Published in final edited form as:

J Chem Phys. 2016 October 21; 145(15): 155102. doi:10.1063/1.4964613.

Effect of the Surface Charge Distribution on the Fluid Phase Behavior of Charged Colloids and Proteins

Marco A. Blanco^{1,2,a)} and Vincent K. Shen¹

¹National Institute of Standards and Technology, Gaithersburg, MD 20899

²Institute of Bioscience and Biotechnology Research, University of Maryland, Rockville, MD 20850

Abstract

A generic but simple model is presented to evaluate the effect of the heterogeneous surface charge distribution of proteins and zwitterionic nanoparticles on their thermodynamic phase behavior. By considering surface charges as continuous “patches”, the rich set of surface patterns that is embedded in proteins and charged patchy particles can readily be described. This model is used to study the fluid phase separation of charged particles where the screening length is of the same order of magnitude as the particle size. In particular, two types of charged particles are studied: dipolar fluids and protein-like fluids. The former represents the simplest case of zwitterionic particles, whose charge distribution can be described by their dipole moment. The latter system corresponds to molecules/particles with complex surface charge arrangements such as those found in biomolecules. The results for both systems suggest a relation between the critical region, the strength of the interparticle interactions, and the arrangement of charged patches, where the critical temperature is strongly correlated to the magnitude of the dipole moment. Additionally, competition between attractive and repulsive charge–charge interactions seems to be related to the formation of fluctuating clusters in the dilute phase of dipolar fluids, as well as to the broadening of the binodal curve in protein-like fluids. Finally, a variety of self-assembled architectures are detected for dipolar fluids upon small changes to the charge distribution, providing the groundwork for studying the self-assembly of charged patchy particles.

I. INTRODUCTION

Strong anisotropic interactions play a key role in driving many thermodynamic and kinetic processes of both newly synthesized complex colloids^{1–4} and naturally occurring biomolecules such as proteins.^{5–7} The specificity and directionality provided by anisotropic interactions is fundamental for determining the biological function of proteins,^{8,9} the properties of functionalized colloids,^{10,11} as well as their propensity to form ordered and disordered dense phases.^{3,12,13} As such, understanding the impact of anisotropic interactions on equilibrium and non-equilibrium properties is highly relevant to systems involving proteins and colloids.

^{a)} mab7@nist.gov.

In the case of protein solutions, there has been increasing interest in studying anisotropic interactions as they provide a means to control liquid–liquid and liquid–solid protein phase separation,^{13–16} as well as the formation of native and non-native protein aggregates.^{17–19} Depending on the bulk protein concentration, specific interactions can lead to different assembly processes. At low protein concentrations, highly specific, anisotropic interactions are responsible for the formation of “lock-and-key” bindings such as those in protein–ligand docking,^{20–22} and long-lived protein oligomers.^{8,23} These interactions are typically characterized by equilibrium dissociation constants (K_D) on the order of μM or smaller values.^{8,21} On the other hand, at sufficiently high protein concentrations, strong attractive interactions lead to bulk phase separation, where the protein-rich phase can be a dense homogeneous liquid,^{6,24} a crystal,^{6,14} or an inhomogeneous solution of amorphous, arrested clusters.^{25,26} This assortment of protein behaviors strongly depends on the protein sequence and solution conditions^{24,27–30} (e.g., pH and the presence of co-solutes), and may lead to the formation of different associated systems ranging from “lock-and-key” protein bindings ($K_D < 1\mu\text{M}$) to short-lived protein clusters ($K_D > 1\text{mM}$);²³ however, it is not yet fully understood how bulk phase separation is related to the physical properties of the solution.

The arrangement of hydrophobic, hydrophilic, and positively and negatively charged amino acids leads proteins to interact with each other through several forces that include solvophobic, hydrogen-bonding, and screened electrostatic interactions. This arrangement of amino acids on the protein surface generally results in a heterogeneous mixture of attractive and repulsive surface “patches”, with different interaction ranges, and whose distribution can wildly vary among different proteins. Although there is no clear definition of the boundaries of these patches, experimental evidence indicates that surface clustering of like amino acids greatly affects protein solubility and biological function.^{27,31} Of particular interest is the case of the arrangement of charged amino acids, as it is more closely related to changes in solution conditions. At sufficiently high ionic strengths, electrostatic forces are mostly screened, and protein–protein interactions become highly short-range compared to the effective diameter (σ) of most proteins. Under such conditions, protein phase behavior and self-assembly are typically independent of the presence of charged patches and changes in pH, but might be greatly affected by the identity of the co-solutes due to depletion and preferential protein–co-solute interactions.^{32–34} By contrast, charged patches become relevant at low ionic strengths, where their effective interaction range is of the same order of magnitude as σ . In this case, small changes in pH and ionic strength can dramatically alter the surface charge distribution, allowing proteins to undergo many of the aforementioned processes.^{13,35–37} A similar situation also holds for solutions of binary mixture of proteins, where liquid–liquid phase separation occurs over a limited pH range as a consequence of differences in the isoelectric points of each protein and the formation of oppositely charged patches between these species.³⁸ In spite of the understanding of the general relevance of charge–charge interactions in all these processes, it is still unknown whether charged patches represent the main driving force for protein self-assembly and phase separation, or simply provide the right balance between short-range attractions and long-range attractions/repulsions to favor a given thermodynamic or kinetic path. A future report will examine the balance of different forces involved in protein–protein interactions and its effect on protein phase behavior; this report focuses on answering a more fundamental question: are charged

patches alone sufficient to drive some, if not all, of the phase separation processes commonly observed in proteins?

Likewise, recent advances in experimental techniques have led to the synthesis of a wide variety of anisotropic colloids with diverse size and surface functionalities^{1–3,39} (e.g., hydrophobic/hydrophilic, metallic, charged, organic). These new types of colloids, commonly referred as “patchy particles”, consist of patterned particles with at least one well-defined patch which provides directionality for interparticle interactions.^{4,40} Note that this definition is also applicable to biomolecules like proteins. The multifunctional nature of patchy particles has opened up the possibility of developing smart or controllable materials that work as building blocks for well-defined, large length-scale structures provided that the formation of disordered phases is frustrated.^{41,42} By adjusting the chemical/physical properties, size, number, and arrangement of the patches, these particles can assemble into various types of architectures, including extended wires,⁴³ films,⁴⁴ and quasi-crystals.^{45,46} As a result, patchy particles have been implemented in applications such as emulsions,⁴⁷ tunable liquid optics,⁴⁸ and targeted drug delivery.⁴⁹

Among the different explored patch functionalities, patchy particles involving mixtures of cationic and anionic patches (e.g., zwitterionic colloids) have attracted interest as stimuli-responsive and bio-mimic materials.^{3,43,50} Due to the attractive-repulsive duality of electrostatic interactions, charged patchy particles offer a high level of specificity for targeted architectures, as they can be designed to avoid undesired assembled structures via long-range repulsions.⁵¹ This specificity can be further controlled by changing solution conditions (e.g., pH or salt content), which can change the overall strength (i.e., the particle net charge) and range of the patch–patch interactions. Moreover, it has been shown that under the influence of an external field (e.g., magnetic or electric), charged patchy particles can be directed to assemble into a variety of structures such as nanocubes,⁵² virus-like capsids,⁵³ spotted vesicles,⁵⁴ staggered chains,⁵⁰ and two-dimensional crystals.⁴³ However, as in the case of protein solutions, it is unclear how the overall arrangement of charged patches may favor the formation of a given assembled structure over other equilibrium and non-equilibrium motifs. Paradoxically, the large number of tunable factors that provides flexibility in designing charged patchy particles, also poses a big limitation for experimentally assessing the generalized effects of the surface charge distribution on the self-assembly and phase separation of these particles. In that regard, computational models have the potential to systematically evaluate the phase behavior of charged colloids over a broad range of patch arrangements and media conditions, and thus to establish general “rules” for the rational design of charged patchy particles.

Many theoretical and computational models have been developed during the last two decades to study the thermodynamic and kinetic behavior of patchy particles.^{36,55–59} These models have provided valuable insight into the effect of specific interactions on protein liquid–liquid phase separation^{60,61} and crystallization.^{62,63} Likewise, they have been fundamental to understanding and controlling the rich range of self-assembled, well-defined structures that patchy colloids can form.^{64–66} However, most of these studies have been mainly devoted to explore how the number of patches, and their size, affect the behavior of anisotropic attractive particles, with particular emphasis on particles with short-range

attractions and a limited number of interactions per patch.^{67–70} By contrast, less attention has been given to particles that exhibit specific, long-range repulsive and attractive interactions such as those in zwitterionic particles. Examples of models for charged patchy particles include those for evaluating the effect of the charge pattern on protein–protein/solvent interactions^{59,71} and the nucleation of protein crystals,⁷² as well as those for studying the self-assembly and gelation of both embedded and induced dipolar colloids.^{73–75} Notably, recent studies on particles with simple charge patterns, termed inverse patchy colloids,^{66,76,77} have illustrated the role of long-range attractions/repulsions on tuning the equilibrium between phases of fluid, solid, and colloidal monolayers. Nonetheless, there remain outstanding questions regarding how the presence of both attractive and repulsive patches will affect the phase behavior of patchy particles in comparison to that of particles with only specific attractions. That is, for a given distribution of cationic and anionic patches, will the equilibrium between the different phases for charged patchy particles be governed by similar underlying factors (e.g., patch size, number) as particles with only attractive patches? Or will the competition between attractions and repulsions lead to a different phase behavior?

Therefore, the aim of this report is to provide a generic but simple computational model to study the rich thermodynamic phase behavior of charged, patchy particles that result from a heterogeneous surface charge distribution. In particular, this work focuses on systems where the particle size is of the same order of magnitude as the screening length or effective charge-charge interaction range, which corresponds to the situation of proteins or nanocolloids at low ionic strength. This model is used here to explore the fluid-phase separation of charged particles (akin to vapor–or liquid–liquid equilibrium) due to changes in the arrangement of charged patches, while future investigations will evaluate the self-assembly of these systems. Two types of charged patchy colloids are studied: dipolar fluids and protein-like fluids. The former system represents the simplest case of zwitterionic particles, and thus it provides the framework to understand how fluid-phase equilibria is affected by factors such as patch size and relative distance between anionic and cationic patches. On the other hand, proteinlike fluids consist of particles with more complex charge distributions than those found in dipolar fluids, and thus they correspond to models intended to mimic charged colloids and charge–charge interactions in proteins. This second type of system allows one to generalize the effect of the surface charge distribution on the fluid phase behavior of charged patchy particles.

II. MODEL AND METHODS

A. Model description

Charged patchy particles are modeled here as spherical particles of diameter σ that interact through a steric repulsion (u^s) and a “patchy” potential (u^c) to capture electrostatic interactions. Although anisotropy in both proteins and colloids might arise from both morphological constraints (e.g., multi-domain proteins or ellipsoidal colloids) and directional interactions, this work is restricted, for simplicity, to spherical particles with orientational-dependent interactions such as globular proteins and patchy colloidal beads. The choice of using patches to represent electrostatic interactions is intended to capture the

clustering of surface charges that is common in proteins,^{78,79} as well as to represent the wide range of functionality patterns that can be designed on to the colloidal surfaces.^{1,4} The pair interaction between particles i and j is then defined as

$$u_{ij}(r_{ij}, \Omega_{ij}) = u^{st}(r_{ij}) + \sum_{a,b \leq n_p} u_{ab}^{cc}(r_{ij}, \Omega_{ab}^{ij}) \quad (1)$$

where r_{ij} corresponds to the center-to-center distance between the pair i - j , and Ω_{ab}^{ij} is the relative orientation between patches a and b of particles i and j , respectively. The summation goes through the total number of electrostatic patches, n_p .

The steric repulsion is modeled by a shifted-force repulsive potential,

$$u^{st}(r) = V^{st}(r) - V^{st}(r_c) - (r - r_c) \left. \frac{\partial V^{st}}{\partial r} \right|_{r=r_c} \quad (2a)$$

$$V^{st}(r) = \left(\frac{\sigma}{r} \right)^{120} \quad (2b)$$

where r_c is the potential cutoff ($V^{st}(r - r_c) = 0$), and is chosen as 1.1σ .

Patch-patch or electrostatic interactions are defined by an isotropic potential (u_{ab}^{rad}), with the strength of the interaction dampened by an angular modulator ($f(\Omega_{ab})$)

$$u_{ab}^{cc}(r, \Omega_{ab}) = u_{ab}^{rad}(r) f(\Omega_{ab}) \quad (3)$$

As mentioned above, the working model is aimed to capture systems where the effective range of charge-charge interactions is comparable to the particle diameter σ . This effective range is built into u_{ab}^{rad} by using a modified shifted-force Lennard-Jones (LJ) potential

$$u_{ab}^{rad}(r) = V_{ab}^{LJ}(r) - V_{ab}^{LJ}(r_c) - (r - r_c) \left. \frac{\partial V_{ab}^{LJ}}{\partial r} \right|_{r=r_c} \quad (4a)$$

$$V_{ab}^{LJ}(r) = \begin{cases} 4\epsilon \left[\left(\frac{\sigma}{r} \right)^{12} - \left(\frac{\sigma}{r} \right)^6 \right] + \epsilon (q_a q_b + 1) & \text{if } r \leq 2^{1/6} \sigma \\ -4\epsilon q_a q_b \left[\left(\frac{\sigma}{r} \right)^{12} - \left(\frac{\sigma}{r} \right)^6 \right] & \text{otherwise} \end{cases} \quad (4b)$$

where ε provides the maximum strength for the patch-patch interaction, and q_a is the charge of patch a and can take values larger (smaller) than zero to represent positively (negatively) charged patches. $r_c = 3\sigma$ is the cutoff distance for the potential. Although a LJ-like energy potential does not correspond to a traditional model for electrostatic interactions such as a Coulomb or a Yukawa-tail potential, the modifications incorporated into eq. 4 ensure the main qualitative feature of the interaction is indeed captured, namely that interactions between two patches will yield attractive (negative) energies if both patches have charges of opposite sign, and repulsive (positive) energies if they have like charges. Note that this modified LJ potential also provides a natural effective interaction range that is less than 2σ , which is consistent with nanoscale particles/molecules at low ionic strength conditions (e.g., $< 10\text{mM}$). Thus, the resulting model preserves the most important characteristics of charge-charge interaction, namely the ability to exert attractions and repulsions beyond steric effects over a distance comparable to the particle size; therefore, the behavior of the working model is anticipated to be general and relevant to the problem evaluated here. Furthermore, the use of a LJ-like potential offers the additional advantage that its calculation requires significantly less computational time than traditional electrostatic models, as there is no need to compute ewald sums or other complex mathematical functions such as exponentials. As such, it allows one to explore systems of particles with a large number of patches and at high concentrations. Nonetheless, for those interested in evaluating the thermodynamic behavior of charged particles with a more rigorous electrostatic potential or at different ionic strength conditions, eq. 4 can be readily replaced by a traditional force model (e.g., a screened coulomb potential) without affecting the treatment of the directional patch-patch interactions.

For the angular dependence, electrostatic patches correspond to conical segments with an opening angle of π (i.e., each patch covers an hemisphere). However, interactions along the surface of the patch are not constant, but smoothly decrease away from the center of the patch. $f(\Omega_{ab})$ is then defined as

$$f(\Omega_{ab}) = \begin{cases} \cos^{\eta_a} \theta_a \cos^{\eta_b} \theta_b & \text{if } |\theta_a|, |\theta_b| \leq \pi/2 \\ 0 & \text{otherwise} \end{cases} \quad (5)$$

where θ_a corresponds to the smallest angle between the normal vector of the patch $\hat{\mathbf{n}}_a$ and the direction of the interparticle separation vector $\hat{\mathbf{r}}_{ij}$ pointing from particle i to j (cf. Fig. 1a). Note $\hat{\mathbf{n}}_a$ is a unit vector measured with respect to a body-fixed frame of reference, but whose orientation changes as the particle rotates. η_a is a variable parameter that controls the angular dependence of the effective interactions between charged patches. By changing η_a , one can explore a wide range of charge profiles, ranging from “Janus”-like patches⁴⁰ (for $\eta_a = 0$) to point-charges in decorated models^{71,72} (for $\eta_a \rightarrow \infty$). Figure 1b illustrates the resulting charge distributions of a single patch as a function of η_a . In order to compare different systems, the effective size of a patch is defined in terms of η_a as the half opening angle δ_a that yields 50% of the maximum patch charge (i.e. $\delta_a = \arccos(0.5^{1/\eta_a})$).

Based on the above description for charged patches, one can further define the particle surface charge distribution ($F(\hat{\mathbf{x}})$) as

$$F(\hat{\mathbf{x}}) = \sum_{a=1}^{n_p} [q_a (\hat{\mathbf{n}}_a \cdot \hat{\mathbf{x}})^{\eta_a} H(\hat{\mathbf{n}}_a \cdot \hat{\mathbf{x}})] \quad (6)$$

where $\hat{\mathbf{x}}$ is an unit vector measured from the center of the particle to any point on the particle surface. $H(x)$ is the Heaviside step function, and is such that it yields a value of 1 if $x > 0$ and 0 otherwise. Eq. 6 provides the mathematical form to evaluate the characteristic moments of the distribution. In particular, the zeroth, first, and second moments (i.e., the net charge Q_0 , the dipole moment $\tilde{\mathbf{Q}}_1$, and the quadrupole moment $\mathbf{Q}_2$) can be expressed via

$$Q_0 = \int_S F(\hat{\mathbf{x}}) dS \quad (7a)$$

$$\tilde{\mathbf{Q}}_1 = \frac{\sigma}{2} \int_S \hat{\mathbf{x}} F(\hat{\mathbf{x}}) dS \quad (7b)$$

$$\mathbf{Q}_2 = \left(\frac{\sigma}{2}\right)^2 \int_S [3\hat{\mathbf{x}}\hat{\mathbf{x}}^T - \hat{\mathbf{x}}\mathbf{I}\hat{\mathbf{x}}^T] F(\hat{\mathbf{x}}) dS \quad (7c)$$

where the integration is done on the particle surface S . The superscript $\mathbf{T}$ indicates the transpose of the vector, and $\mathbf{I}$ corresponds to the identity matrix. The prefactor of $\sigma/2$ scales $\tilde{\mathbf{Q}}_1$ and $\mathbf{Q}_2$ by the particle size.

Following Eq. 2–6, the working model provides a simple description of continuous surface charge distributions, which can be used to represent a variety of complex and exotic surface patterns. By varying the number of positive and/or negative charged patches, as well as the effective size of each patch, one can control the net charge and higher order moments of the surface charge distribution. Likewise, given the continuous nature of this model, it can be readily implemented in molecular dynamics simulations to study the effect of the charge distribution on non-equilibrium processes of charged particles with heterogeneous charge distributions (e.g., kinetics of nucleation and self-assembly). Although this work focuses on fluid–fluid phase equilibrium, a future report will explore the dynamic behavior of such systems.

B. Computational method

Flat-histogram sampling methods are used to evaluate the thermodynamic behavior and fluid phase separation of charged patchy particles. Specifically, Wang-Landau-Transition Matrix Monte Carlo^{64,80} (WL-TMMC) simulations are performed in the grand-canonical ensemble.

WL-TMMC is a powerful method that provides all thermodynamic properties (e.g., free-energy, potential energy, pressure) and structural information as a function of density at fixed volume and temperature. As simulations are performed in the grand-canonical ensemble, density fluctuations directly related to macroscopic transitions can be readily obtained from a single simulation, with minimum effect from system size.⁸¹

Grand canonical WL-TMMC is used to calculate the probability of observing N particles for a given chemical potential μ , temperature T , and volume V (i.e., the macrostate probability distribution, $\Pi(N/\mu, T, V)$). Specific implementation details of WL-TMMC are provided elsewhere^{64,80} and summarized here in the Supplementary Information. Simulations are initialized by running Wang-Landau with the update factor set to unity. Every time a flatness criteria of 80% is met, this factor is multiplied by 0.5 and the histogram of visited macrostates is reset. The collection matrix, needed for Transition Matrix Monte Carlo, is updated as soon as the update factor is smaller than 1×10^{-6} . The simulation then switches to Transition Matrix Monte Carlo when the update factor is smaller than 3×10^{-8} , with the TMMC biasing function calculated from the collection matrix and subsequent updates every 10^6 trials. A simulation is considered converged if it swept 25 times. A sweep is achieved if each macrostate has been visited from a different macrostate at least 100 times. After a simulation has swept at least two times, canonical ensemble averages for the quantities used in this work (see below) are accumulated and updated every 10^3 trials. In order to reduce computational time, the entire density range for a given isotherm (i.e., the range of N macrostates, from 0 to N_{max}) is divided into 12 windows, with overlap between neighboring windows of no less than five particle numbers. The size of each window is selected to balance computational load by decreasing window size with increasing density range by a power scaling factor of 3. $\Pi(N/\mu, T, V)$ for the complete N range is reconstructed by matching the free-energy of neighboring windows at the middle of the overlapping region, while the two largest and smallest macrostates at each window are discarded when neighbor windows are present.

Patchy particles have long been recognized for their ability to self-assemble into a variety of high-order macrostructures. Due to the large length-scale correlations of these structures, simulations of patchy particles are challenging when only traditional MC moves are considered.⁸¹ In order to overcome these limitations and improve sampling, the following MC trials are employed (see Table I): (i) rigid-body translations and rotations; (ii) particle insertion/deletion; (iii) orientational-bias⁸¹ (OBMC); (iv) multi-first particle insertion⁸² (MFI); and (v) grand canonical aggregation-volume bias⁸³ (AVB). AVB is a specific method for enhancing sampling in strongly associating systems. In grand canonical AVB, insertions and deletions are only performed in relation to the vicinity (i.e., the interacting or “bonded” region) of a target particle, and thus the phase space of the corresponding trial is greatly reduced. Similarly, both MFI and OBMC moves are employed to ensure that the most likely state is selected (from a list of proposed configurations) during the trial. Insertions and deletions are carried out by coupling MFI with OBMC. For insertions, MFI is used to select the position for inserting the new particle from k_1 proposed positions (based on steric interactions). OBMC is then employed to choose the best particle orientation (from k_2 possibilities) based only on patch–patch interactions. A similar scheme is followed in the case of deletions, with the difference that the probability to remove a given particle now

depends on $k_1 - 1$ and $k_2 - 1$ “alternative” configurations for MFI and OBMC, respectively. A total of 10 proposed configurations are evaluated during either MFI and OBMC (i.e., $k_1 = k_2 = 10$). Additionally, moves of type (iii)-(v) are concatenated into a single grand canonical trial move, and implemented as follows. A target particle is randomly selected, and those particles belonging to its “in” (bonded) region are identified. The “in” region corresponds to a spherical shell with inner and outer radii $r_{in} = 0.99\sigma$ and $r_{out} = 1.5\sigma$, respectively. Then, an insertion/deletion is attempted within the *in* region using the MFI/OBMC scheme described above. Specific details about the different type of moves, the resulting acceptance criteria, and their implementation in WL-TMMC are provided in the Supplementary Information. Table I summarizes the different MC trials used in this work, as well as their corresponding probabilities for attempting one of these moves.

During a simulation, canonical ensemble averages are collected for the potential energy, the center-to-center distance distribution, and the cluster-size distribution. Here, a particle is considered to be part of a cluster if it is within a cutoff distance of 1.5σ from one or more of the other particles belonging to the cluster. This cut-off distance ensures that the separation between two particles is within the well of the interaction energy (in the case of interactions between oppositely charged patches), as well as is in agreement with traditional cluster criteria for clustering of fluids interacting with Lennard-Jones-like potentials.⁸⁴ For an observable A , the canonical average (denoted $\langle A \rangle_{NVT}$) can be calculated as

$$\langle A(N) \rangle_{NVT} = \frac{\sum_{i=0}^{N_{trial}} A_i \delta(N_i - N)}{\sum_{i=0}^{N_{trial}} \delta(N_i - N)} \quad (8)$$

where N_i is the number of particles at the trial i , $\delta(x)$ is the Dirac delta function, and N_{trial} is the total number of sampled configurations during the course of the simulation.

After the simulation is finished and $\Pi(N/\mu, T, V)$ is calculated and normalized (see Supplementary Information), grand canonical averages of the observable A are readily obtained as a continuous function of the average density $\langle N \rangle_{\mu VT}$ via

$$\langle A(\langle N \rangle_{\mu VT}) \rangle = \sum_{n=0}^{N_{max}} \langle A(n) \rangle_{NVT} \Pi(n|\mu, T, V) \quad (9a)$$

$$\langle N \rangle_{\mu VT} = \sum_{n=0}^{N_{max}} n \Pi(n|\mu, T, V) \quad (9b)$$

Note that the state condition of the average properties in Eq. 9 can be controlled via reweighting the macrostate distribution to different values of μ . That is,

$$\ln \Pi(N|\mu, T, V) = \ln \Pi(N|\mu_0, T, V) + (\mu - \mu_0) N \quad (10)$$

where $\Pi(N|\mu_0, T, V)$ is the macrostate distribution obtained from the simulation at a chemical potential μ_0 , and μ is the target thermodynamic state.

C. Second virial coefficient

Effective interactions between macromolecules are typically characterized by the second virial coefficient B_2 . This quantity can be experimentally probed for most systems of interest,^{85–87} and thus it plays a central role in many molecular models and theories for phase separation and self-assembly of colloids and proteins.^{14,88,89} B_2 is formally related to strength of the intermolecular interaction between a pair of particles via

$$B_2 = -\frac{2\pi}{\Omega^2} \int \int (e^{-u_{12}/k_B T} - 1) r_{12}^2 dr_{12} d\Omega_1 d\Omega_2 \quad (11)$$

where u_{12} is defined in eq. 1, and corresponds to the interaction energy between two particles and is a function of the separation distance between both particles (r_{12}) and the orientation of each particle with respect to a fixed frame of reference (Ω_j). $\Omega = \int d\Omega_j$ represents the whole orientational space for each particle. As described by eq. 11, negative values of B_2 indicate net attractive interactions, while positive values represent net repulsive pair interactions. Of particular relevance is the case where only steric interactions are considered, as B_2 corresponds to the excluded volume of the particles (for the working model that is $B_2 \approx 2\pi\sigma^3/3$)

Here, theoretical values of B_2 are calculated by implementation of the direct Mayer Sampling method.⁹⁰ Briefly, this method calculates eq. 11 using a biased Monte Carlo approach, performing importance-sampling based on those configurations relevant to the integral for the system of interest, and comparing the results against a reference system for which B_2 is known (akin to a free-energy perturbation of the reference system). Thus, for the working model, the selected reference system correspond to an isotropic fluid that interact through a shifted-force Lennard-Jones potential (i.e., via eq. 4 with $q_a q_b = -1$). Sampling is performed for two particles in an infinite volume, where trials are attempted to displace or rotate one particle at a time. Trials are accepted/rejected based on a Metropolis criterion, with a probability proportional to the absolute value of the integrand in eq. 11. Each simulation consists of a short “equilibration period” of 5×10^7 trials to adjust step sizes for the trials to achieve 50% acceptance rate. Thereafter, simulation is followed by a sampling period of 5×10^8 trials. Specific details regarding this method are provided in Ref. 90.

III. RESULTS AND DISCUSSION

The phase behavior of charged patchy particles is studied as a function of the intrinsic surface charge distribution. Specifically, the effects of patch size and overall patch arrangements on fluid–fluid phase separation are investigated. The fluid phase boundaries

are calculated using WL-TMMC for a series of patchy systems with different surface charge distributions. Simulations are performed as described above in a cubic box of $V = 729\sigma^3$ with periodic boundary conditions. For each system, binodal curves are constructed by determining phase coexistence points at a minimum of 5 different temperatures.

Uncertainties on estimated densities along the coexistence curve are calculated from block averages of the collection matrix followed by propagation of error (see Supplementary Information). Unless otherwise stated, the critical density and temperature are obtained by assuming that the coexistence states are related to the critical point by the law of rectilinear diameters.⁹¹ For a given system, the particle patches have the same size and they are assigned a charge of unity. That is, η_a is identical for all patches and $q_a = 1e$ or $-1e$ with e being the elementary charge. Additionally, only systems with negligible net charge are considered (i.e., $Q_0 = 0$ within uncertainty from numerical integration of Eq. 7). In what follows, the phase behavior of two different model systems (namely, dipolar and protein-like fluids) is illustrated.

A. Dipolar fluids

Dipolar fluids correspond to the simplest systems that exhibit anisotropic interactions.^{73,74,92} They consist of particles carrying a dipole moment as a result of two opposite (arrangement of) charges. From a practical perspective, the charge distribution of dipolar fluids is effectively characterized by its first moment (i.e., $\tilde{\mathbf{Q}}_1$). As such, these fluids represent an ideal model system to evaluate the effect of the dipole moment on the phase separation of charged particles. In the context of the working model, dipolar fluids are represented here as spherical particles decorated by two oppositely charged patches (Fig. 1c). Following Eq. 6 and 7, two factors directly control the magnitude of the dipole moment: the size of the patch (given by the value of δ_a as defined above), and the relative distance between the center of both patches (denoted here as d); the effects of both factors are systematically investigated. Figure 2 illustrates the phase coexistence curves for a series of dipolar fluids with different values of δ_a and d .

Similar to what has been reported for attractive patchy particles,^{57,93,94} the range of temperatures for which dipolar fluids exhibit fluid–fluid phase separation depends strongly on the size and relative position of the charged patches, which is consistent with changes in the overall strength of interactions. Smaller and/or overlapping patches result in weaker net interactions, and thus phase separation occurs at lower T . However, unlike other patchy systems,⁵⁷ the shape of the coexistence curve cannot be described by the law of rectilinear diameter (cf. Fig. S1 in Supplementary Information). At high temperatures (i.e., near criticality), the density difference between the coexisting phases exhibits a small temperature dependence, with the dilute phase having a relatively high density (e.g., $\rho\sigma^3 \approx 0.35$). On the other hand, at low temperatures, the density of the dilute phase shows a strong temperature dependence. These two behaviors result in a binodal curve with two different curvatures. Notoriously, the change in the curvature as a function of temperature is more prominent for large patch sizes and large patch separations. This behavior was also observed in simulations at smaller and larger volumes (see Fig. S1 in Supplementary Information), which implies that the change in curvature is representative of the macroscopic behavior of dipolar fluids rather than a size-effect artifact. Structural analysis of the dilute phase along the binodal

curve suggests that particle self-assembly at high T might be the cause of this behavior (see below). In order to approximately locate the critical temperature, the macrostate distributions were determined for a set of temperatures in the vicinity of the critical point using a T -spacing of no larger than $0.05 \epsilon/k_B$. The critical temperature T_c was estimated to be the midpoint temperature between two temperatures, the lowest T yielding an unimodal distribution and the highest T yielding a bimodal distribution. Error bars in T_c are defined by the difference between these two temperatures. Likewise, the critical density ρ_c corresponds to the midpoint density of the two stable phases at the highest T yielding a bimodal distribution, with error bars estimated from the uncertainties on those densities. Using the calculated critical points to normalize the phase envelopes (cf. Fig. S2), changes in the shape of the binodal curve become more evident, with a broadening of the equilibrium curves as either the patch size or patch separation distance decreases.

Interestingly, among all evaluated dipolar fluids, fluid phase separation was only observed over a limited range of patch sizes and patch separations (i.e., $\delta_a > 40^\circ$ and $d/\sigma > 0.7$), where the range of available d values decreases as δ_a decreases. Outside this range of geometries, fluid phase separation is frustrated by spontaneous particle self-assembly into a variety of high-order structures such as linear and entangled chains, as well as lamellar phases (see Fig. S3 in Supplementary Information). Given the propensity of particles with directional interactions to self-associate into arrested states,^{56,73,95} these findings are not completely surprising. Although a study of the self-assembly of charged particles is beyond the scope of this work and will be considered in a future report, the formation of these ordered structures motivated a further structural analysis along the coexistence curves. As such, the structural behavior of the dipolar fluids is investigated via analysis of the cluster-size distribution. In general for all evaluated systems, the cluster distribution shows that the monomer remains the most likely thermodynamic state during phase separation (see Fig. S4), as one may anticipate for a fluid–fluid transition akin to vapor–liquid equilibrium. However, near T_c , the size distributions for the dilute phase also reveal a larger amount of intermediate-size clusters (> 20 particles per cluster) when compared to that of the dense phase. Visual inspection of the structures at densities close to the binodal curve suggests the formation of gel-like, fluctuating clusters. It has been long recognized the propensity of different fluids to form metastable clusters near the critical region on a homogeneous phase transition,^{83,96} but it is somehow counterintuitive that such structures are observed more often in the dilute phase. In fact, comparison of the average cluster size for both phases at equilibrium (Fig. 3) shows that the number of particles per cluster in the dilute phase is consistently greater than those of a reference isotropic fluid at equivalent temperatures (solid line in the figure). By contrast, the cluster size along the dense phase remains similar to that of the reference system, and below those of the dilute phase for large patch sizes and high temperatures (blue circles in Fig. 3). Here, the reference fluid consists of shifted-force Lennard-Jones particles interacting with a potential similar to that in Eq. 4 with $q_a q_b = -1$. From the perspective of fluid phase behavior, the results in Fig. 3 may indicate a close relation between the fluctuating clusters and the shape of the binodal curves. For instance, the presence of these structures might retard macroscopic phase separation due to the formation of regions with very high local density, and thus causing the relative high densities of the dilute phase. Furthermore, the crossover between the average cluster size of both

phases observed at large patch sizes (e.g., blue symbols in Fig. 3) seems to be related to the apparent double curvature of the coexistence curves. Nonetheless, it is unclear if the formation of these clusters is the direct cause of the anomalous shape of the binodal curves observed for large patch sizes, or rather both phenomena are the consequence of an unknown underlying factor that is not captured by the parameters evaluated here.

In order to relate the phase behavior of dipolar fluids to the charge distribution, the critical temperature is compared to the magnitude of the dipole moment (Q_1) in Fig. 4a. The results show a clear relationship between these two quantities. When the system consists of Janus-like, dipolar particles (i.e., evenly charged hemispheres), both the critical temperature and dipole moment are maximized. As the effective size of the patches decreases at fix patch distance, T_c shows a monotonic decrease following a non-linear dependence. Although a similar behavior is also observed when d is reduced, resulting T_c values exhibit small differences from the trend obtained in the former case (see inset in Fig. 4a). These deviations become more evident as the distance between patches gets smaller (or the overlapping between patches becomes larger). In the case of ρ_c , a dependence with respect to Q_1 is also observed, with significant changes in the critical density for small dipole moments -e.g., at small values of δ_a and/or d (cf. Fig. 2). Changes in both T_c and ρ_c with respect to the size and position of the patches suggest that the magnitude and the direction of the dipole moment (e.g., whether or not $\tilde{Q}_1$ goes through the center of the particle) play an important role in controlling the phase behavior of this type of charged particles. However, performing a systematic quantification of the latter aspect of $\tilde{Q}_1$ is not possible for this model, as the range of conditions where “homogeneous” phase separation is stable with respect to self-assembly is very limited (see above). Nonetheless, the results in Fig. 4 clearly illustrate the effect of the charge distribution on the phase separation of charged particles, and provide a phenomenological boundary for assessing the thermodynamic stability of particles with simple surface charge arrangements.

The behavior of the critical point with respect to the dipole moment (and thus, the charge distribution) can also be explained from the perspective of the number of possible “bonds” a patch can form, or the so-called patch valence. For large patch sizes and no patch overlap (i.e., $d = \sigma$), the valence can be high provided the long-range nature of charge interactions. That is, there is a large number of particles in the first interaction shell of a given particle, which facilitate the formation of thermodynamically stable states at higher densities. As the effective size of the patches decreases by decreasing either δ_a or d , the number of interacting particles is significantly reduced, resulting in both lower T_c and ρ_c . That is, it requires stronger intermolecular interactions in order to phase separate. Such effects can be indirectly observed by evaluating the second virial coefficient (B_2) at the critical temperature (cf. Fig. 4b). As defined in eq. 11, B_2 captures the strength of interactions between a pair of particles averaged over all the orientational degrees of freedom, and thus it implicitly accounts for any restriction in the orientation between particles as a result of changes in the patch valence. For large patch sizes, the results in Fig. 4b show that dipolar fluids exhibit a weakly attractive net interaction given by $B_2(T_c) \approx 0$. This value, however, rapidly decreases as either δ_a or d decreases. This behavior of the second virial coefficient is in agreement with the above description based on patch valence, and suggests the existence of a strong entropic

barrier for small and/or overlapping patches that needs to be overcome. Similar arguments have been used to describe the behavior of the critical region as a result of changes in the patch coverage for one- and two-patch attractive fluids with a similar range of interactions than that used here ($\sim 1.5\sigma$).^{93,94}

Nonetheless, the intrinsic competition between attractive and repulsive long-range interactions in the working model seems to lead to a more pronounced effect than that observed in attractive patchy systems based on the results in Fig. 4b. That is, when comparing systems with equivalent charge distributions but different degrees of patch overlap, those systems with smaller patch distances require stronger pair interactions to phase separate despite yielding similar values of Q_1 . As such, this implies that changes in the binodal curve due to changes in the charge distribution may be influenced by the entropy of the system rather than the energy. To provide further support for this point, the entropy of the system along the coexistence curve was evaluated (see Fig. S5 in the Supplementary Information). Particularly, the methodology described by J. R. Errington *et al.*⁹⁷ to calculate excess entropies from $\ln \Pi(N)$ was implemented here. The excess entropy S^{ex} , defined as the entropy of the system minus that of an ideal gas at the same T and ρ , provides information regarding the effect of interparticle interaction on both the orientational and translational entropy. The results in Fig. S5 illustrate an increase of the excess entropy of both phases at equilibrium as δ_a and/or d decreases. Furthermore, the difference in the excess entropy of phases $\Delta S^{ex} (= S_{dense}^{ex} - S_{dilute}^{ex})$ changes from negative values for large patches to positive values for small and/or overlapping patches. Given that anisotropic interactions are the main contribution to the energy, S^{ex} is heavily weighted by favorable orientations between particles. Thus, positive values of S^{ex} indicates an increase of the orientational entropy and larger entropic barriers for the phase transition, as it was anticipated from Fig. 4.

B. Protein-like fluids

In the previous section, the fluid phase behavior of particles with simple surface charge distributions was described in terms of the dipole moment. However, many colloidal and biological systems such as proteins present a more complex charge arrangement, where it is not evident that the charge distribution can be described by its first moment alone. In order to generalize how the charge arrangement affects the fluid-phase equilibrium, the behavior of particles with multiple charged patches is evaluated. For clarity, such systems are referred to as protein-like fluids (PLFs). PLFs are represented here by spherical particles decorated by 12 patches, where these patches are placed in a “disco ball” pattern (i.e., they are located along rows equally spaced by latitude as illustrated in Fig. 1d). The size of each patch is such that $\delta_a = 30^\circ$, which results in significant overlap between neighboring patches at distances smaller than $\delta_a\sigma$. The charge distribution for this system is then controlled by changing the identity or charge of each patch, while keeping the same number of positively and negatively charged patches to yield $Q_0 \approx 0$. Although surface charges in proteins and multivalent colloids might in principle exhibit a wider range of patterns, the particular choice of patch size and arrangement for PLFs provides simplicity and generality. An interesting consequence is that neighboring patches of like charges effectively behave as a single patch, and thus allows one to explore a richer set of surface patterns. In spite of the simplicity of this geometry, the number of distinguishable combinations of charge

arrangements is close to one hundred, which is problematic for computationally evaluating the phase behavior of all these systems. Instead, a representative number of charge patterns are only considered for simulations. The pool of studied systems consists of both random charge arrangements and selected surface patterns. Table S1 in Supplementary Information summarizes the set of evaluated systems, as well as the intrinsic properties of their charge distributions. Fluid–fluid phase diagrams are calculated via WL-TMMC simulations as described in Section II. Unlike dipolar fluids, binodal curves of PLFs can be described by the law of rectilinear diameters. Thus, it is the method of choice to estimate the critical point for all the evaluated systems. Figure 5 shows illustrative examples of the coexistence curves for PLFs.

The phase equilibrium curves for PLFs exhibit wide variation among the different charge arrangements considered here (cf. Fig. 5). Although the size, number, and location of all patches are identical for all PLFs, changing the identity of each patch (while preserving the neutrality of the particle) can dramatically alter both the position of the critical point and the shape of the phase envelope. This assortment of behaviors ranges from coexistence regions that are skewed towards high densities to broad binodal curves that resemble the behavior of isotropic particles. Furthermore, for some of the evaluated charge patterns, the formation of ordered, arrested structures akin to solid phases (e.g., crystallization) frustrate or even prevent phase separation between the dilute and dense fluid phases (see Table S1). Interestingly, when plotting the binodal curves in reduced units (Figure 6), the wide range of behaviors fall into distinct groups, where each group exhibits a different level of broadening in terms of the width of the reduced phase diagram.

As mentioned above, the choice of patch size and their arrangement allows for neighboring patches with the same charge to act as a single, “super” patch. In this context, two patches are considered to be neighbors if the distance between their centers is equal to $\delta_a\sigma$. As such, the different type of phase behaviors observed in Fig. 6 seems to be qualitatively related to the average size of these super patches. For instance, when all patches with the same charge are located in the same hemisphere, the charge pattern resembles that of a Janus-like particle with a narrow coexistence curve characteristic of dipolar fluids. That is, criticality occurs at relatively high densities (e.g., $\rho_c\sigma^3 > 0.4$), and metastable clusters are present in the dilute phase of the coexistence region (see previous section). Similar behaviors are also observed for other charge patterns resulting in a single net patch, although they show a slightly broader coexistence region (e.g., compare blue circles and purple diamonds in Fig. 6). As the size of the “super” patches is reduced, there is a change in the shape of the equilibrium curves, with a widening of the reduced coexistence regions. Particularly, major differences are observed along the dense phase due to the critical density decreasing and the binodal curve skewing towards lower densities (cf. Fig. 5 and 6).

Although the above description seems to represent the general behavior of PLFs, not all of the evaluated systems with the same average size for the super patches fall within the same category. That is, some of the PLFs with small average net patches behave like those with slightly larger super patches, and viceversa. At this point, the underlying reason behind this variability is unclear, but a possible explanation might lie in the nature of electrostatic interactions and the particular charge arrangement of each PLF. Similar to the case of dipolar

fluids with $d < \sigma$, the relative position of oppositely charged net patches may alter the energetic and entropic balance between attractive and repulsive interactions (e.g., via changes in the patch valence), leading to deviations between similar systems. Nonetheless, these results are consistent with the work of Bianchi *et al.*^{67,95} for short-range attractive patchy particles, in that changing the arrangement of attractive sites, while maintaining the number of patches fixed, leads to different critical behaviors.

Additionally, the effect of the different moments of the charge distribution on the fluid–fluid equilibrium was investigated. Specifically, the relation between the critical temperature of PLFs and the magnitude of the dipole moment (Q_1) was analyzed in the context of the different type of coexistence curves described above (Figure 7). Similar to dipolar fluids, a clear trend is observed between the critical temperature and Q_1 , where T_c exhibits a monotonic, non-linear dependence with respect to the dipole moment. As one may anticipate, those systems with the largest “super” patches yield the largest values of Q_1 , and thus the highest values of T_c . However, small changes in the charge arrangement for these PLFs (e.g., comparing Janus-like particles vs. particles with a “baseball”-like pattern) lead to small variations in the dipole moment but large changes in T_c . By contrast, for PLFs with small super patches (red triangles and green squares in Figs. 6 and 7), the behavior of T_c vs. Q_1 is reversed in that large changes in Q_1 yield small changes in the value of the critical temperature. Interestingly, PLFs within these two categories fall within the same range of T_c (i.e., $k_B T_c / \epsilon$ between 0.2 and 0.3), but access different ranges of dipole moments. While the charge arrangements of those PLFs with slightly narrower phase envelopes (red triangles in Fig. 6) correspond to low-to-intermediate values of Q_1 , systems with a broader coexistence region are limited to only intermediate values of the dipole moment (e.g., Q_1 between 1 and $2 e \sigma$). Notably, for a fixed value of the dipole moment, PLFs within the latter category consistently exhibit lower critical temperatures than their counterpart with narrower critical regions. Such behavior is in agreement with previous arguments regarding energetic/entropic imbalance. That is, some charge patterns may enhance intrinsic competition between electrostatic forces, and thus stronger particle–particle interactions are needed in order to phase separate (i.e., lower values of T_c). For instance, if one considers a charge arrangement such as negatively charged patches surrounded by positively charged sites (akin to a soccer-ball pattern), most, if not all, of the orientations between particles might result in equally favorable attractive and repulsive patch–patch interactions. However, those patterns that favor repulsive interactions generally involve disperse charge distributions, hence the relative high dipole moment despite the lower T_c .

In addition, the phase behavior of PLFs was further investigated in terms of higher-order moments of the charge distribution, namely the quadrupole moment (see Table S1). Although no apparent relation was observed between the quadrupole moment and the fluid–fluid phase equilibrium, the combination of low values for the dipole and quadrupole moments seem to be related to the formation of high density structures. As such, those PLFs where the fluid coexistence regions are either frustrated or prevented by these ordered, arrested solid phases exhibit low values for both moments of the charge distribution.

IV. CONCLUSIONS

The results for both dipolar and protein-like fluids provide valuable insight into how the charge distribution may affect the fluid phase behavior of proteins and charged colloids, and illustrate the strong correlation between the critical point, the strength of the interparticle interactions, and the dispersion of charged patches observed through the dipole moment. As such, lower stability with respect to a homogeneous phase transition (e.g., high T_c) is obtained for systems that exhibit stronger attractive patch–patch interactions as a consequence of clustering of cationic or anionic patches or larger patch sizes (i.e., larger values of Q_1). Likewise, the size and position of the charged patches seem to be also related to the shape of the binodal curve in that smaller “net” patches yields broader phase envelopes. When analyzing these results in the context of attractive patchy systems,^{67,93} similarities between both attractive and charged patchy particles can be drawn in terms of the effect of the geometry of the patches on the phase behavior. That is, if one only considers the size and number of patches, the fluid phase behavior could be explained from the standpoint of the patch valence. For instance, increasing number of attractive charge–charge interactions per patch (i.e., larger net patch size) leads to a higher critical temperature, but preserves the shape of the binodal curve.^{55,57,94} However, these similarities only hold for systems with low patch coverage and/or when competition between long-range attractions and repulsions are null or at least negligible (e.g., for diametrically opposed patches in dipolar fluids or when neighboring patches of like charge act effectively as a single super patch in PLFs). Arguments for patch valence are mainly based on energetic contributions to the free-energy of the system, and may underestimate changes in the orientational entropy that arise from energetic penalties. As a result, systems where long-range repulsions are prominent tend to exhibit lower critical temperatures and broader phase envelopes than what one might anticipate.

Similarly, the above results suggest that liquid–liquid phase separation of proteins might be highly influenced, if not driven, by specific charge–charge interactions. Although the working model does not fully represent proteins since short-range attractive forces (e.g., hydrophobic and hydrogen-bond interactions) are not considered here, it provides a good representation of the protein charge distribution near neutral pH. At physiological conditions, the majority of proteins present a moderate dipole moment ($3 - 10 \text{ e nm}$),⁹⁸ with a very broad liquid–liquid phase envelope that is generally metastable with respect to crystal solubility;^{99–101} that is the case of globular proteins such as lysozyme^{102,103} and different crystallins.^{24,25} Typically, fluid phase separation of these proteins can be experimentally observed at very low temperatures and/or in the presence of high concentration of co-solutes, such as ammonium sulfate or PEG, that enhance attractive protein-protein interactions.^{33,34} Specifically, the use of these excipients facilitates probing liquid-liquid phase separation by bringing the position of the critical point to accessible temperatures (e.g., T_c higher than the freezing point of the solvent).¹⁰⁴ Although the driving forces for phase separation in the presence of these excipients may not be charge-charge interactions, different studies have shown that the main characteristics of the resulting coexistence curves remain unaltered by reducing the concentration of excipients to conditions where electrostatic interactions are relevant.^{105,106} In fact, many theoretical models do require

incorporation of charge-charge interactions for adequately fit experimental liquid-liquid binodal curves.^{106,107} Interestingly, if one considers the average size of a globular protein (~ 4 nm), those PLFs with broad coexistence curves and similar values for the dipole moment (e.g., triangles and squares in Figs. 6 and 7) qualitatively capture the main characteristics of experimental protein phase behavior. This suggests that the distribution of surface charges might be determining factor in defining such features of the coexistence curves. On the other hand, large dipole moments (> 20 e nm) are mainly observed for large proteins with a high propensity to form protein complexes,^{98,108} as well as for membrane proteins.¹⁰⁹ For such cases, phase separation is generally frustrated by protein oligomerization, and high protein concentrations and/or confinement conditions (e.g., lipid cubic phases) are required for the formation of dense macrophases.^{110–112} Additionally, in many cases, the presence of ionic surfactants or other polyelectrolyte molecules help modulate the formation of ordered phases.¹¹⁰ Even though the mechanism for liquid phase separation and crystallization of these proteins under such conditions is not well understood, one might anticipate that the equilibrium between phases is driven by the formation of microphases that enhance dipolar interactions based on the characteristics of these proteins and the role of polyelectrolytes. In that regard, the model presented here might help to understand the phase behavior of these systems, as many of these features naturally arise due to the high disparity in the surface charge distribution (cf. Figs. 3 and 5).

Finally, this work on dipolar fluids provides the groundwork for studying the self-assembly of charged patchy particles. As it was illustrated in Fig. S3, these particles can exhibit a variety of architectures that ranges from chains to bilayers, where all these structures form upon small changes in the charge distribution. In particular, these ordered structures were observed for systems with low patch coverage (e.g., small patches and/or large overlapping between patches), which indicates why self-assembly was not detected in PLFs over the evaluated conditions. Many of these self-assembled structures have also been recognized in attractive patchy particles,⁵⁶ but it is remarkable how the inclusion of long-range repulsion enhances the sensitivity of patchy particles to form different ordered clusters given the small set of patch arrangements explored here. As such, these preliminary results open an important question regarding which are the thermodynamic and structural boundaries for the formation of these self-assembled architectures in terms of those factors evaluated here (i.e., patch size and location), as well as additional factors such as the range of the interactions and the imbalance between cationic and anionic patches.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

Harold W. Hatch, Nathan A. Mahynski, Joseph E. Curtis, and Steven D. Hudson are thanked for many helpful and stimulating discussions, as well as revisions to the manuscript.

References

1. Yi GR, Pine DJ, Sacanna S. *J Phys Condens Matter*. 2013; 25:193101. [PubMed: 23611897]

2. Pawar AB, Kretschmar I. *Macromol Rapid Commun.* 2010; 31:NA.
3. Wang A, Shi W, Huang J, Yan Y. *Soft Matter.* 2016; 12:337. [PubMed: 26509717]
4. Glotzer SC, Solomon MJ. *Nat Mater.* 2007; 6:557. [PubMed: 17667968]
5. Piazza R. *Curr Opin Colloid Interface Sci.* 2000; 5:38.
6. Dumetz AC, Chockla AM, Kaler EW, Lenhoff AM. *Biophys J.* 2008; 94:570. [PubMed: 18160663]
7. Amin S, Barnett GV, Pathak JA, Roberts CJ, Sarangapani PS. *Curr Opin Colloid Interface Sci.* 2014; 19:438.
8. Keskin O, Gursoy A, Ma B, Nussinov R. *Chem Rev.* 2008; 108:1225. [PubMed: 18355092]
9. Xu D, Lin SL, Nussinov R. *J Mol Biol.* 1997; 265:68. [PubMed: 8995525]
10. Chen Q, Whitmer JK, Jiang S, Bae SC, Luijten E, Granick S. *Science.* 2011; 331:199. [PubMed: 21233384]
11. Tanaka T, Komatsu Y, Fujibayashi T, Minami H, Okubo M. *Langmuir.* 2010; 26:3848. [PubMed: 19835405]
12. Sacanna S, Pine DJ, Yi GR. *Soft Matter.* 2013; 9:8096.
13. Raut AS, Kalonia DS. *Mol Pharm.* 2015; 12:3261. [PubMed: 26237070]
14. Rosenbaum DF, Zukoski CF. *J Cryst Growth.* 1996; 169:752.
15. George A, Wilson WW. *Acta Crystallogr D Biol Crystallogr.* 1994; 50:361. [PubMed: 15299385]
16. Curtis RA, Blanch HW, Prausnitz JM. *J Phys Chem B.* 2001; 105:2445.
17. Bemporad F, De Simone A, Chiti F, Dobson CM. *Biophys J.* 2012; 102:2595. [PubMed: 22713575]
18. Weiss WF, Young TM, Roberts CJ. *J Pharm Sci.* 2009; 98:1246. [PubMed: 18683878]
19. Cohen SI, Vendruscolo M, Dobson CM, Knowles TP. *J Mol Biol.* 2012; 421:160. [PubMed: 22406275]
20. Morrison JL, Breitling R, Higham DJ, Gilbert DR. *Bioinformatics.* 2006; 22:2012. [PubMed: 16787977]
21. Pfaff SJ, Chimenti MS, Kelly MJS, Arkin MR. *Methods Mol Biol.* 2015; 1278:587. [PubMed: 25859978]
22. Holdgate G, Geschwindner S, Breeze A, Davies G, Colclough N, Temesi D, Ward L. *Methods Mol Biol.* 2013; 1008:327. [PubMed: 23729258]
23. Blanco MA, Sahin E, Robinson AS, Roberts CJ. *J Phys Chem B.* 2013; 117:16013. [PubMed: 24289039]
24. McManus JJ, Lomakin A, Ogun O, Pande A, Basan M, Pande J, Benedek GB. *Proc Natl Acad Sci.* 2007; 104:16856. [PubMed: 17923670]
25. Annunziata O, Pande A, Pande J, Ogun O, Lubsen NH, Benedek GB. *Biochemistry.* 2005; 44:1316. [PubMed: 15667225]
26. Stradner A, Sedgwick H, Cardinaux F, Poon WCK, Egelhaaf SU, Schurtenberger P. *Nature.* 2004; 432:492. [PubMed: 15565151]
27. Kramer RM, Shende VR, Motl N, Pace CN, Scholtz JM. *Biophys J.* 2012; 102:1907. [PubMed: 22768947]
28. Curtis RA, Ulrich J, Montaser A, Prausnitz JM, Blanch HW. *Biotechnol Bioeng.* 2002; 79:367. [PubMed: 12115400]
29. Dumetz AC, Chockla AM, Kaler EW, Lenhoff AM. *Biochim Biophys Acta - Proteins Proteomics.* 2008; 1784:600.
30. Wang W, Singh S, Zeng DL, King K, Nema S. *J Pharm Sci.* 2007; 96:1. [PubMed: 16998873]
31. Warwicker J, Charonis S, Curtis RA. *Mol Pharm.* 2014; 11:294. [PubMed: 24283752]
32. Minton AP. *Curr Opin Struct Biol.* 2000; 10:34. [PubMed: 10679465]
33. Wang Y, Annunziata O. *J Phys Chem B.* 2007; 111:1222. [PubMed: 17266278]
34. Dumetz AC, Lewus RA, Lenhoff AM, Kaler EW. *Langmuir.* 2008; 24:10345. [PubMed: 18702478]
35. Dorsaz N, Filion L, Smallegange F, Frenkel D. *Faraday Discuss.* 2012; 159:9.
36. Hloucha M, Lodge JFM, Lenhoff AM, Sandler SI. *J Cryst Growth.* 2001; 232:195.

37. Li W, Persson BA, Morin M, Behrens MA, Lund M, Zackrisson Oskolkova M. *J Phys Chem B*. 2015; 119:503. [PubMed: 25494398]
38. Xu Y, Mazzawi M, Chen K, Sun L, Dubin PL. *Biomacromolecules*. 2011; 12:1512. [PubMed: 21413681]
39. Liu Y, Liu B, Nie Z. *Nano Today*. 2014; 10:278.
40. Zhang Z, Glotzer SC. *Nano Lett*. 2004; 4:1407.
41. Muthukumar M. *Science* (80-). 1997; 277:1225.
42. Zaccarelli E. *J Phys Condens Matter*. 2007; 19:323101.
43. Gangwal S, Pawar A, Kretzschmar I, Velev OD. *Soft Matter*. 2010; 6:1413.
44. Cho JH, Gracias DH. *Nano Lett*. 2009; 9:4049. [PubMed: 19681638]
45. van der Linden MN, Doye JPK, Louis AA. *J Chem Phys*. 2012; 136:054904. [PubMed: 22320761]
46. Chen Q, Bae SC, Granick S. *Nature*. 2011; 469:381. [PubMed: 21248847]
47. Glaser N, Adams DJ, Böker A, Krausch G. *Langmuir*. 2006; 22:5227. [PubMed: 16732643]
48. Bucaro MA, Kolodner PR, Ashley Taylor J, Sidorenko A, Aizenberg J, Krupenkin TN. *Langmuir*. 2009; 25:3876. [PubMed: 19708158]
49. Champion JA, Katare YK, Mitragotri S. *Proc Natl Acad Sci U S A*. 2007; 104:11901. [PubMed: 17620615]
50. Chen Q, Diesel E, Whitmer JK, Bae SC, Luijten E, Granick S. *J Am Chem Soc*. 2011; 133:7725. [PubMed: 21513357]
51. Tien J, Terfort A, Whitesides GM. *Langmuir*. 1997; 13:5349.
52. Yang J, Elim HI, Zhang Q, Lee JY, Ji W. *J Am Chem Soc*. 2006; 128:11921. [PubMed: 16953633]
53. Daniel MC, Tsvetkova IB, Quinkert ZT, Murali A, De M, Rotello VM, Kao CC, Dragnea B. *ACS Nano*. 2010; 4:3853. [PubMed: 20575505]
54. Christian DA, Tian A, Ellenbroek WG, Levental I, Rajagopal K, Janmey PA, Liu AJ, Baumgart T, Discher DE. *Nat Mater*. 2009; 8:843. [PubMed: 19734886]
55. Wertheim MS. *J Chem Phys*. 1987; 87:7323.
56. Bianchi E, Blaak R, Likos CN. *Phys Chem Chem Phys*. 2011; 13:6397. [PubMed: 21331432]
57. Kern N, Frenkel D. *J Chem Phys*. 2003; 118:9882.
58. Wilber AW, Doye JPK, Louis AA, Noya EG, Miller MA, Wong P. *J Chem Phys*. 2007; 127:085106. arXiv:0606634 [cond-mat]. [PubMed: 17764305]
59. Roberts CJ, Blanco MA. *J Phys Chem B*. 2014; 118:12599. [PubMed: 25302767]
60. Liu H, Kumar SK, Sciortino F. *J Chem Phys*. 2007; 127:084902. [PubMed: 17764289]
61. Jacobs WM, Oxtoby DW, Frenkel D. *J Chem Phys*. 2014; 140doi: 10.1063/1.4878836
62. Li X, Gunton JD, Chakrabarti A. *J Chem Phys*. 2009; 131:115101. [PubMed: 19778150]
63. Fusco D, Headd JJ, De Simone A, Wang J, Charbonneau P. *Soft Matter*. 2014; 10:290. [PubMed: 24489597]
64. Hatch HW, Mittal J, Shen VK. *J Chem Phys*. 2015; 142:164901. [PubMed: 25933785]
65. Preisler Z, Vissers T, Smallenburg F, Munaoi G, Sciortino F. *J Phys Chem B*. 2013; 117:9540. [PubMed: 23902159]
66. Noya EG, Kolovos I, Doppelbauer G, Kahl G, Bianchi E. *Soft Matter*. 2014; 10:8464. [PubMed: 25234070]
67. Bianchi E, Tartaglia P, Zaccarelli E, Sciortino F. *J Chem Phys*. 2008; 128:144504. [PubMed: 18412456]
68. Zaccarelli E, Sciortino F, Tartaglia P. *J Chem Phys*. 2007; 127:174501. [PubMed: 17994821]
69. Bianchi E, Capone B, Kahl G, Likos CN. *Faraday Discuss*. 2015; 181:123. [PubMed: 25924842]
70. Kalyuzhnyi YV, Docherty H, Cummings PT. *J Chem Phys*. 2011; 135doi: 10.1063/1.3604819
71. Yigit C, Heyda J, Dzubiella J. *J Chem Phys*. 2015; 143doi: 10.1063/1.4928077
72. Staneva I, Frenkel D. *J Chem Phys*. 2015; 143:194511. [PubMed: 26590546]
73. Schmidle H, Hall CK, Velev OD, Klapp SHL. *Soft Matter*. 2012; 8:1521.
74. Tavares J, Teixeira P. *Mol Phys*. 2011; 109:1077.

75. Kogler F, Velev OD, Hall CK, Klapp SHL. *Soft Matter*. 2015; 11:7356. [PubMed: 26278680]
76. Bianchi E, Kahl G, Likos CN. *Soft Matter*. 2011; 7:8313.
77. Bianchi E, Likos CN, Kahl G. *Nano Lett*. 2014; 14:3412. [PubMed: 24842542]
78. Wada A, Nakamura H. *Nature*. 1981; 293:757. [PubMed: 7290210]
79. Strickler SS, Gribenko AV, Gribenko AV, Keiffer TR, Tomlinson J, Reihle T, Loladze VV, Makhataдзе GI. *Biochemistry*. 2006; 45:2761. [PubMed: 16503630]
80. Shen VK, Siderius DW. *J Chem Phys*. 2014; 140:244106. [PubMed: 24985617]
81. Frenkel, D.; Smit, B. *Acad Press*. New York: 2002. p. 638
82. Vlugt TJH, Martin MG, Smit B, Siepmann JI, Krishna R. *Mol Phys*. 1998; 94:727.
83. Chen B, Siepmann JI, Oh KJ, Klein ML. *J Chem Phys*. 2001; 115:10903.
84. Yasuoka K, Matsumoto M. *J Chem Phys*. 1998; 109:8451.
85. Blanco MA, Sahin E, Li Y, Roberts CJ. *J Chem Phys*. 2011; 134:225103. [PubMed: 21682538]
86. Schaink HM, Smit JAM. *Phys Chem Chem Phys*. 2000; 2:1537.
87. Tessier PM, Lenhoff AM, Sandler SI. *Biophys J*. 2002; 82:1620. [PubMed: 11867474]
88. Moon YU, Curtis RA, Anderson CO, Blanch HW, Prausnitz JM. *J Solut Chem*. 2000; 29:699.
89. Guo B, Kao S, McDonald H, Asanov A, Combs LL, Wilson WW. *J Cryst Growth*. 1999; 196:424.
90. Singh J, Kofke D. *Phys Rev Lett*. 2004; 92:220601. [PubMed: 15245206]
91. Zollweg JA. *J Chem Phys*. 1972; 57:1021.
92. Ganzenmüller G, Patey G, Camp PJ. *Mol Phys*. 2009; 107:403.
93. Sciortino F, Giacometti A, Pastore G. *Phys Chem Chem Phys*. 2010; 12:11869. arXiv:1007.5402. [PubMed: 20714597]
94. Giacometti A, Lado F, Largo J, Pastore G, Sciortino F. *J Chem Phys*. 2010; 132:174110. [PubMed: 20459159]
95. Bianchi E, Largo J, Tartaglia P, Zaccarelli E, Sciortino F. *Phys Rev Lett*. 2006; 97:168301. arXiv: 0605701 [cond-mat]. [PubMed: 17155440]
96. Dussi S, Rovigatti L, Sciortino F. *Mol Phys*. 2013; 111:3608.
97. Errington JR, Truskett TM, Mittal J. *J Chem Phys*. 2006; 125doi: 10.1063/1.2409932
98. Felder CE, Prilusky J, Silman I, Sussman JL. *Nucleic Acids Res*. 2007; 35:512.
99. Godfrin PD, Valadez-Pérez N, Castaneda-Priego R, Wagner N, Liu Y. *Soft Matter*. 2014
100. Asherie N. *Methods*. 2004; 34:266. [PubMed: 15325646]
101. Asherie N, Lomakin A, Benedek GB. *Phys Rev Lett*. 1996; 77:4832. [PubMed: 10062642]
102. Muschol M, Rosenberger F. *J Chem Phys*. 1997; 107:1953.
103. Lettieri S, Li X, Gunton J. *Phys Rev E*. 2009; 79:031904.
104. Broide M, Tominc T, Saxowsky M. *Phys Rev E*. 1996; 53:6325.
105. Wang Y, Lomakin A, Latypov RF, Laubach JP, Hideshima T, Richardson PG, Munshi NC, Anderson KC, Benedek GB. *J Chem Phys*. 2013; 139doi: 10.1063/1.4811345
106. Katsonis P, Brandon S, Vekilov PG. *J Phys Chem B*. 2006; 110:17638. [PubMed: 16942109]
107. Platten F, Valadez-Perez NE, Castaneda-Priego R, Egelhaaf SU. *J Chem Phys*. 2015; 142doi: 10.1063/1.4919127
108. Upadhyayula S, Bao D, Millare B, Sylvia SS, Habib KMM, Ashraf K, Ferreira A, Bishop S, Bonderer R, Baqai S, Jing X, Penchev M, Ozkan M, Ozkan CS, Lake RK, Vullev VI. *J Phys Chem B*. 2011; 115:9473. [PubMed: 21682315]
109. Takashima S. *Biophys Chem*. 2001; 94:209. [PubMed: 11804731]
110. Brown DA, London E. *Biochem Biophys Res Commun*. 1997; 240:1. [PubMed: 9367871]
111. Baumgart T, Hammond AT, Sengupta P, Hess ST, Holowka DA, Baird BA, Webb WW. *Proc Natl Acad Sci*. 2007; 104:3165. [PubMed: 17360623]
112. Landau EM, Rosenbusch JP. *Proc Natl Acad Sci U S A*. 1996; 93:14532. [PubMed: 8962086]

FIG. 1.

(a) Schematic representation for the interaction between patches. Each patch is defined by its normal surface vector $\hat{\mathbf{n}}$. Two patches are considered to interact if the characteristic angle of one patch θ_a (given by the opening angle between $\hat{\mathbf{n}}_a$ and the interparticle distance $\mathbf{r}_{ij}$) intersects with that of the other patch (i.e., θ_b). (b) Illustrative example of the charge distribution for a single patch ($f(\Omega) = \cos^{\eta_a} \Omega$) for different values of η_a . Ω corresponds to the solid angle between $\hat{\mathbf{n}}_a$ and a reference frame as the particle is rotated. (c) Schematic representation of selected dipolar fluids, where the charge distribution is characterized by the effective patch size δ and the patch separation d . (d) Schematic representation of selected protein-like fluids, where the charge distribution is characterized by the magnitude of the dipole and quadrupole moments (Q_1 and Q_2 , respectively). In panels (c) and (d), blue (red) patches represent positive (negative) charges.

FIG. 2.

Phase coexistence of dipolar fluids in the T - ρ plane for different sizes and arrangement of charged patches. (a) Different patch sizes δ_a ranging from 90° to 40.7° at fixed patch separation of $d = \sigma$. (b) Different patch-patch separation distances, d , at fixed patch size of $\delta_a = 75.5^\circ$. Curves are given in normalized units with T scaled by the characteristic temperature ϵ/k_B , where k_B is the Boltzmann factor and ϵ defined in Eq. 4. ρ is normalized by the particle size σ . Error bars along the binodal curve correspond to 95% confidence intervals in calculated densities, while those in the critical point are obtained from neighbor isotherms as described in the main text.

FIG. 3.

Average cluster size as a function of reduced temperature for different dipolar fluids. Symbols correspond to the average size along each of the equilibrium phases: (squares) dense phase; and (circles) light phase. Color coding represent different dipolar fluids as indicated in the figure. Lines correspond to the average cluster size for an isotropic reference fluid (i.e., an attractive, shifted-force Lennard-Jones fluid).

FIG. 4.

(a) Dependence of the critical temperature T_c on the magnitude of the dipole moment Q_1 for dipolar fluids; inset corresponds to a magnified section of the curve. Dashed line in the inset is guide to the eye for the cases where $d/\sigma = 1$. (b) Second virial coefficient B_2 as function of Q_1 ; B_2 is evaluated at the critical temperature and reported relative to the hard-sphere value (i.e., $B_2^* = 3B_2/2\pi\sigma^3$). Symbols in both panels represent all dipolar fluids considered here: (black circles) different patch sizes at fix $d/\sigma = 1$; (red squares) different values of d/σ for $\delta_a = 75.5^\circ$; (green triangles) different values of d/σ for $\delta_a = 60^\circ$; and (blue diamonds) different values of d/σ for $\delta_a = 50.9^\circ$. Error bars corresponds to 95% confidence intervals are smaller than the size of the symbols for most systems and are not visible at the scale of the figure.

FIG. 5.

Illustrative examples of the fluid–fluid coexistence regions in the T - ρ plane for protein-like fluids. Each curve corresponds to a PLF with a different dipolar moment Q_I . Error bars along the binodal curve correspond to 95% confidence intervals in calculated densities, while those in the critical point are obtained from fitting coexistence curves to the law of rectilinear diameters.

FIG. 6.

Coexistence curves in terms of reduced temperature T/T_c and density ρ/ρ_c . Symbols correspond to the different shapes of binodal curves obtained from PLFs. Average super patch size of: (green squares) 2.5 to 4.5 patches; (red triangles) 4.5 patches; (blue circles) 6 patches; and (purple diamonds) 6 patches. Error bar corresponds to 95% confidence intervals for calculated equilibrium densities and fitted critical points.

FIG. 7.

Critical temperature T_c for protein-like fluids as a function of the magnitude of the dipole moment Q_1 . For clarity, colored symbols are provided for comparison with the different type of coexistence regions observed in Fig. 6. 95% confidence intervals are smaller than the size of the symbols and are not visible at the scale of the figure.

TABLE I

Monte Carlo trial moves and their probability of selection.

Trial	Probability
Rigid-body translation	0.35
Rigid-body rotation	0.20
Orientational-bias	0.15
Insertion or deletion	0.26
Grand canonical-Aggregation-volume bias	0.04