducing swelling. The hydration of hydrophilic groups and water molecules and the unique internal pores of the network structure cause the polymer network to extension and enhance this water absorption capacity. The phantom network model developed by James and Guth suggests that the resulting low concentration of cross-linking points is beneficial for the large movements of binding points [27]. In addition, Flory established a mathematical expression for the swelling capacity of hydrogels based on elastic theory [28]

$$Q^{5/3} = \frac{\left(\frac{1}{2} V_u S^{\frac{1}{2}}\right) + \left(\frac{1}{2} - X_1\right) / V_1}{V_e / V_0} \quad (1)$$

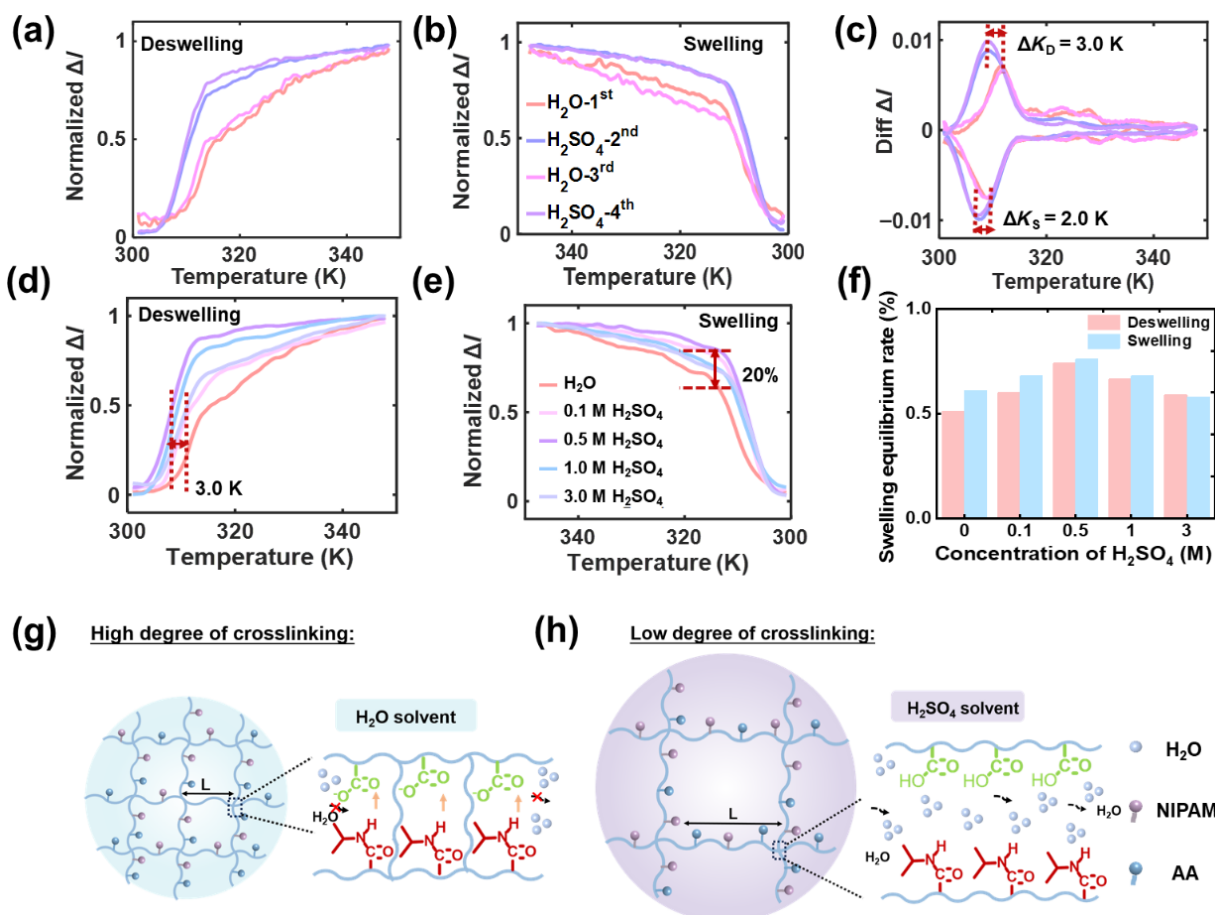


Figure 4 Resolving transient phase transition behavior tuned by differentiated hydrogen-bonding networks within single nano-hydrogel. (a) and (b) Normalized plasmonic intensity variation versus temperature during deswelling and swelling event of single nano-hydrogel in different solution treatments. The nanohydrogel is treated with DIW, and then 0.5 M H₂SO₄ solution to supply protons for hydrogen-bonding formation. The above process is repeated twice to verify its reversibility of hydrogen-bonding networks within single nano-hydrogel. (c) Differential result of plasmonic intensity variation in A and B. (d) and (e) Effect of proton concentration for deswelling and swelling dynamics of a single nano-hydrogel during a representative VPT cycle. (f) Regulation of swelling equilibrium rate of a single nano-hydrogel by proton concentration. (g) and (h) Schematic illustrations of hydrogen-bonding formation and polymer networks within a nano-hydrogel in DIW and proton solution, respectively.

where Q is the rate of water adsorption, the first term of the molecule in the formula $\frac{1}{2} V_0 S^{1/2}$ represents osmotic pressure, the second term $\left(\frac{1}{2} - X_1\right) / V_1$ represents the affinity of the hydrogel with water, and the sum of the two terms represents the water absorption capacity, the denominator V_0 / V_1 represents crosslinking density.

Therefore, when the crosslinking density decreases, the intermolecular force between the polymer chains is significantly weakened, making the network structure of the nanogels looser. This structural change provides a more favorable channel for the entry of water molecules, which greatly enhances the water absorption capacity of nanogels, resulting in a significant increase in the degree of expansion and particle size [29]. At the same time, due to the reduced space limitation for water molecules to move in the network, nanogels exhibit higher deformation flexibility (Figs. 4(g) and 4(h)), which is directly reflected in the solution environment that a single nanogel has a relatively high VPT. However, due to the looseness of the network structure, its VPTT decreased slightly. Upon increasing the sulfuric acid concentration to 3.0 M, the intermolecular interactions within the system underwent substantial modifications. At this time, the formation

rate of hydrogen-bonds between protonated carboxyl groups and copolymers far exceeds the diffusion rate of water molecules, resulting in a large number of water molecules trapped in the nanogels in a short period of time, breaking the original dynamic balance and forming a non-uniform swelling state. This non-uniform swelling state limits the further expansion of the nano-hydrogel network, so that its expansion capacity is moderately reduced compared to the lower sulfuric acid concentration [30]. The H₂SO₄ treatment simultaneously introduces two synergistic effects, protonation and ionic strength. We further performed control experiments by immersing the nanohydrogels in KCl solutions. The results show that KCl treatment similarly induces a significantly VPT signal, accompanied by a slight decrease in VPTT, which is consistent with the thermodynamic trend observed in the H₂SO₄ system (Figs. S9 and S10 in the ESM). However, the two systems exhibit a difference in reversibility, the optical signal of the nanohydrogels in the KCl system fails to return to its initial state, whereas the H₂SO₄ system displays completely reversible network recovery. This comparison indicates that ionic strength weakens the intramolecular interactions through the charge screening effect of the COO⁻, and enhances the VPT response amplitude, but is insufficient to achieve reversible network self-

repair. The superior reversibility imparted by the acidic environment arises from the fact that carboxyl protonation not only eliminates intrachain charge screening but also introduces dynamically reconfigurable hydrogen-bonding networks, thereby enabling the nanohydrogels with pH-modulated structural recoverability.

Regulating the AA content in PNIPAM-based nanogels serves as an effective strategy for achieving broad-range tunability of their LCST. This property is of significant importance for expanding the applications of single-polymer systems in biomedical fields and beyond. The normalized Raman spectra obtained through confocal methods indicate that the signal at $2850\text{--}3050\text{ cm}^{-1}$ corresponds to the C–H stretching band in PNIPAM, while the signal at 1650 cm^{-1} corresponding to the C=O stretching vibration peak of –COOH increases, indicating an increase in the AA monomer content in the copolymer network (Fig. 5(a)) [31].

Nanohydrogels containing 5% AA (a hydrophilic component) exhibit an abrupt change in optical signal (Fig. 5(b)), and the copolymerization of NIPAM with AA increases the VPTT of the nanohydrogels (Figs. 5(c) and 5(d)) and Figs. S16(a) and S16(b) in the ESM). In contrast, the volume jump phenomenon disappears in NIPAM-based nanogels with a higher AA content (60%) (Fig. S15

in the ESM), a phenomenon further confirmed by DLS characterization (Fig. S13 in the ESM). The interactions of the hydrophobic portions of the polymer are increased, the surrounding water structure is reduced, and hydrophobic interactions dominate (Fig. S6 in the ESM). When hydrophilic monomer AA is introduced into the network, the carboxyl groups are randomly distributed throughout the network, increasing the LCST of the nano-hydrogel. Incorporating higher AA content enables intermolecular hydrogen bonding between AA and adjacent NIPAM monomers, with only a fraction of hydrophilic monomers engaging in water molecule capture. Further increases in AA content promote continuous AA–NIPAM interactions, forming robust intermolecular hydrogen bonds that hinder nano-hydrogel network collapse (Fig. 5(e) and Fig. S7 in the ESM).

This study reveals the core role of hydrogen bond nanoconfinement in regulating the phase transition behavior of P(NIPAM₉₅-co-AA₅) nanohydrogels and its dynamic reversibility. First, we demonstrate that the dynamic exchange of water molecules with polymer networks reversibly drives the reorganization of hydrogen-bonded networks, and this process does not destroy the structural integrity of the network. More importantly, by precisely regulating the pH environment, we

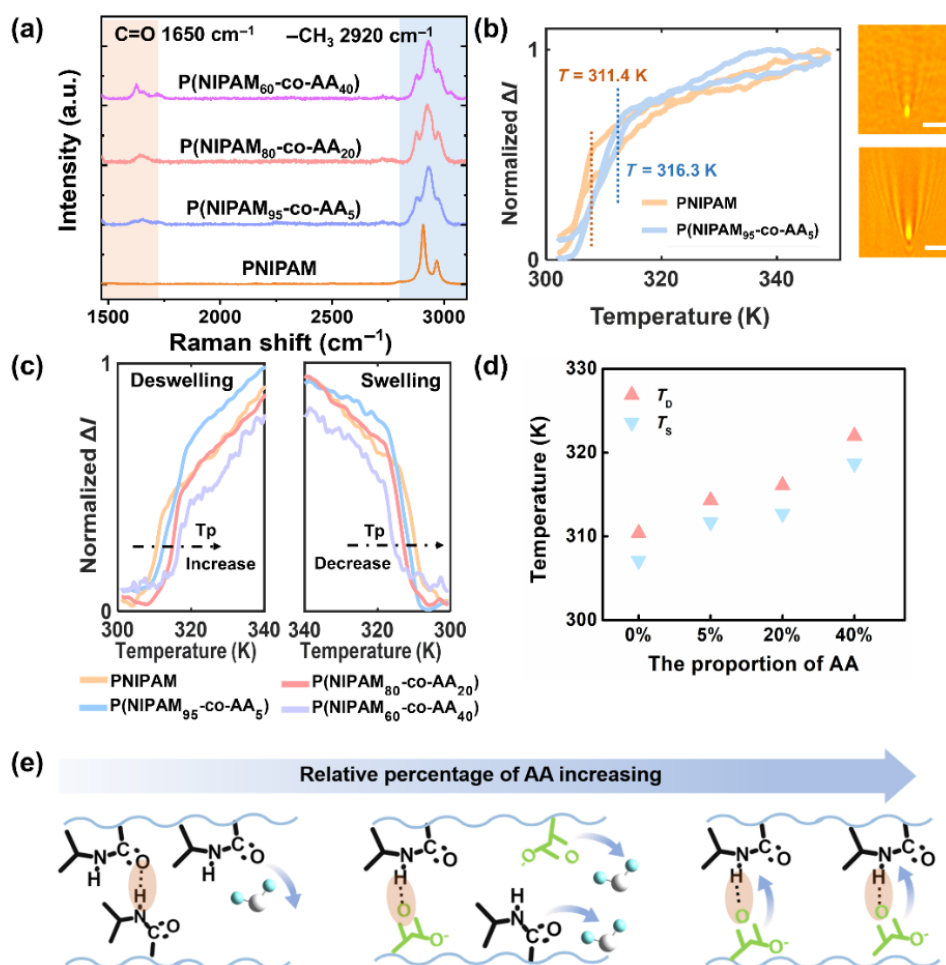


Figure 5 Probing heterogeneous VPT behavior of single PNIPAM nano-hydrogel with different AA contents. (a) Structure characterization by Raman spectra indicating relationship between carbonyl group signal at 1650 cm^{-1} of AA and methyl signal at $2850\text{--}3050\text{ cm}^{-1}$ of NIPAM. (b) Dynamics for transient volume phase transition event of single pure PNIPAM nano-hydrogel and P(NIPAM₉₅-co-AA₅) nano-hydrogel during thermal cycling. Insets show plasmonic images of PNIPAM and P(NIPAM₉₅-co-AA₅) nano-hydrogel and scale bars are 3 and 5 μm , respectively. (c) Deswelling and swelling dynamics of single PNIPAM nano-hydrogel with different AA contents in a representative VPT cycle. (d) VPTT of single PNIPAM nano-hydrogel with different AA contents. (e) Hydrogen-bonding evolution diagram of single nano-hydrogel contain with different AA contents.

realized the “programming” of the hydrogen-bonded nanoconfinement strength, at a specific acid concentration, the osmotic pressure-driven hydration and the protonated carboxyl-mediated physical cross-linking achieve the best synergy, which induces a larger volume expansion and the lowest VPTT. However, too strong hydrogen bonds (at high AA content or high acid concentration) will freeze the network dynamics and inhibit the phase transition. These findings shed light on a universal strategy to precisely design hydrogel responsive behavior by balancing hydrophilic/hydrophobic interactions and osmotic pressure.

The deswelling/swelling reproducibility of single P(NIPAM₉₅-co-AA₅) nanohydrogel is finally evaluated (Fig. S17 in the ESM). Over 70 consecutive heating-cooling cycles, a recoverable VPT of single nanohydrogel is observed, confirming their unique reversibility. To further investigate the effect of nanohydrogel size on VPT behavior, all three size populations of nanohydrogels exhibit typical VPT behavior. Notably, with increasing particle size, the volume change of nanohydrogels during the VPT process is significantly enhanced (Fig. S12 in the ESM). The size of nanohydrogels is a key parameter regulating their VPT response characteristics, which provides an experimental basis for the performance optimization of nanohydrogels designed based on size.

4 Conclusion

In summary, we have established a localized thermoregulation-coupled surface plasmon resonance imaging strategy, enabled by an integrated optical tweezer system, to interrogate the transient volume phase transition dynamics of single nano-hydrogels under laser-focused thermal modulation. This single-entity *operando* approach resolves intermediate states and equilibrium thermodynamics during volume phase transition event. The intrinsic VPT temperature of individual nano-hydrogels governed by hydrogen-bond nanoconfinement within polymer networks is quantified, independent of external thermal modulation parameters. Furthermore, by correlating transient hydration states with polymer-water hydrogen-bond reorganization during VPT, we elucidated the nanoscale mechanistic interplay between water-molecule dynamics and phase transition energetics. By introducing pH-tunable hydrogen-bonding interaction, we decoded heterogeneous VPT behaviors through programmable control of three-dimensional polymer crosslinking densities at the single-nanogel level. This spatiotemporal thermal-field manipulation strategy not only establishes a universal methodology to correlate nanoconfinement effects with VPTT modulation but also provides foundational insights for engineering stimulus-responsive nanomaterials. It also opens avenue for probing nano-confined phase transitions in adaptive intelligent systems, which promotes from flexible nanoelectronics to precision biomedicine. Future work will focus on applying these basic design principles to specific device construction and *in vitro* biological testing.

Electronic Supplementary Material: Supplementary material (further details of materials, characterization, experimental, SEM images of nano-hydrogels and other experimental figures) is available in the online version of this article at <https://doi.org/10.26599/NR.2026.94908979>.

Data availability

All data needed to support the conclusions in the paper are

presented in the manuscript and the Electronic Supplementary Material. Additional data related to this paper may be requested from the corresponding author upon request.

Acknowledgements

Financial support of this work from the National Natural Science Foundation of China (NSFC, Nos. 22574073, 22222406, and 22174062) was acknowledged.

Declaration of competing interest

The authors declare no conflict of interest.

Author contribution statement

H. W. designed research. N. P. performed research. X. Y. G., D. H., and B. J. conducted the sample synthesis and Raman measurement. W. W. provided the guidance on this work and revised manuscript. N. P. and H. W. wrote the paper. All authors have given approval to the final version of the manuscript.

Use of AI statement

None.

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