

Programmable Anisotropy and Percolation in Supramolecular Patchy Particle Gels

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Abstract

Patchy particle interactions are predicted to facilitate the controlled self-assembly and arrest of particles into phase-stable and morphologically-tunable “equilibrium” gels, which avoids the arrested phase separation and subsequent aging that is typically observed in traditional particle gels with isotropic interactions. Despite these promising traits of patchy particle interactions, such tunable equilibrium gels have yet to be realized in the laboratory due to experimental limitations associated with synthesizing patchy particles at high yield. Here, we introduce a supramolecular metal-coordination platform consisting of metallic nanoparticles linked by telechelic polymer chains, which validates the predictions associated with patchy particle interactions, and facilitates the design of equilibrium particle hydrogels through limited valency interactions. We demonstrate that the interaction valency and self-assembly of the particles can be effectively controlled by adjusting the relative concentration of polymeric linkers to nanoparticles, which enables the gelation of patchy particle hydrogels with programmable local anisotropy, morphology, and low mechanical percolation thresholds. Moreover, by crowding the local environment around the patchy particles with competing interactions, we introduce an independent method to control the self-assembly of the nanoparticles, thereby enabling the design of highly anisotropic particle hydrogels with substantially reduced percolation thresholds. We thus establish a canonical platform that facilitates multifaceted control of the self-assembly of the patchy nanoparticles *en route* to the design of patchy particle gels with tunable valencies, morphologies, and percolation thresholds. These advances lay important foundations for further fundamental studies of patchy particle systems, and for designing tunable gel materials that address a wide range of engineering applications.

Keywords

patchy particles, self-assembly, supramolecular chemistry, equilibrium gels, bioinspired materials

Introduction

The route to dynamic arrest in particle gels has profound consequences on their emergent structure and mechanical properties, and has important implications for understanding self-assembly in biology and optimizing the design of functional soft materials. For particles with isotropic and reversible interactions such as colloids interacting *via* depletion interactions, *dynamic arrest* (at volume fractions ϕ below the glass transition) commonly occurs through an arrested spinodal decomposition, following quenching into a two-phase coexistence region.¹⁻² In contrast, for particles with limited-valency interactions, theoretical studies have predicted that arrest can occur through equilibrium associations, due to the emergence of interaction anisotropy and suppression of the two-phase region to higher interaction strength ε and lower ϕ (Fig. 1A).³⁻⁷ Unlike isotropic particle gels, which continuously age with time due to their underlying thermodynamic instability, these “patchy” particle gels can thus undergo “*equilibrium arrest*” and remain thermodynamically stable⁷ and thus avoid temporal aging effects. Patchy particle arrest therefore represents a promising method for designing soft particle gels for materials applications requiring long-term stability, with relevance in structural and biomedical engineering. Moreover, theoretical studies have predicted that the morphology and the density of such patchy particle gels should also be tunable by controlling the valency (and therefore the anisotropy) of particle interactions,^{2,6} enabling the design of stable soft materials with a broad range of microstructures. In fact, growing evidence suggests that this exact strategy is utilized during the assembly of soft and stable biological materials with tunable microstructures, such as graded-index protein lenses in squid eyes⁸ and degradation-resistant egg whites in duck eggs,⁹ among others.¹⁰⁻¹¹

Despite these promising traits of patchy particle gels, experimental validation of their properties remains limited. Although advances in molecular engineering now permit the design of a myriad of patchy particles with precise interaction sites and valencies,¹²⁻²³ these systems remain difficult to synthesize and assemble at requisite scales for designing patchy particle gels. Thus, patchy particle arrest has so far only been studied in a narrow range of experimental systems with fixed valencies, most prominently in discotic clay particles,³ but also in molecular systems, such as DNA stars^{2, 7, 24-26} and peptides.²⁷ Notably, the lack of an accessible experimental particle platforms in which the particle patch valency can be tuned has prevented access to the design of gels with the wide ranges of morphologies and percolation behaviors predicted by theory.^{6, 8} A patchy particle-based material system with tunable valency would not only facilitate bio-inspired designs of stable particle gels with tunable morphologies and percolation thresholds,⁸ but also allow deeper studies of the fundamental physics of patchy particle self-assembly in a variety of environments.² Thus, in this work, we show that a polymer-metal nanoparticle composite gel system governed by supramolecular metal-coordination interactions²⁸ provides an experimental realization of a patchy particle system with tunable effective interaction valency, and permits study of the self-assembly and equilibrium arrest of particles at a variety of morphologies and percolation thresholds.

Results and Discussion

Our gel system consists of 1-arm catechol-functionalized poly(ethylene glycol) (1cPEG) chains, which are grafted onto Fe₃O₄ nanoparticles (NPs), and 4-arm nitrocatechol-functionalized poly(ethylene glycol) (4nPEG) telechelic linker chains (Fig. 1B). The catechol ligand coordinates strongly onto the Fe₃O₄ NP surface to facilitate a highly water-stable 1cPEG-NP complex (Fig. 1C),²⁹ but the ligand can be dynamically exchanged by the stronger binding nitrocatechol ligand on the 4nPEG (Fig. 1D).³⁰⁻³¹ Upon introducing the 4nPEG to an aqueous solution of 1cPEG-NP, the ligand exchange process can therefore induce bridging attractions between the initially-repulsive NPs, which drives formation of a supramolecular patchy particle hydrogel (SPPH) in a state of arrest (Fig. 1E).²⁸ As such, we may expect that the interaction valency, and thus the average coordination number M (the number of connected nearest neighbors per particle)^{5, 8} in the SPPH, be tunable in a straightforward manner by simply adjusting the *ratio of the number of the 4nPEG linkers to the number of the NPs*, $r = N_{4nPEG}/N_{NP}$. We

can assume r will then be correlated to, but generally larger than, M , due to the many competitive binding events that may also occur upon introducing 4nPEG linkers to the NPs. For instance, in addition to inducing high M associations between NPs, the linkers can also remain unbound, form loops (with both ends of the polymers binding to the same NPs), form dangling ends (with one unbound end), and also multi-valent interactions (with multiple linkers binding two NPs, a phenomenon which can drastically slow down dissociation).³² These numerous possibilities and degrees of freedom makes it difficult to *a priori* determine the coordination number in a patchy hydrogel system of a given linker-to-NP ratio r – this may be more feasible through simulation techniques, as demonstrated successfully in the references,^{8, 33} which would contribute to our design framework. Nevertheless, in this work, we demonstrate that this simple parameter r can adequately serve as an indirect macroscopic handle to access various phase behaviors expected from patchy particles of different interaction valencies, and to design “equilibrium” gels with controlled anisotropy, morphology, and percolation behaviors.

Using prototypical concentrations of $n_l = 10$ wt. % 4nPEG linkers, and $\phi_{NP} = 1.93$ vol. % Fe_3O_4 NPs following prior work on a similar system²⁸, we characterize the SPPH and test for the most distinctive hallmarks of equilibrium arrest, namely temporal equilibration of both the mechanical and structural properties of the gel.^{2-5, 8-9} For a typical gel volume of $V = 200 \mu\text{L}$, these species concentrations correspond to $N_{4nPEG} = 2.0 \times 10^{-6}$ mol and $N_{NP} = 3.5 \times 10^{-8}$ mol (given $D \approx 7$ nm), thus corresponding to $r = \frac{N_{4nPEG}}{N_{NP}} = \frac{10.81n_l}{\phi_{NP}} = 56$ (derivation in the SI). Time-dependent viscoelastic measurements show that, upon mixing the 4nPEG and NPs in an aqueous solution and raising the temperature to 55 °C, gelation (as defined by the point when the storage modulus G' exceeds the loss modulus G'') is observed within 10 minutes (Fig. S4A), followed by a steady evolution of the moduli until the moduli reach equilibrium values after approximately 8 hours (Fig. 1F, S4B). This gelation process is mediated by the ligand exchange and bridging of 1cPEG-NP complexes by 4nPEG (Fig. 1D, 1E); analogous control systems with 4-arm PEGs functionalized with weaker affinity ligands that cannot displace the 1cPEG graft on the NPs as effectively are not able to create stiff gels (Fig. S5). Step strain measurements of the SPPH at 25 °C reveal slow and broadly-distributed stress relaxation modes, which can be succinctly captured by a Kohlrausch-Williams-Watts (KWW) model of the form $G = G_0 \exp[-(t/\tau)^\alpha]$ with a stretching exponent of $\alpha \approx 0.31$ (parameters in Table S3) consistent with the theoretically predicted $\alpha \approx 0.33$ for a deeply-arrested equilibrium associating system (Fig. 1G).³⁴⁻³⁵ Importantly, stress relaxation measurements after 24 and 48 hours of aging nearly overlap with each other, again in support of the negligible rheological aging in our patchy gel, in contrast to the substantial rheological aging observed in typical colloidal gels.³⁻⁴ Characterization of the time-dependent structure factor $S(q)$ the SPPH *via* small-angle x-ray scattering (SAXS) tells a similar story (Fig. 1H). $S(q)$ is obtained from scattering measurements by dividing the scattered intensity $I(q)$ by a spherical form factor (see Methods). A pronounced interparticle peak is observed at $q \approx 0.05 \text{ \AA}^{-1}$, corresponding to an interparticle distance of $D_{NP-NP} = 2\pi/q = 12.5$ nm, which represents the combined diameters of the particle (~ 7.0 nm, Fig. S2B) and the bridging polymer (~ 5.5 nm, Fig. S1B), and is in good agreement with the proposed microstructure of the SPPH (Fig. 1E). The upturn in $S(q)$ at low q indicates the formation of local aggregates with fractal dimensions of $D_f = 2.7$ (as determined by the slope), consistent with commonly observed values in polymer gels³⁶⁻³⁷ and biological patchy gels.⁸ Characterization of $S(q)$ at even lower q *via* ultra-small-angle x-ray scattering (USAXS) reveals that this fractal regime extends up to the local mesh size of $\xi \sim 200$ nm (Fig. S6), suggesting that $D_f = 2.7$ originates from intra-mesh surface scattering of NP aggregates.³⁷ Importantly, unlike arrested spinodal decomposition gels which exhibit continuous structural aging with time,^{1, 3-4} our SPPHs exhibit no evidence of structural aging as manifested by the overlap in the $S(q)$ measurements after 24 and 48 hours of aging, in agreement with mechanical measurements (Fig. 1F, 1G), and providing strong evidence of equilibrium arrest in the SPPH.

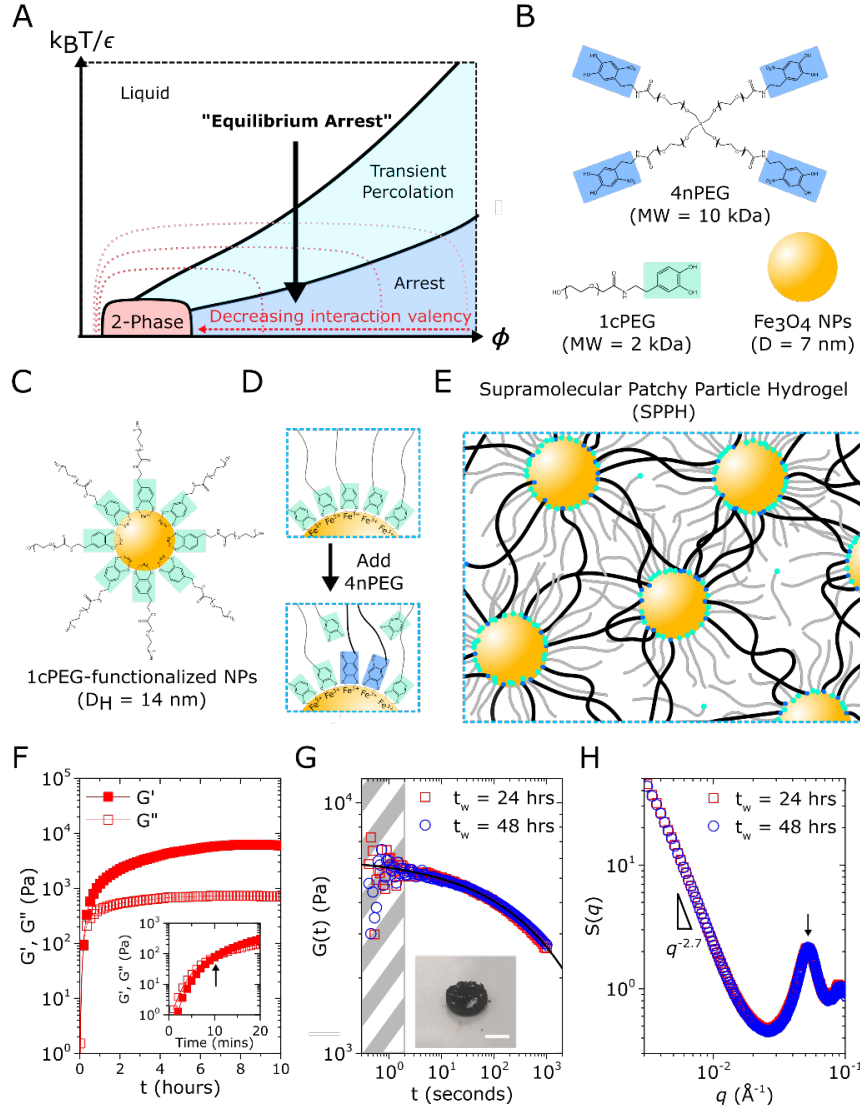


Figure 1. Design of phase-stable patchy particle hydrogels through supramolecular interactions. A) Schematic phase diagram of attractive patchy particles as a function of effective temperature $k_B T/\epsilon$ and volume fractions ϕ . The reduction of interaction valency causes the two-phase coexistence curve to shrink to smaller ϕ and higher ϵ .⁵ This contraction opens routes for patchy particles to become arrested at arbitrarily low densities through an equilibrium association-dissociation process (“equilibrium arrest”) rather than an arrested spinodal decomposition.^{2, 35} B) Structures of the Fe_3O_4 NPs, 1cPEG, and 4nPEG. C) Schematic of the 1cPEG-stabilized Fe_3O_4 NP complex, which exhibits a narrow distribution in the hydrodynamic diameter around $D_H \approx 14$ nm (Fig. S3A). D) Illustration of the ligand exchange process between 1cPEG and 4nPEG that occurs at the surface of the Fe_3O_4 NP. E) Schematic of the supramolecular patchy particle hydrogel (SPPH). Grey lines with teal caps represent 1cPEG, and black lines with blue caps represent 4nPEG linkers. F) Time-dependent evolution of the storage modulus G' and loss modulus G'' during assembly of the SPPH system as a result of the ligand exchange process at 55 °C measured *via* small-amplitude oscillatory shear (SAOS). (Inset) Gelation, defined as the point where $G' > G''$, occurs at 10 minutes (arrow). G) Relaxation modulus $G(t)$ of the SPPH at 25 °C measured at wait times of $t_w = 24$ hours and $t_w = 48$ hours after gelation. The solid line is a fit of the KWW equation to both sets of data. The noisy data in the shaded region at $t \leq 2$ seconds are within the finite noise time of the instrument and are discarded from the fitting procedure. (Inset) Representative macroscopic image of the SPPH gel at $v = 56$. Scale bar = 1 cm. H) Static structure factor $S(q)$ of the SPPH (obtained by dividing the scattered intensity $I(q)$ by a spherical form factor, see Methods) at wait times of $t_w = 24$ hours and $t_w = 48$ hours after gelation.

Another distinct hallmark of arrested patchy particle systems is the controllability of their self-assembly and phase behavior *a priori* by the external tuning of the particle patch valency (Fig. 2A).^{2, 5, 8} We demonstrate such control in our SPPH, wherein the average coordination number M is controlled

indirectly by tuning the number of 4nPEG linkers between the particles through the parameter $r = N_{4nPEG}/N_{NP}$. Through electron microscopy and rheology, we show that at low r , the system remains in the sol state as NP self-assembly is anisotropic and sparse (due to small number of linkers); above a percolation threshold of r , the system undergoes gelation and can become progressively denser and stiffer with increasing r ; and at higher r , the dense gel undergoes precipitation due to the increasing isotropy of the NP self-assembly, and the expected re-emergence of the two-phase coexistence curve at high valencies (and weaker repulsive interactions) (Fig. 2A).^{2, 5}

We demonstrate the controllability of the self-assembled microstructure of the SPPH through r *via* scanning and transmission electron microscopy (SEM / TEM). SEM microscopy images of the SPPH gels (preparation details in the Methods) show dramatic differences at $r = 7.5, 28.0$, and 56.0 , with a notable increase in the density of dark spots at lower r values (Fig. 2B, S9A, S9B, S9E). These dark spots represent electron-density-deficient (and thus NP-deficient) regions, which is corroborated by backscattered electron micrographs of the same systems (Fig S9C, S9D). Thus, the SEM images provide corroboration of the expected structures of the SPPH as a function of r (Fig. 2A). TEM images of the NP assemblies reveal the microscopic origins of these morphologies observed in SEM. For TEM imaging, the SPPHs were imaged in the solution state *via* significant dilution followed by air-drying on a carbon grid (see Methods). Notwithstanding the effects of the air-drying procedure on the microstructure of the system, a clear difference between the $r = 3.0$ and the $r = 56.0$ can be seen (Fig. 2C, S8A, S8B). A sheet-like single layer of NPs is observed for $r = 3.0$, whereas NP aggregation is observed for $r = 56.0$, evidenced by the sample opacity due to lack of beam penetration. Similar NP aggregations are observed at even higher valencies facilitated by $r = 112$ (Fig. S8C). We note that sheet-like layers are an important anisotropic structural motif in the SPPH – although they are not visible in the SEM micrographs discussed thus far (Fig 2B), we revisit this issue later and show that these sheet-like features do become salient and visible in SEM upon introducing a network-stabilizing competing ion. Overall, the TEM images provide microscopic evidence of the onset of anisotropy at lower r and vice versa, corroborating the different microstructures of the gels observed *via* SEM. These demonstrations of programmable morphologies in the SPPH is consistent with predicted behavior of patchy particle systems, where the interaction valency dictates the anisotropy of interactions, and thus the density of the resulting self-assembled particle network.^{2-3, 5-6, 8-9}

Rheological characterizations show that the SPPH system is able to access a wide array of rheological states, as a direct consequence of the different microstructural self-assembly of the NPs at different r . Linear viscoelastic measurements show that with increasing r at constant $\phi_{NP} = 1.93\%$ – that is, with the same density of cross-linking nodes (the nanoparticles), but with varying density of linkers – the SPPH transitions from a viscoelastic fluid ($r = 4.5$) to a viscoelastic gel ($4.5 < r \leq 56$), and then transitions back to a softer gel at higher interaction valencies ($r = 112$) (Fig. 2D). This behavior is concisely illustrated by the frequency-averaged phase angle $\overline{\delta(\omega)}$ of each system: with increasing r , the SPPH transitions from a viscous liquid ($\overline{\delta(\omega)} \approx 90^\circ$) to an elastic solid ($\overline{\delta(\omega)} \approx 0^\circ$) and back towards the viscous liquid state (Fig. 2E). The stiffening of the SPPH at $\phi_{NP} = 1.93\%$ with increasing r is consistent with the expected behavior of a patchy system with varying interaction valencies – for example, the phantom network theory shows that for a valency-controlled network with the same node density, the modulus scales with $(M - 2)/M^{38}$ – as is the re-entrant softening at higher r which is indicative of an onset of phase separation. At an even higher linker-to-NP ratio $r = 168$, the SPPH is no longer homogeneous and visibly phase separates into viscoelastic sol and gel phases (Fig. 2F; rheological characterization of constituents in Fig. S10B). This provides further proof that the re-entrant softening of the SPPH at $r = 112$ originates from phase separation, as expected from the emergence of the two-phase coexistence at high interaction valency.⁵ The r -dependent state behaviors of the SPPH is therefore consistent with systems composed of patchy particles at different valency (Fig. 1A), and suggest a direct relationship between the linker-to-NP ratio, the tunable local anisotropy, the

programmable morphology of the self-assembling patchy particle network, and ultimately the final global state of the SPPH system.^{2-3, 5-6, 8-9}

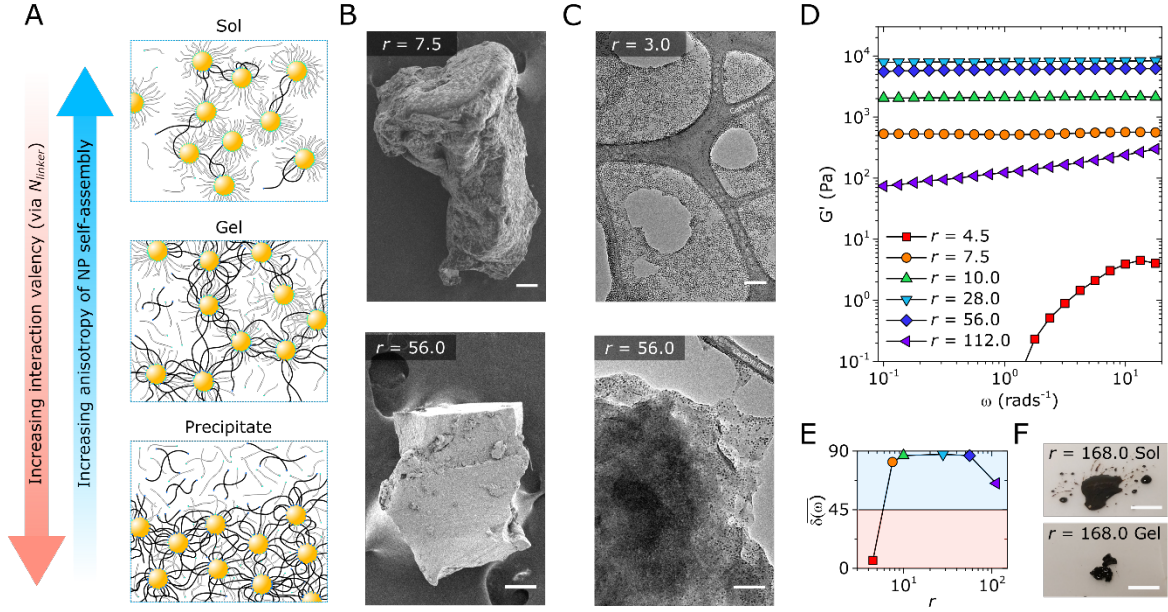


Figure 2. Systematic control of self-assembly, morphology, and rheological state behavior of the SPPH obtained by varying the linker-to-NP ratio parameter r . A) Illustration of the effect of varying N_{linker} via the linker-to-NP ratio r , and therefore the interaction anisotropy, by introducing different amounts of 4nPEG linkers to the 1cPEG-NP complex at constant ϕ_{NP} and volume (same graphical representations as Fig. 1E). B) Representative SEM micrographs of the SPPH in the gel state at $\phi_{NP} = 1.93\%$ and at $r = 7.5$ and $r = 56.0$. Scale bar = 100 μm . C) Representative TEM micrographs of diluted SPPH solutions at $r = 3.0$ and $r = 56.0$. The thick strips in the micrographs are the holey carbon grids on which the samples were dried (see Methods). Scale bar = 100 nm. D) Storage modulus $G'(\omega)$ of the SPPH at $\phi_{NP} = 1.93\%$ and various r (corresponding rheograms of the loss modulus $G''(\omega)$ shown in Fig. S10A). The viscoelastic gel state at $4.5 < r \leq 56$ shows an almost frequency-independent response in $G'(\omega)$ and $G''(\omega)$, consistent with the broad stress relaxation spectra observed in Fig. 1G. E) Frequency-averaged phase angle $\bar{\delta}(\omega)$ of the SPPH as a function of r (symbols corresponding to those in Fig. 2D). As hypothesized in Fig. 2A, increasing r causes the SPPH system to transition from a viscoelastic liquid (shaded in red) to a viscoelastic solid gel (shaded in blue). The onset of phase separation is observed at $r = 112$. F) Macroscopic images of the system at $r = 168$, exhibiting visible phase separation into viscoelastic solid and viscoelastic liquid states. Scale bar = 1 cm.

The valency-dependent rheological state behaviors of our SPPH system can be systematically explored through gelation experiments at different polymer linker concentrations n_l and NP concentrations ϕ_{NP} , the results of which are summarized by a state diagram of r^{-1} as a function of ϕ_{NP} (Fig. 3A). The state diagram shows that high linker-to-NP ratios r ($N_{4nPEG} \gg N_{NP}$) lead to the precipitation and collapse of the gel phase across a wide range of ϕ_{NP} , which is consistent with the predicted emergence of the two-phase coexistence (Fig. 1A) and the densification of morphologies in the SPPH (Fig. 2A, 2B, 2C) at high interaction valencies. Therefore, at a given linker concentration n_l , the percolation threshold for the gel phase as a function of ϕ_{NP} is dictated by the point at which $r = \frac{10.81n_l}{\phi_{NP}}$ is suppressed below the threshold for precipitation (roughly when the iso- n_l lines cross the $r \approx 100$ precipitate-gel boundary with increasing ϕ_{NP} , Fig. 3A). This observation suggests that the NP percolation threshold may be tunable by adjusting the interaction valency *via* the linker concentration n_l , and indeed, rheology measurements of the SPPH across different n_l and ϕ_{NP} validate this hypothesis (Fig. 3B, S13). The frequency-averaged storage modulus $\overline{G'}(\omega)$ is obtained by averaging $G'(\omega)$ from $\omega = 0.1 \text{ rad s}^{-1}$ to $\omega = 42.2 \text{ rad s}^{-1}$ for the different SPPH (full SAOS spectra in Fig. S13). The mechanical percolation thresholds ϕ_c of the NPs of the SPPH at different n_l (Fig. 3C) are obtained by fitting the data to a classic

percolation model, $\overline{G'(\omega)} = K(\varphi - \varphi_c)^\gamma$, where K is a pre-factor and γ is a scaling exponent related to the dimensionality of the percolating system.^{36, 39-41} As hypothesized, the NP percolation threshold φ_c is correlated to linker concentration n_l , in excellent agreement with the micrographs showing that low values of r drive local anisotropy in NP self-assembly and low-density SPPH morphologies (Fig. 2B, 2C). The relationship between the dimensionality exponent γ and n_l tells a similar story (Fig. 3D). All obtained exponents are below $\gamma \cong 2$, indicating that NP percolation in SPPH is analogous to percolation of resistor networks or gels with isotropic forces,^{39, 41} in good agreement with prior studies on polymer-bridged NPs, and PEG networks.^{36, 40} Importantly, γ also varies systematically with n_l , and decreases from $\gamma \approx 1.7$ to $\gamma \approx 1.2$ as the linker concentration is lowered from $n_l = 15.0\%$ to $n_l = 1.8\%$. This trend is indicative of a progressive reduction in the dimensionality of the percolating system from 3-D to values close to 2-D (per numerical simulation estimates, $\gamma \approx 1.5 \sim 2.0$ for 3-D and $\gamma \approx 1.1 \sim 1.3$ for 2-D),^{36, 39-41} in excellent agreement with the expected onset of anisotropy at lower linker concentrations and v (Fig. 2A, 2B, 2C). These results confirm that controlling the NP interaction valency provides programmable anisotropy, morphology, and percolation threshold of the SPPH, thus corroborating theoretical predictions^{2, 6, 8} and advancing our ability to design structurally versatile biomimetic patchy particle systems.⁸

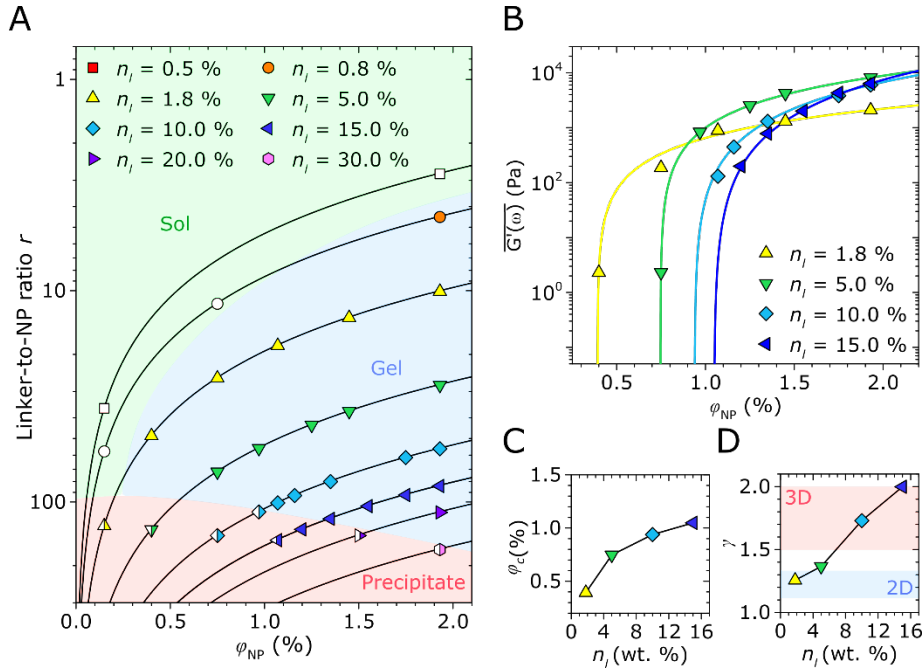


Figure 3. Systematic control of rheological states and percolation thresholds in the SPPH. A) r^{-1} - φ_{NP} state diagram of the SPPH for different linker concentrations n_l (wt. %). The state diagram is divided into sol, gel, and precipitate regions (see Fig. 2A) by categorizing SPPH systems based on the following criteria: sol for systems which are visibly fluids with no observed gelation (Fig. S11); gel for systems with measurable plateaus in the storage moduli $G'(\omega)$ (Fig. 2D, 3B, S13); precipitate for systems which are either visibly phase-separated (see Fig. 2F), or behave as fluids at the particular r but are observed to form gels at lower r . Individual sol, gel, and precipitate systems are also marked as open, filled, and half-filled symbols, respectively. The solid lines connecting the symbols represent iso- n_l lines in the v - φ_{NP} space. Note that r is equivalent to $10.81 n_l / \varphi_{NP}$ (see SI). B) Frequency-averaged storage moduli $\overline{G'(\omega)}$ plotted as a function of φ_{NP} for SPPH with different linker concentrations n_l (wt. %). Solid lines indicate fits to the percolation model $\overline{G'(\omega)} = K(\varphi - \varphi_c)^\gamma$ (fitting parameters in Table S4). C) Percolation threshold φ_c and D) dimensionality exponent γ as functions of linker concentration n_l , with symbols corresponding to the data shown in Fig. 3B. Red and blue shaded regions in D) illustrate the range of predicted γ values *via* numerical simulations for 3-D and 2-D percolation, respectively.^{36, 39-41}

Lastly, we demonstrate a strategy to control the self-assembly and percolation behaviors of the SPPH *independently* from the aforementioned method of varying linker concentration n_l . Specifically, we hypothesized that introducing weak cross-linking species to compete with the NPs for the 4nPEG

linkers may reduce the number of linkers that form between NPs, such that the asymptotic increase in r with increasing N_{4nPEG} at low φ_{NP} (Fig. 3A) may be avoided. Our hypothesis is motivated by patchy particle assembly in biological settings, which often takes place in crowded viscoelastic environments such as the cellular cytosol which have many competing weak interactions.^{8, 42-43} Thus, to simulate such a crowded competitive macromolecular environment for the SPPH, we introduce Fe^{3+} ions to the 4nPEG linkers ($n_l = 10$ wt. %) and NP mixture. In addition to competing with the Fe_3O_4 NP surface for the nitrocatechol groups in 4nPEG (thus reducing r), the Fe^{3+} ions also transiently cross-link the 4nPEG linkers *via* di-functional coordination bonds to form a viscoelastic fluid (Fig. 4A). We refer to this system as a *supramolecular patchy particle hydrogel with competing interactions* (SPPH-CI). SEM micrographs of the SPPH-CI show that the competing ions induce striking changes in the morphology of the system compared to the SPPH. Notably, we observe highly anisotropic striations at mesoscopic length-scales in the SPPH-CI *via* SEM (Fig 4B, S16). This shows that the SPPH-CI system is able to retain the sheet-like layers of NPs – previously observed in the regular SPPH *via* TEM (Fig. 2C) – at mesoscopic length-scales due to the presence of the transient ion network. We also observe regular porosity in the microstructure of the SPPH-CI samples, which appears to gain prominence with increasing φ_{NP} (Fig. S14), and can be attributed to NP-rich domains. Lastly, TEM micrographs of the diluted SPPH-CI show clear evidence of assortative self-assembly⁴⁴ leading to a clear separation of NP-rich and ion-rich domains (Fig. S15), justifying the proposed microstructure of the SPPH-CI (Fig. 4A).

The onset of these highly anisotropic structures is also correlated with a much earlier onset of mechanical percolation of the NP network in the SPPH-CI compared to the regular SPPH, as demonstrated by rheological measurements of the SPPH-CI as a function of φ_{NP} for $n_l = 10.0$ % (Fig. 4C). Evidence of a contribution to the elastic modulus from the slowly-relaxing NP network emerges at $\varphi_{NP} = 0.15$ % (arrow), far below the critical percolation threshold of $\varphi_c \sim 1.1$ % predicted for the regular SPPH at $n_l = 10$ wt. % (Fig. 3B, 3C). This finding is corroborated by the observation of an emergent NP mesostructure at $\varphi_{NP} = 0.15$ % in the SPPH-CI observed *via* USAXS (Fig. S17). The obvious separation of timescales between the ion and NP relaxation modes in Fig. 4C allows us to capture the individual contributions of the ion and NP networks to the viscoelastic moduli, *via* the fractional Zener model (FZM, Fig. S18A). Fitting results to the FZM are shown as solid and dashed lines in Fig. 4C, tabulated in Table S2, and shown individually in Fig. S18D. This operation allows us to obtain the viscoelastic moduli of just the NP network between $\omega = 0.1 - 42.2$ rads^{-1} (*i.e.* the dashed lines in Fig. S18D) and fit the frequency-averaged moduli to the percolation model $\overline{G'(\omega)} = K(\varphi - \varphi_c)^\gamma$. We find that $\varphi_c \approx 0.2$ % and $\gamma \approx 1.16$ for the NPs in the SPPH-CI (Fig. S18B). These percolation parameters are lower than those from all other regular SPPHs studied, indicative of highly anisotropic self-assembly. The separation of timescales between the ion and NP relaxation modes also allows us to illustrate the individual contributions of the ion and NP components to the overall moduli of the system based on the FZM predictions in Fig. S18D (Fig. 4D). The timescales for the ion and NP relaxations are estimated to be at $\omega = 10^3$ rads^{-1} and 10^{-2} rads^{-1} , respectively, but we note that the trend is not sensitive to the particular ω estimates chosen for the ion and NP systems (Fig. S18E). The results show that the viscoelastic response of the 4nPEG + Fe^{3+} transient network matrix dominates the overall viscoelastic response of the SPPH-CI system at low φ_{NP} (Fig. 4D), which suggests that the introduced Fe^{3+} ions successfully compete away a majority of 4nPEG linkers compared to the binding sites on the surfaces of the NP. Therefore, competing ion interactions suppress the increase in the linker-to-NP ratio r at low φ_{NP} which would otherwise cause the collapse of the gel phase due to the high interaction valency associated with high r (Fig. S19). The suppressed r also facilitates a highly anisotropic and low-density self-assembly of NPs, in agreement with our earlier hypothesis. These findings highlight the potentially complementary role of crowding and competition in facilitating anisotropic self-assembly of patchy building-blocks in biology,^{8-9, 42} and provides a bio-inspired approach to independently tailor the interaction valency, morphology, and density of patchy particle assemblies in engineered soft materials.

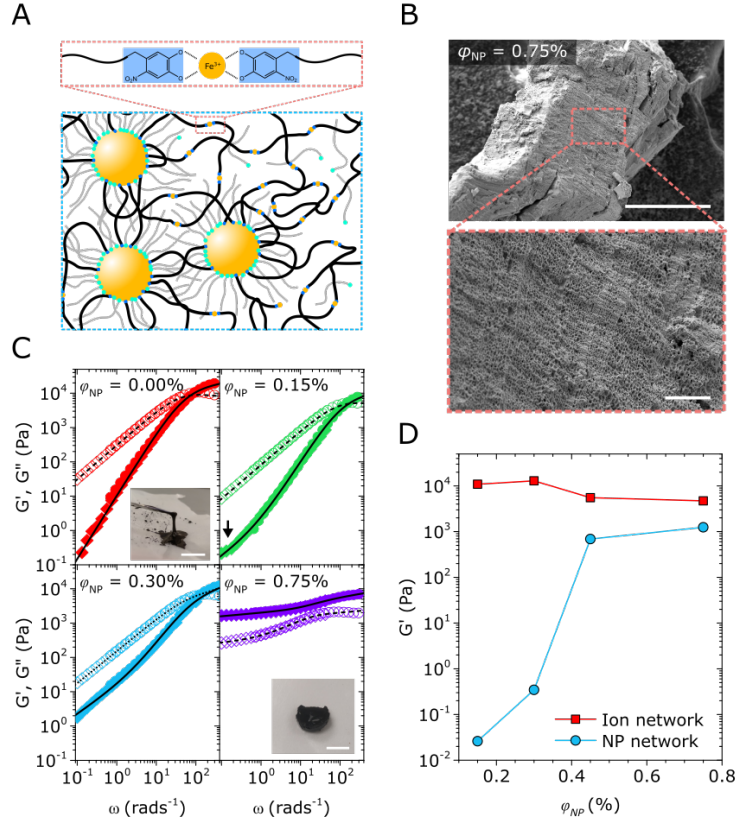


Figure 4. Independent control of the valency of particle-particle interactions in the SPPH ($n_l = 10$ wt. %) by introducing competing interactions. A) Schematic of the crowded SPPH with competing ion interactions (SPPH-CI). The Fe^{3+} ions introduced in the gel compete with the Fe_3O_4 NPs for the 4nPEG linkers to form di-functional coordination complexes (red box). B) Representative SEM micrographs of the SPPH-CI system at $\phi_{NP} = 0.75\%$, at different magnifications. Top panel scale bar = 100 μm ; bottom panel scale bar = 10 μm . Anisotropic striations are observed throughout the sample, which are also observed in the $\phi_{NP} = 0.45\%$ gel and in different samples of the $\phi_{NP} = 0.75\%$ gel (Fig. S16). C) Evolution of the storage modulus $G'(\omega)$ (solid symbols) and loss modulus $G''(\omega)$ (open symbols) of the SPPH-CI with increasing ϕ_{NP} , at 4nPEG concentrations of $n_l = 10$ wt. % (see Fig. S18C for additional ϕ_{NP} values). The data is obtained from SAOS measurements at 5 $^{\circ}\text{C}$ (circles) and 25 $^{\circ}\text{C}$ (diamonds), and shifted to a reference temperature of 25 $^{\circ}\text{C}$. Solid and dashed lines indicate fits of the dynamic moduli to the FZM. The insets in the $\phi_{NP} = 0.00\%$ and $\phi_{NP} = 0.75\%$ panels show representative images of the SPPH-CI system at the respective NP concentrations, demonstrating a stark transition from a mobile viscoelastic liquid to a rubbery gel. Scale bar = 1 cm. D) The elastic contributions of the Fe^{3+} ion network and the Fe_3O_4 NP network to the overall viscoelasticity of the SPPH with competing interactions, as a function of ϕ_{NP} .

Conclusion

To conclude, we have presented a supramolecular polymer-bridged patchy particle hydrogel system that can serve as an experimental platform for designing particle gels which are not only phase-stable, but also offer tunability in the particle-particle interaction valency, thus providing access to a wide range of morphologies and percolation behaviors that have been predicted by theory. We show that the valency of the particle-particle interaction can be effectively tuned by adjusting the number of polymer linkers per particle, and also independently by adding competing interactions to the system. The precise quantitative relation between r and the coordination number M remains unclear, however – we anticipate that additional simulations would provide critical insight into the M of the SPPH, and facilitate greater rational control over the design of the anisotropic supramolecular hydrogels. Beyond our SPPH system, we anticipate the findings in this work to be salient for a wide range of gel systems with controllable cross-link functionalities, such as DNA gels,²⁴ metal-organic cage gels,³³ protein gels,^{8, 11} bridged particle gels,^{36, 45-51} and multifunctional polymer gels.⁵² We anticipate these findings will accelerate the design of bio-inspired programmable soft materials for structural and biomedical

applications, and inspire deeper studies of the physics of patchy particle self-assembly in diverse environments.

Methods

Polymer synthesis. Unless specifically stated otherwise, all chemicals are purchased from Sigma-Aldrich. 4-arm PEG N-Hydrosuccinimide (10 kDa, JenKem Technology) was functionalized with nitrocatechol using a variation of previously reported methods to create 4-arm nitrocatechol-PEG (4nPEG).⁵³ Nitrodopamine end-groups were first synthesized by dissolving 1.00 g of dopamine hydrochloride and 1.45 g of sodium nitrite in 30 mL of water. The reaction vessel was then placed into an ice bath, and 5.0 mL of 20% w/w sulfuric acid was slowly added under vigorous stirring. The yellow precipitate was collected through vacuum filtration, washed with cold water once and cold methanol thrice, and vacuum dried. 4-arm PEG N-Hydrosuccinimide was then functionalized with the collected nitrodopamine salt by dissolving 1.0 g of 4-arm PEG N-Hydrosuccinimide and 0.24 g of the nitrodopamine salt (2 x equivalent of N-Hydrosuccinimide) in 12 mL of DMF. The reaction vessel was then moved to an oil bath at 55 °C, and 0.2 mL of TEA (> 2 x equivalent of N-Hydrosuccinimide) was added to initiate the coupling. The reaction was left to proceed under N₂ overnight. Afterwards, the product was extracted with chloroform and dried using sodium sulfate. The product was then precipitated in diethyl ether thrice, and vacuum dried. The functional efficiency of the 4nPEG was typically around ~88 % (see Calculations section).

1-arm PEG N-Hydrosuccinimide (2 kDa, JenKem Technology) and 4-arm PEG N-Hydrosuccinimide (10 kDa, JenKem Technology) were functionalized with catechol to create 1-arm catechol-PEG (1cPEG) and 4-arm catechol-PEG (4cPEG) following the same method, but without the conversion step from dopamine to nitrodopamine.

Nanoparticle synthesis. Oleylamine-stabilized Fe₃O₄ NPs with an average diameter of 7 nm were synthesized by reductive decomposition of an iron salt in a heat-up method.⁵⁴⁻⁵⁵ In a typical synthesis, 1.05 g of iron(III) acetylacetonate (99%, Strem Chemicals) was dissolved in the mixture of 15 mL of benzyl ether (99%, Acros Organics) and 15 mL of oleylamine (98%) in a 100 mL, three-necked, round-bottomed flask. Benzyl ether is the solvent and oleylamine serves as both the reducing agent and stabilizing ligand. The mixture was vigorously stirred and degassed under vacuum at room temperature for 1 hour, following by heating (ramp rate of ~7 °C/min) at 120 °C for 1 hour. During the temperature ramp, the flask was backfilled with nitrogen at 90 °C. The mixture was then quickly heated under inert atmosphere to 210 °C (heating rate of ~15 °C/min) and held for 1 hour. Upon cooling to 50 °C, 50 mL of ethanol was added to the dark brown reaction product, followed by subsequent centrifugation and redispersion in THF. The oleylamine-functionalized Fe₃O₄ NPs dispersed in THF are then functionalized with 1cPEG by adding 1cPEG to the solution at a 1cPEG:Fe₃O₄ mass ratio of 3:1. The solution was heated to 55 °C and ligand exchange was allowed to proceed for 24 hours. The resulting solution was then precipitated in hexane thrice before drying under vacuum.

SPPH gel synthesis. SPPHs gels are made by mixing aqueous solutions of 4nPEG, 1cPEG-NP and HCl to make a final volume of 200 μ L and pH of ~ 2.2, with varying amounts of 4nPEG and 1cPEG-NP depending on the n_i and ϕ_{NP} (note ϕ_{NP} refers to volume of the Fe₃O₄ core, not the entire 1cPEG-NP complex). An acidic environment for the SPPH is chosen due to the fact that a pH of ~2 facilitates stable mono-coordinations between nitrocatechol and iron on the Fe₃O₄ surface.⁵⁶ At higher pH of ~ 5.2 where affinities are stronger and bis-coordinations dominate,⁵⁶ we found that the NPs showed some signs of etching, manifested by an emergence of a Fe³⁺ ion relaxation mode even in the absence of Fe³⁺ ions added (Fig. S20). Therefore, an acidic environment is used to prevent any trace amounts of Fe³⁺ ions in the 1cPEG-NP solution from participating in the cross-linking of the gel. SPPH with competing ions (SPPH-CI) are made by mixing aqueous solutions of 4nPEG, 1cPEG-NP, FeCl₃ (added with a stoichiometric ratio of 2 nitrocatechol to 1 iron) and NaAc buffer to make a final volume of 200 μ L and a pH of ~ 5.2 to facilitate bis-coordinations (Fig. 4A). Although the etching effect found in the regular

SPPH at this pH will likely contribute to an increased availability of Fe^{3+} than the stoichiometric amount added, this does not affect the gelation of the system,⁵⁶ and the etching effect on the size of the NPs was negligible as indicated by USAXS scattering intensities of the SPPH-CI compared to the regular SPPH (Fig. S17, Table S1). For a typical sample, the mixture is sealed and placed in a 55°C oven for 24 hours to create a gel.

Nuclear Magnetic Resonance (NMR). ^1H spectra of 4nPEG was obtained using a Varian 300 MHz spectrometer on a 4nPEG sample dissolved in CDCl_3 (10 mg / mL).

Dynamic Light Scattering (DLS). DLS measurements made using an Anton-Paar Litesizer 100 Particle Size Analyzer. The number-weighted size distribution of 4nPEGs (Fig. S1B) were obtained on samples at 10 wt. % dilutions in water. The number-weighted size distribution of 1cPEG-functionalized NPs (Fig. S3C) were obtained on much more diluted samples (0.01 wt. %) due to the black opaque color of the specimen. Measurements were repeated three times to ensure reproducibility of data.

Thermal Gravimetric Analysis (TGA). Mass profiles of dry 1cPEG-functionalized Fe_3O_4 NPs were characterized using a TA Instruments Q500 TGA. After equilibration at 25 °C, the oven temperature was ramped to 500 °C at 10 °C/min under nitrogen gas flow-rates of 60 mL/min.

Scanning Electron Microscopy (SEM). All SEM images were obtained using a Zeiss Crossbeam 540 field-emission SEM operating at 3-9 kV using currents ranging from 500-1000 pA. Unless stated otherwise, all images are obtained *via* secondary electron scattering mode. Prior to imaging, samples were chemically fixed with glutaraldehyde - a broadly reactive molecule commonly used to fix biological samples due to its ability to cross-link amine and hydroxyl groups. The samples are then dried using tetramethylsilane (TMS) to preserve as much of the hydrated morphology as possible under high vacuum.⁵⁷⁻⁵⁸ To do so, hydrated specimens were first fixed in 2% glutaraldehyde then dehydrated by substituting water with a series of ethanol concentrations (35%, 45%, 55%, 65%, 75%, 85%, 95%, and 100%). The specimens were then transitioned through gradations of Ethanol and TMS, (50%/50%, 20%/80% and 100% TMS) and then allowed to air dry under the fume hood. While this fixation and drying procedure caused affine shrinkages to the sample, salient microstructural elements were retained after the fixation and drying steps, allowing for qualitative but informative imaging of the gels in their native state (Fig. S21).

Transmission Electron Microscopy (TEM). TEM images of the synthesized NPs were obtained using a FEI Talos F200X G2. Samples were prepared by dispersing Fe_3O_4 NPs in hexane, and then dropping a diluted solution onto a TEM grid. The TEM grid was allowed to air dry for several hours. Size distribution of the particles were obtained through pixel analysis of the images *via* ImageJ. TEM images of the SPPHs were obtained using a FEI Tecnai G2 Spirit Twin TEM operating at 120 kV. Samples were prepared by synthesizing the SPPHs at 1/1000 the original concentration. This diluted solution was then dropped and air-dried onto 200 mesh carbon-coated copper holey grids from Ted-Pella, as the holes allow for imaging of the organic PEG phase.

Ultra-Small-Angle X-ray Scattering (USAXS). USAXS and SAXS intensities were collected using the USAXS instrument at 9-ID beamline at the Advanced Photon Source (APS). Samples were mounted in cells with sample thickness of ~ 1 mm and with 3M Scotch tape on either side. Due to variations in the thickness of the actual samples, only relative intensities are reported in the paper. X-ray energy was 21 keV (wavelength = 0.5905 Å), and the beam size was 0.6 x 0.6 mm for USAXS and 0.1 x 0.6 mm for SAXS. Data collection was 90 seconds for USAXS and 15 seconds for SAXS. Data were subtracted from a blank cell with scotch tapes, reduced, and then merged together using software (*Indra*, *Nika*, and *Irena*) provided by the beamline.⁵⁹⁻⁶⁰ Slit smearing inherent to the USAXS instrument was removed using desmearing routines in *Irena* to generate pinhole equivalent data for analysis.

Time-dependent Small-Angle X-ray Scattering (SAXS). Time-dependent SAXS measurements were carried out using a pin hole camera at the small-angle XPCS beamline 8-ID-I at the APS. Samples

were mounted in cells with sample thickness of 1.5 mm and were sealed with thin Kapton (or was it polycarbonate) windows on either side. Due to variations in the thickness of the actual samples, only relative small-angle scattering intensities are reported in the paper. Monochromatic x-rays at an energy of 7.35 keV was used and the beam size was 15 μm x 15 μm , with a nominal flux of 10^9 photons/s, which is then attenuated by a factor of 14. A LAMBDA (Large Area Medipix Based Detector Array) pixel array detector with a pixel size of 55 μm and array size of 516 x 1556 was used. The measurements were checked for radiation damage by controlling the dose using filters and an optimum dose was determined for the measurements.

Linear Rheology. All viscoelastic measurements of the hydrogels were conducted using Anton-Paar's MCR-302 stress-controlled rheometer, with a 10 mm parallel plate geometry and a gap size of 1 mm. All gels are loaded, trimmed, and sealed with mineral oil prior to characterization to prevent dehydration. Time-dependent small-amplitude oscillatory shear (SAOS) experiments for gelation measurements in Fig. 1F were conducted at an angular frequency $\omega = 5 \text{ rads}^{-1}$ at an amplitude of $\gamma = 1 \%$. All other SAOS experiments are conducted at an amplitude of $\gamma = 1 \%$. Step strain experiments for relaxation measurements in Fig. 1G were conducted at an amplitude of $\gamma = 1 \%$. Large-amplitude oscillatory shear (LAOS) measurements are conducted at an angular frequency $\omega = 5 \text{ rads}^{-1}$ to ensure that $\gamma = 1 \%$ is within the linear regime (Fig. S4C).

Supporting Information

The Supporting Information file contains information on fitting parameters, calculation and analysis schemes, NMR/DLS characterization of polymers and nanoparticles, and supplementary rheological data, scattering curves, and microscopy images of the SPPH and SPPH-CI system.

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Author Contributions

J.S, G.H.M, and N.H-A designed the study and interpreted the results, M.H.R and B.B.L synthesized and characterized nanoparticles. J.S and J.I performed USAXS characterization and analysis. J.S and D.M performed SEM characterization and analysis. J.S. performed all other experimental procedures. J.S., G.H.M, and N.H-A drafted the manuscript with input from J.B.T as well as all other authors. G.H.M and N.H-A co-supervised the study.

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