A unique route of colloidal phase separation yields stress-free gels

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Phase separation often leads to gelation in soft and biomatter. For colloidal suspensions, we have a consensus that gels form by the dynamical arrest of phase separation. In this gelation, percolation of the phase-separated structure occurs before the dynamical arrest, leading to the generation of mechanical stress in the gel network. Here, we find a previously unrecognized type of gelation in dilute colloidal suspensions, in which percolation occurs after the local dynamical arrest, i.e., the formation of mechanically stable, rigid clusters. Thus, topological percolation generates little mechanical stress, and the resulting gel is almost stress-free when formed. We also show that the selection of these two types of gelation (stressed and stress-free) is determined solely by the volume fraction as long as the interaction is short-ranged. This universal classification of gelation of particulate systems may have a substantial impact on material and biological science.

INTRODUCTION

Gelation is a consequence of the dynamical arrest of network-forming phase separation, which occurs in a broad class of soft and biomatter, such as colloidal suspensions (1–6) and protein solutions (7). Gels have many technological applications, including foods (8), aerogels (9), composites (10), drag delivery systems (11), and flexible electronics (12). Moreover, gelation phenomena have biological significance. Gelation (or aggregation) of biomolecules in living cells causes neurodegenerative diseases or cellular response to external environments (13–16). Despite its interdisciplinary importance, a general mechanism of gelation remains elusive.

For studying the physical mechanism of gelation, a colloid-polymer mixture is an ideal model system since it offers not only the three-dimensional (3D) single-particle resolution under confocal microscopy but also the tunability of interparticle potentials (Fig. 1A) (17–20). Systematic studies on the phase diagram have established that gas-liquid phase separation induces gelation (1, 4–6, 18, 21, 22). Various kinetic pathways of gelation have been identified, depending on the volume fraction of colloids, \( \phi_c \) (21). For \( \phi_c \gtrsim 0.1 \), it was found that spinodal decomposition leads to gelation due to the dynamic arrest by glass transition (1–6, 18, 21, 23). Gas-liquid phase separation leads to a large difference in the dynamics between the dilute-gas and dense-liquid phases. When the volume fraction of the dense liquid phases reaches the glass-transition line, the colloidal-rich phase is dynamically arrested, and the gel is formed. Because of the large difference in the dynamics between the two phases, the colloidal-rich phase cannot catch up with the domain deformation speed induced by phase separation and behaves as a viscoelastic body. Thus, we identify this type of phase separation leading to gelation as “viscoelastic phase separation (VPS)” (2, 3). In VPS, the network structure can generally be formed even if it is a minority phase, contrary to the common sense of ordinary phase separation (24). Its pattern is not determined by the interfacial tension of thermodynamic origin but by the mechanical force balance condition. The network structure formed in this manner is under self-generated tension (2, 3, 25–27).

We refer gelation via VPS to “VPS gel (VPS-G).” The final state formed by gelation has elasticity related to the microscopic structures (19, 28).

On the other hand, for a very low colloid volume fraction, it was proposed that diffusion-limited cluster aggregation (DLCA) takes place, and the resulting clusters may eventually percolate after a long time (21, 29–32). This type of cluster-forming phase separation has also been studied intensively (33–35). In a practical time window of experiments, the lower bound \( \phi_c \) of gelation (with percolation) is around 0.05, whereas a nonequilibrium cluster state (without percolation) continues to exist below this limit (6, 36).

Thus, a fundamental question is how the transition occurs from cluster phase separation to VPS with an increase in \( \phi_c \). Confocal microscopy observation of gels and gelation at single-particle–level resolution has provided crucial microscopic information about gelation [see, e.g., (6, 19, 20, 28, 37–42)]. However, the conventional experimental protocol initiates gelation ex situ; it mostly allows access only to postgelation structures. Accordingly, the kinetic process of gelation has been challenging to access. We have recently overcome this limitation by developing a novel protocol of in situ single-particle imaging (43–45). Single-particle–level observation and analysis of the entire process of gelation have shown that at rather high \( \phi_c \) (\( \gtrsim 0.1 \)), a system undergoes VPS: A percolated network of the colloidal-rich phase is formed and then coarsens with time until dynamical arrest due to either the formation of isostatic structures (44) or crystallization (43). Thus, the dynamic arrest of VPS and the emergence of elasticity are not due to glass transition but by rigidity percolation, which occurs after topological percolation (43, 44). The link between percolation of rigid, load-bearing units and elasticity of gels has also been pointed out by simulations (46–48) and experiments (49). Its relationship to the yielding of gels has also been discussed (50, 51). However, all these studies were made on gelation at \( \phi_c > 0.1 \). For a dilute suspension, the microscopic kinetic pathway (particularly, the early stage) of gelation has been mostly unknown, although it is known that gel is formed by percolation of clusters (21, 29–35).

Here, we unveil how phase separation occurs to form dilute gels by in situ single-particle imaging and hydrodynamic simulations. Our unique experimental protocol allows us to initiate phase separation in situ and thus follow the entire gelation processes of dilute colloidal suspensions at a single-particle level. We find the following

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kinetic pathway for the gelation of dilute suspensions. For \( \phi_c \leq 0.1 \), phase separation initially leads to the formation of isolated, chain-like clusters due to many-body hydrodynamic interactions of short-range nature (i.e., squeezing effect). Then, these clusters “fold” to form compact, rigid, load-bearing structures, similar to the folding of polymers. Then, these rigid isostatic clusters percolate to form a gel. Thus, local mechanical relaxation mostly finishes already when percolation happens. Briefly, topological percolation generates little mechanical stress. We call this type of gel “stress-free gel (SF-G),” although, strictly speaking, stress can be generated later, particularly around junction points, to approach a more stable state gradually. For \( \phi_c \geq 0.1 \), on the other hand, percolation occurs before clusters mechanically relax toward rigid, stable structures. Thus, topological percolation generates substantial mechanical stress in the network. Relaxation of the self-generated mechanical stress proceeds after percolation while accompanying the network coarsening, until the dynamic arrest. We call this type of gel VPS-G, as we mentioned above. We also find that we can classify gelation of hard sphere–like colloids universally into these two types, SF-G and VPS-G, as long as the interaction is short range.

**RESULTS AND DISCUSSION**

**Experiments and phase diagram**

We used sterically stabilized spherical colloids of poly(methyl methacrylate) (diameter, \( \sigma = 2.21 \) \( \mu \)m; polydispersity, 5\%). We suspended colloids in a mixture of \textit{cis}-decalin and cyclobromohexane so that its density and refractive index match those of colloids. Polystyrene was added to the colloidal suspension to induce depletion attractions between colloids. We used two polymers (polystyrene) with different molecular weights of \( 3.8 \times 10^6 \) and \( 4.3 \times 10^5 \) Da. The polymer-colloid size ratios \( \xi = 2 r_g / \sigma \) (where \( r_g \) is the radius of gyration of polymer) for these two polymers were 7 and 2\% (see Fig. 1A). We screened the surface charges of colloids sufficiently by adding salt [tetrabutylammonium bromide (TBAB)]. We estimated the Debye length of a system saturated with TBAB as several nanometers or less. Thus, we may regard our systems as hard spheres attracted with short-range depletion interactions. See Materials and Methods on the details of our samples.

We prepare colloid-polymer mixtures with various volume fractions (\( \phi_c \)) and attraction strengths (\( \Delta = \beta U_0 \), where \( U_0 \) is the potential depth and \( \beta = 1 / k_B T \) is the inverse of the thermal energy) for these two polymers were 7 and 2\% (see Fig. 1A). We screened the surface charges of colloids sufficiently by adding salt [tetrabutylammonium bromide (TBAB)]. We estimated the Debye length of a system saturated with TBAB as several nanometers or less. Thus, we may regard our systems as hard spheres attracted with short-range depletion interactions. See Materials and Methods on the details of our samples.

We used a specially designed salt-reservoir cell (see Materials and Methods and figs. S2 and S3 for the details). In the conventional experimental protocol, a mixture of colloids, polymers, salt, and solvents is prepared in the unstable state and then shaken to mix them mechanically. Then, the decay of the mixing flow leads to phase separation. Thus, in most cases, this protocol only allows access to postgelation structures formed under uncontrolled mixing flow, and thus, the kinetic pathway of gelation has been mostly unknown. We recently developed a novel protocol of in situ single-particle imaging using a nonperturbative initiation of phase separation by gentle salt injection. This protocol enables us to experimentally follow the gelation processes from the very beginning.

![Fig. 1. Phase diagram and a typical kinetic pathway to a dilute gel. (A) Schematic explanation of depletion attractions in colloid-polymer mixtures. U(r) is the interaction potential, \( r \) is the distance between the centers of mass of the two colloids, and \( \beta = 1 / k_B T \) is the inverse of the thermal energy. (B) Phase diagram of a colloid-polymer mixture with the attraction range of \( \xi = 7\% \) on the plane of the polymer concentration \( c_p \) and the colloidal volume fraction \( \phi_c \). Triangles, circles, and squares represent states of homogeneous fluids, clusters, and gels, respectively. See fig. S1 for the phase diagram at \( \xi = 2\% \). (C) 3D reconstructions of the gelation process observed in a sample with \( \phi_c = 0.046 \), \( c_p = 2.2 \) mg/g, and \( \xi = 7\% \). Clusters are colored according to the radius gyration of each cluster \( R_g \) (see Materials and Methods) normalized by a half of system length, \( L_{sys} R_g^{norm} = R_g / L_{sys} \). Isolated colloids and colloids in very small clusters are rendered as small points. The movies of the full processes are available in movies S1 and S2.](http://advances.sciencemag.org/)


2 of 10
at a single-particle resolution without suffering from harmful flow effects due to mixing (see fig. S3) (43, 44). It is crucial especially for studying gelation in dilute colloidal suspensions since weak flow in the sample cell strongly perturbs the gelation process. In our protocol, we confirm that the drift due to residual flow is negligible more than 3000 s, as shown in fig. S3C. The absence of drift along z direction also confirms almost perfect density matching. We show an example of the gelation process at a dilute colloidal suspension ($\phi_c = 0.046$) in Fig. 1C and movies S1 and S2.

**The lower bound volume fraction of VPS-G**

First, we study the lower bound composition of VPS-G. VPS has two characteristic features: (i) percolation of the colloid-rich phase in the early stage of phase separation and (ii) the resulting action of the self-generated internal stress in the coarsening process of the percolated network. We detect percolation by comparing the size of the largest cluster connected by bonds with the system size (see Materials and Methods and fig. S4 for the details). In Fig. 2A, we can see that the percolation time ($\tau_{perc}$) steeply increases from 30 to $9.7 \times 10^3$ s as $\phi_c$ decreases from 0.17 to 0.046 (see fig. S4 on the estimation of the percolation time).

This behavior leads to the violation of feature (i) with a decrease in $\phi_c$. We also evaluate the effect of mechanical stress on particle configurations by (i) choosing colloidal particles with only two nearest (bonded) neighbors and (ii) computing the angle between the two bonds ($\theta_{bond}$). Without the action of internal tension, all open-angle bonds should relax to closed ones as a consequence of structural relaxation toward a lower energy compact state (the upper case of Fig. 2B). If internal tension stretches the open-angle segments, then $\theta_{bond}$ may be kept at large angles near 180° (the lower case of Fig. 2B). Figure 2 (C and D) shows that there are distinct peaks in the distribution of $\theta_{bond}$. The $\theta_{bond}$ distribution of a gel sample with $\phi_c = 0.083$ has a sharp peak ("closed angle") in $50^\circ < \theta_{bond} < 70^\circ$ and a broad peak ("open angle") in $70^\circ < \theta_{bond} < 180^\circ$ (Fig. 2C). This border angle of $\theta_{bond} \sim 70^\circ$ roughly corresponds to the angle below, which the two neighbors are bonded (i.e., the three particles form a compact triangular structure; see the upper picture of Fig. 2B). Without the action of mechanical tension, the open-angle configuration should smoothly relax to a closed-angle one. In a sample of $\phi_c = 0.083$, we observe this behavior (Fig. 2C). In contrast, in a sample with $\phi_c = 0.17$, configurations of open angles remain even in the late stage and another peak appears in a region $140^\circ < \theta_{bond} < 180^\circ$ (Fig. 2D). This large-angle peak ("stretched angle") is a clear sign of mechanical stretching due to the self-generated internal mechanical stress, which is characteristic of VPS (3, 26, 52). This stretching is a consequence of percolation before the relaxation of open configurations to compact ones.

We show the temporal change in the fraction of open bonds during phase separation in Fig. 2E for various colloid volume fractions $\phi_c$, various polymer concentrations $c_p$, and the two polymer-colloid size ratios $\xi = 7$ and 2%. The result shows that for $\phi_c \geq 0.12$, open-angle bonds survive for a long time, indicating that gels form before mechanical relaxation and are under the action of the self-generated mechanical tension. In contrast, for $\phi_c \leq 0.083$, the fraction of open-angle bonds rapidly decays with time, indicating that mechanical stress decays before percolation (i.e., the formation of gels starts after mechanical stress decay).
Spherical particles interacting with short-range isotropic attractions (53C). Both BD and FPD simulations show that the presence of hydrodynamic interactions significantly increases the lifetime of bonds with open angles (τ_{open}) from τ_{open} = 337 ± 39 s, which is about two orders of magnitude longer than the Brownian time of colloids, which is the time required for a colloid to diffuse over its radius (τ_{B} = 2.3 s) (Fig. 2A). If only short-range attractions and thermal fluctuations determine the local compaction dynamics, then τ_{open} should be the order of a few τ_{B}, which significantly differs from the observed value. This disagreement suggests that hydrodynamic interactions strongly slow down the local compaction process. Numerical simulations with [fluid particle dynamics (FPD) (25, 26, 45, 54)] and without hydrodynamic interactions [Brownian dynamics (BD)] (see Supplementary Text on the details) clearly show that the presence of hydrodynamic interactions increases τ_{open} by a factor of 10 or more (the inset of Fig. 3A). So, we conclude that hydrodynamic interactions strongly affect the compaction dynamics in t < τ_{open}. Thus, we define “early stage” by the condition of t < τ_{open}.

Folding of an elongated cluster
In the early stage of SF-G formation, particles are connected with open angles, forming single-stranded clusters, and then these single-strand clusters gradually transform into compact ones (Figs. 2E and 3A and fig. S5A). A critical question is whether the condensation is brought by the motion of individual particles toward a local equilibrium liquid droplet or by the correlated motion retaining the chain-like structure toward a nonequilibrium solid cluster. This question is also related to the origin of the nonergodicity of dilute nonequilibrium condensates (55). Here, we note that the conventional ex situ experiments can hardly address this question. To answer this question unambiguously, we apply high-speed scanning with in situ single-particle imaging, in which the time resolution of 3D imaging is 1.5 s [faster than the Brownian time of colloids (τ_{B} = 2.3 s)]. Single-stranded chains fold into multiple-stranded ones (Fig. 3B and movies S3 and S4). The extremely low probability of bond rearrangement indicates the absence of an intermediate liquid state. To the best of our knowledge, this is the first demonstration that linearly arranged spherical particles interacting with short-range isotropic attractions (without permanent connections) can spontaneously fold while keeping the 1D connectivity toward a compact structure.

To elucidate what feature of the interparticle interaction leads to the unique folding process, we run numerical simulations with an initial configuration in which 10 colloids form a straight chain. We use short-range Asakura-Oosawa (AO) and long-range Lennard-Jones (LJ) potentials (see Fig. 3C). Both BD and FPD simulations with short-range attractions lead to the folding of chains. However, hydrodynamic interactions in FPD tend to stabilize a single-stranded structure and make the final structure more elongated (compare Fig. 3, D and E). If we make FPD simulations with the long-range LJ potential, on the other hand, a single-stranded chain of colloids "continuously" takes a more compact, spherical shape without being trapped in a metastable solid state to form a liquid-like droplet (Fig. 3F). That is, they quickly lose the memory of the initial 1D connectivity. A comparison of these numerical simulation results indicates that the short-range nature of the attractive potential (see Fig. 3C) is crucial for the polymer-like folding process. We can see the difference even more evidently by checking how the cluster shape becomes more compact and spherical: In Fig. 3G, we plot the temporal change of the aspect ratio σ_{y}/σ_{x}, which is smaller for a more anisotropic shape and becomes 1 for a spherical shape (see Supplementary Text on the definition).

So, the folding process toward a nonergodic state is a consequence of the interplay between short-range attractions and hydrodynamic interactions. Note that hydrodynamic interactions have two roles: They prevent the formation of compact clusters and help form elongated chain-like clusters. Then, they also prevent rapid liquid-like collapsing of the clusters and, instead, lead to the unique polymer-like folding process.

Stabilization mechanism of folded clusters
Next, we study how local structures can be stabilized in the post-folding regime. We recently found by in situ single-particle imaging that VPS-G gains macroscopic elasticity when rigid isostatically percolated structures form (44). The Maxwell criterion for isostaticity of \langle N_{C} \rangle ≥ 6, where N_{C} is the particle contact number, is a minimum requirement for mechanical stability (56). In all of our samples showing SF-G, the folding of chain-like colloidal clusters leads to an increase in \langle N_{C} \rangle toward ~6 (Fig. 4A and fig. S5A) while keeping the preexisting bonds (Fig. 4B). Then, local structures are dynamically arrested when \langle N_{C} \rangle approaches 6 (Fig. 4A). So, the isostaticity may be responsible for the stabilization of particle configurations not only for VPS-G but also for SF-G.

Here, we directly check for SF-G whether the formation of isostatic structures couples with the emergency of local solidity. To probe the solidity of a cluster, we measure the relative displacement of a pair of particles initially bonded, Δd(t_{0}, Δt), instead of the mean square displacement of individual particles. First, we identify all bonded pairs at t = t_{0} as (i, j). Then, the distance between the particles in each pair is tracked as a function of Δt from t_{0} as d_{i,j}(t_{0} + Δt) = |\mathbf{r}_{i}(t_{0} + Δt) − \mathbf{r}_{j}(t_{0} + Δt)|, where \mathbf{r}_{i}(t) is the position of colloid k at time t. We consider only (i, j) pairs bonded at t = t_{0} and do not care about the bonding status after t = t_{0}. We note that d_{i,j}(t_{0} + Δt) is affected by neither translational nor rotational motion of a cluster and thus extracts the relative displacement of a pair of particles inside isolated clusters. Last, the averaged relative displacement of particle pairs is calculated as Δd(t_{0}, Δt) = \langle d_{i,j}(t_{0} + Δt) − d_{i,j}(t_{0}) \rangle, where the average is taken over all (i, j). The result of this analysis is shown in Fig. 4B, showing the stability of bonds in clusters at least after t_{0} ≥ 174 s.
To further evaluate the solidity of clusters, we define the cooperativity angle, \( \theta_{\text{coop}} \), which characterizes the degree of the coherency of particle motion. First, we calculate the cage displacement of colloid \( i \) as \( \delta \text{cage}_i = \sum_i \delta \text{cage}_j(i) \), where \( \delta \text{cage}_i \) is the displacement of colloid \( j \) bonded to colloid \( i \) (i.e., colloid \( j \) is a member of particles forming the cage of colloid \( i \)), and \( \delta \text{cage}_j(i) \) takes a value of 0 and 1, respectively, for successful and unsuccessful tracking of particle \( j \) after \( \Delta t \). Then, the cooperativity angle of colloid \( i \) is calculated as the angle between \( \delta \text{cage}_i \) and \( \delta \text{cage}_j \). Last, \( \theta_{\text{coop}} \) is obtained by averaging \( \theta_{\text{coop}} \) over all colloids in clusters whose mass \( M \geq 10 \). If a cage in a cluster is completely solid, i.e., if the particles in a cluster move together as a rigid solid body, then \( \theta_{\text{coop}} \) becomes zero. In Fig. 4A, we see the monotonic decay of \( \theta_{\text{coop}} \) with time. Figure 4C also shows that the distribution of the cooperativity angle has a peak, and its position shifts toward a lower angle with time. This shift is the signature of the increase in the cooperativity of particle motion, i.e., solidity. These results indicate that the emergence of isostatic structures is accompanied by that of cooperativity in the particle motion, i.e., local solidity. This solidification is realized by polymer-like folding while keeping the bond connectivity.

Hence, we may conclude that the establishment of local isostaticity is responsible for the freezing of local structures in an out-of-equilibrium
solid state for not only VPS-G but also SF-G. Here, note that SF-G has apparent similarity to DLCA at a mesoscopic level, having the same fractal dimension of 1.85 [fig. S5B and (32)]. Because the fractal dimension is far below three, nonequilibrium clusters formed in the early stage of SF-G (and DLCA) can effectively fill the space as the cluster size grows in the late stage (fig. S5C), leading to percolation in SF-G even at $\phi_0 < 0.1$. Unlike SF-G, however, DLCA assumes sticky attractions that do not allow the change of the bond-bond angles and ignores hydrodynamic interactions. Thus, we conclude that the physical mechanism is fundamentally different between SF-G and DLCA and DLCA is not a relevant mechanism of cluster formation in the volume fraction we studied.

**A unified picture of gelation**

On the basis of the understanding of the fundamental mechanism of SF-G mentioned above, we now draw a unified picture of gelation. In both VPS-G and SF-G, hydrodynamic interactions enhance the formation of single-stranded chain-like clusters in the early stage. We may regard the chain-like structure as a “precursor” of gelation. In SF-G ($\phi_0 \leq 0.1$), single-stranded chains cannot percolate before folding occurs, i.e., $\tau_{perc} > \tau_{open}$ due to the low volume fraction of colloids (Fig. 5, A and B). We can confirm this in Fig. 5 (A and B), where we plot the fraction of single-stranded segments in all particles (not for the system volume), $\phi_{ss}$, and the average particle contact number, $\langle N_C \rangle$, as a function of time. Here, $\phi_{ss}$ is defined as follows: First, we identify single-stranded clusters as particles with $N_C = 2$ and $\theta_{bond} > 70^\circ$ and particles bonded with these particles. Then, the number of particles in single-stranded clusters is normalized by the total number of particles in the scanned volume. The results in Fig. 5 (A and B) show that single-stranded chains can fold to form isostatic clusters before percolation. We emphasize that the folding process is intrinsically stress-free because the clusters are isolated. As clusters formed in this way have a fractal dimension less than two (fig. S5B), the meeting probability between clusters is rather high. Thus, cluster-cluster aggregation eventually leads to percolation (fig. S5C). However, this happens only in the postfolding regime. Thus, topological percolation generates little mechanical stress.

In contrast, in VPS-G ($\phi_0 \geq 0.1$), single-stranded chain-like structures spontaneously percolate before their folding starts, i.e., $\tau_{perc} < \tau_{open}$ (Fig. 5C). So, the network structure just after percolation is far from local structural stabilization, and thus, the local particle configurations tend to become more and more compact with time. This compaction, while keeping the network connectivity, generates mechanical stress inside the network. Thus, topological percolation generates substantial mechanical tension in the network, unlike SF-G. Last, when stable isostatic structures formed in the network percolate, the coarsening is arrested, resulting in a frozen gel state with elasticity (44).

![Fig. 5. Difference between SF-G and VPS-G.](http://advances.sciencemag.org/)

(A to C) Comparison between the percolation time and the characteristic time of local compaction for SF-G and VPS-G. (A) $\phi_0 = 0.046$ (SF-G). (B) $\phi_0 = 0.083$ (SF-G). (C) $\phi_0 = 0.17$ (VPS-G). All samples have $c_p = 2.2$ mg/g and $\xi = 7\%$. $\phi_{ss}$ represents the fraction of single-stranded segments. The blue and yellow vertical dotted lines indicate $\tau_{open}$ and $\tau_{perc}$, respectively (see also Fig. 2A). In SF-G (A and B), colloids percolate after the decay of $\phi_{ss}$. In VPS-G (C), on the other hand, the percolation time coincides with the time when the fraction of single-stranded domains becomes 50% of all particles. (D) to (F) Analysis of stretched segments in VPS-G. (D) Temporal change of $\phi_{stretch}$, the fraction of colloids with $140^\circ < \theta_{bond} < 180^\circ$ in colloids with $N_C = 2$ (see Fig. 2B, right bottom). The symbols in (D) are the same as in Fig. 2E. This result for stretched bonds is similar to the one for open bonds in Fig. 2E, but the threshold angle is different (see Fig. 2B). We can see that the fraction of stretched bonds does not decrease with time but that of open bonds initially decreases. (E and F) 3D images of stretched segments during a process of VPS-G formation. The images are taken at $t = 2400$ s during gelation at $\phi_0 = 0.17$, $c_p = 2.2$ mg/g, and $\xi = 7\%$. Red particles represent strongly stretched segments with $N_C = 2$ and $140^\circ < \theta_{bond} < 180^\circ$. Blue particles form closed angles (or have $N_C \geq 3$). In (E), the image is sliced from the full volume for the better visibility of network structures. In (F), only stretched segments are visualized in the entire volume of scanning.
The difference between SF-G and VPS-G can be seen clearly by looking at how the fraction of the stretched bonds with $140^\circ < \phi_{\text{bond}} < 180^\circ$, $\phi_{\text{stretch}}$, changes with time (Fig. 5D). For VPS-G, $\phi_{\text{stretch}}$ gradually increases with time, whereas for SF-G, it quickly decreases. The absence in the decay of $\phi_{\text{stretch}}$ in VPS-G indicates that highly elongated bonds initially formed just after aggregation start to be stretched before compaction. The monotonic increase in $\phi_{\text{stretch}}$ with time means that self-generated tension creates more stretched bonds. Thus, after the percolation, many bonds are more and more stretched by self-generated tension after the percolation in VPS-G, as shown in Fig. 5 (E and F). In contrast, there are few stretched bonds in SF-G.

The mechanism of SF-G also explains why the pathway to the equilibrium state (liquid droplets) is blocked. In the initial stage, hydrodynamic interactions prevent the direct gathering of particles that leads to the formation of compact droplets (see Fig. 6A, top). Then, the polymer-like folding proceeds to form compact clusters without passing a liquid-like state. In the later stage, stabilization via isostaticity freezes internal dynamics. Thus, SF-G restricts access to liquid-like phase separation. This is the case for colloids interacting with short-range attractions. As we numerically show in Fig. 3, long-range LJ attractions may allow the pathway to liquid-like states. Thus, our result suggests that the "long-range" nature of the attraction may play critical roles in the formation of liquid-like droplets. This conclusion is supported by the finding of Lu et al. (5) that liquid-like droplets form exclusively in the presence of long-range attractions.

The type of gelation is determined by which of percolation and folding is faster. Figure 2A shows that a crossover between the characteristic time scale of percolation, $\tau_{\text{perco}}$, and that of folding, $\tau_{\text{open}}$, takes place around $\phi_c \sim 0.1$. Thus, this crossover composition determines the border between VPS-G and SF-G. Figure 6B shows that we can classify all gelation kinetic pathways into VPS-G and SF-G pathways by only one control parameter, $\phi_c$: All the kinetic pathways can be collapsed onto the two master curves on the plane of the normalized average cluster mass $M^*$ (see Materials and Methods for its definition) and the average contact number $\langle N_c \rangle$. The classification is independent of the potential depth $\Delta$, i.e., $\xi$, and the range of the potential, $\xi$, as long as the attractive interaction is short-ranged. This result indicates that the attractive potential affects only the thermodynamics, i.e., the shape of the phase diagram, but not the selection of the kinetic pathways of gelation.

Conclusions
In conclusion, we have established a new type of gel, SF-G, and identified a universal kinetic route to SF-G. In situ single-particle imaging and hydrodynamic simulations unveil the fundamental mechanism of SF-G, in which the network forms with a unique interplay between short-range attractions and hydrodynamic interactions. We can universally classify gelation to SF-G and VPS-G based solely on $\phi_c$. Our finding may have a substantial impact on the fundamental understanding of the kinetics of demixing-induced gelation of colloidal suspensions, protein solutions, and emulsions (2–4, 7, 18). Moreover, our finding unveils how phase separation undergoes percolation in dilute suspensions (57). We have also shown that the concept of DLCA needs to be modified under the influence of hydrodynamic interactions. From an application viewpoint, the percolation critically affects the elasticity and electronic conductivity. Thus,
the mechanisms of SF-G and VPS-G should be useful for designing gel materials (8–12). SF-G may also allow us to form a stable soft colloidal gel free from network shrinkage since it does not suffer from the self-generated internal mechanical stress, which is inevitable for VPS-G (26) and causes network shrinkage (9, 52). The colloid volume fraction separating between SF-G and VPS-G also provides the border of the settling behavior between cluster deposition and collective settling under gravity (9).

Our work also reveals how the character of the interparticle attractions affects aggregation, phase separation, and gelation. We note that the above classification should be applied only to colloids with short-range attractions. For example, we have shown that the aggregation process of colloids is quite different between the short-range and long-range attractions under the influence of hydrodynamic interactions. Note that the concept of rigidity becomes vague for long-range interactions. The effect of Coulomb repulsions is also an interesting issue. Long-range Coulomb repulsions are known to modify the phase diagram and local structures markedly: A combination of short-range attraction and long-range repulsion results in the formation of small equilibrium clusters (58) or a unique structural organization in the network (59). Strong Coulomb repulsions expand a glass state to a very low volume fraction (60–62). How the nature of interactions modifies the gelation behavior is an interesting topic for future investigation, particularly in connection to biological phase separation (see below).

Last, we mention an implication of our findings on biomolecular condensation in living cells. Biomolecular condensates are usually in a liquid state but can be solid aggregates (or gels) in neurodegenerative diseases or under environmental stresses, such as ultraviolet exposure, energy depletion, and pH change (13–16, 63). Thus, a critical question is what molecular features cause gelation (14, 15, 64). Protein molecules often consist of poorly-folded “polymer-like” domains [intrinsically disordered regions (IDRs)] and highly-folded “colloid-like” domains. So far, the polymer-like feature of proteins has mainly been considered to understand biomolecular condensates (14, 15, 63–65). Only recently, the colloid-like feature has attracted attention (66). A recent mutational study by Franzmann et al. (16) provides a hint on the roles of the polymer-like and colloid-like features in phase separation. They showed that native prion proteins (with IDRs) undergo liquid phase separation in living cells, whereas the removal of IDRs from prion proteins leads to gelation. We may regard polymer-like IDRs with charged domains as the sources of long-range interactions and the gelling mutants as colloids with short-range interactions. Our study shows that colloids with short-range attractions should directly form gels upon phase separation, without passing through a liquid state. On noting the similarity between colloid and globular protein phase separation (7), this finding could be useful for understanding a physical mechanism of biomolecular condensates. However, since interprotein interactions are far more complex than intercolloid ones (67), its validity should be carefully examined considering the complexity of interactions (e.g., electrostatic, anisotropic, and multicomponent characters).

**Materials and Methods**

**Samples**

We prepared poly(methyl methacrylate) colloids with the steric stabilizer of methacryloxypropyl terminated poly(dimethylsiloxane) labeled with rhodamine isothiocyanate for fluorescent microscopy by following (68, 69). The diameter of colloids, $\sigma$, is estimated as 2.21 µm with its polydispersity of 5% from our previous paper (43). Colloids were suspended in a mixture of cis-decalin and cyclohexane so that its density matches that of colloids. The precision of density matching in our experiments is $10^{-4}$ g/ml, in which the aggregation process was free from gravity effect more than $1.0 \times 10^4$ s. Polystyrene was added to the colloidal suspension to induce depletion attractions between colloids. We used two polystyrenes with different molecular weights of $3.8 \times 10^6$ and $4.3 \times 10^5$ Da. The Flory scaling from (6) estimates the radius gyration ($r_g$) of $3.8 \times 10^6$ and $4.3 \times 10^5$ Da as 78 and 26 nm, respectively. These sizes correspond to the normalized attraction range, $\xi = 2r_g/\sigma$, as 7% and 2%, respectively (see Fig. 1A). To screen the surface charges of colloids, we added a sufficient amount of salt (TBAB) into colloid + polymer mixtures. We estimated the Debye length of a system saturated with TBAB as several nanometers or less (70). Note that colloid + polymer mixtures at $\xi = 2\%$ showed monotonic aggregation.

**Confocal microscopy**

Time-lapsed 3D images were acquired by a laser-scanning confocal microscope (Leica TCS SP5, Leica Microsystems) with an 8000-Hz resonant scanner. The wavelength of the excitation laser was 532 nm. We fixed the pixel resolution as 192 nm per pixel. In standard in situ experiments, the volume of 123 µm by 123 µm by 125 µm was acquired every 30 s (early stage) or 90 s (late stage). In a high-speed scan, the volume of 123 µm by 24.5 µm by 22.9 µm was scanned every 1.5 s. The typical data size of one in situ measurement was 100 gigabytes. Coordinates of colloids were extracted from 3D images by a center-of-mass method with highly efficient algorithm and implementation on a workstation computer (71). We tracked each colloid as a function of time by the Crocker and Grier method (71, 72).

**Glass capillary cell**

To confirm the phase boundary between the fluid and nonequilibrium condensates, we mixed all components (colloids + polystyrene + solvent + TBAB) ex situ and put it into a glass capillary cell. We confirmed that samples near the gelation boundary are in a fluid state, from the absence of coarsening more than $2.0 \times 10^3$ s.

**Salt-reservoir cell**

For in situ single-particle imaging, samples were prepared in salt-reservoir cells (43). As shown in fig. S2, the cell has a colloidal chamber, membrane filter, and reservoir with an incubation window. Initially, both solvents in the colloidal chamber and reservoir contained no TBAB. Thus, colloids were stably dispersed by strong electrostatic repulsions from unscreened surface charges in the beginning. Then, we exchanged the solution in the reservoir quickly through the incubation window for the solution containing salt (TBAB) with the saturation level. Then, we immediately sealed the window to avoid evaporation of the solvent. We made these operations on a microscope stage. Then, TBAB quickly diffused into the colloidal chamber through the membrane filter, screened the surface charges of colloids, and triggered aggregation of colloids via short-range depletion attractions by the preexisting polymers. The diffusion of TBAB into the scanning volume is less than 60 s (fig. S3). We define $t = 0$ for each sample as the time when $N_C = 1.0$.

**Detecting bonds**

We regard two colloids to be bonded if $\left| \mathbf{r}_i - \mathbf{r}_j \right| < d_{\text{bond}}$, where $r_i$ is the coordinate of colloid $k$ and $d_{\text{bond}}$ is the threshold distance for the
bond detection. For $\xi = 2$ and 7%, we set $d_{\text{bond}} = 1.043 \sigma$ and 1.099$\sigma$, respectively, by taking the first minima of the radial distribution function $g(r)$, the polydispersity of colloids, the radius gyration of polystyrene, and the precision of coordinate determination into account.

### Analysis of cluster structures

Clusters were identified by connecting all bonds. For each cluster, we computed the mass of the cluster ($M$) and its gyration radius ($R_g$). We evaluate the fractal dimension ($d_f$) of a cluster by fitting the relation of $M \sim R_g^{d_f}$ for clusters of $M \geq 10$. The averaged, normalized cluster masses were calculated for each time step as $M' = \Sigma M_i^2 / (\Sigma M_i)^2$, where $M_i$ is the mass of cluster $i$. If all colloids are included in one network, then $M' = 1$. If all colloids stay as monomers, then $M' = N^{-1} \sim 0$, where $N$ is the total number of colloids in the scanned volume. In the analysis of effective volume fraction ($\phi_{\text{eff}}$), we replace each cluster with a solid sphere whose radius is equal to the radius of gyration of the cluster. Then, the effective volume fraction is calculated as $\phi_{\text{eff}} = \Sigma (\frac{4}{3} \pi R_i^3) / V$, where $R_i$ is the radius of gyration of cluster $i$ and $V$ is the system volume.

### Analysis of the percolation time and the lifetime of open-angle configuration

The percolation time ($\tau_{\text{perc}}$) is defined as the time when $R_{\text{max}}/L_{\text{sys}}$ exceeds 0.8, where $R_{\text{max}}$ is the radius of gyration of the biggest cluster and $L_{\text{sys}}$ is a half of the system length. The lifetime of an open-angle configuration ($\tau_{\text{open}}$) is extracted from the relation of $\phi_{\text{open}}(t) \propto \exp (-t/\tau_{\text{open}})$, where $\phi_{\text{open}}(t)$ is the fraction of configurations of $\theta_{\text{bond}} > 70^\circ$ among the colloids with $N_C = 2$. For SF-G and VPS-G samples used in our experiments, the fitting ranges were 0 s $\leq t \leq 480$ s and 0 s $\leq t \leq 90$ s, respectively. For BD and FPD simulations, the fitting ranges were 1.6$\tau_L \leq t \leq 10.2\tau_L$ and 4.2$\tau_L \leq t \leq 100\tau_L$, respectively.

### Supplementary Materials

Supplementary material for this article is available at http://advances.sciencemag.org/cgi/content/full/6/41/eabb8107/DC1

### References and Notes


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Acknowledgments: We thank M. Tateno for discussion. Funding: This study was partially supported by Grants-in-Aid for Specially Promoted Research (JP25000002 and JP20H05619) and Scientific Research (A) (JP18H03675) from the Japan Society for the Promotion of Science (JSPS). S.A. also thanked the support from JSPS KAKENHI grant nos. JP19H02579 and JP19H05321. Author contributions: H.Ta. supervised the study. H.Ta. and H.Ts. designed the project. H.Ts. performed the experiments. S.A. performed the numerical simulations. All authors discussed the results and contributed to the writing of the manuscript. Competing interests: The authors declare that they have no competing interests. Data and materials availability: All data needed to evaluate the conclusions in the paper are present in the paper and/or the Supplementary Materials. Additional data related to this paper may be requested from the corresponding authors.

Submitted 19 March 2020
Accepted 18 August 2020
Published 7 October 2020
10.1126/sciadv.abb8107

A unique route of colloidal phase separation yields stress-free gels
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Sci Adv 6 (41), eabb8107.
DOI: 10.1126/sciadv.eabb8107