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Jonas Wessén, Tanmoy Pal and Hue Sun Chan

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Jonas Wessén, a) Tanmoy Pal, a) and Hue Sun Chan

AFFILIATIONS
Department of Biochemistry, University of Toronto, 1 King’s College Circle, Toronto, Ontario M5S 1A8, Canada

a)Authors to whom correspondence should be addressed: jonas.wessen@utoronto.ca and tanmoy.pal@utoronto.ca

ABSTRACT
Phase separation of several different overall neutral polyampholyte species (with zero net charge) is studied in solution with two oppositely charged ion species that can form ion pairs through an association reaction. Hereby, a field theory description of the system, which treats polyampholyte charge sequence dependent electrostatic interactions as well as excluded volume effects, is given. Interestingly, analysis of the model using random phase approximation and field theoretic simulation consistently shows evidence of a re-entrant polyampholyte phase separation at high ion concentrations when there is an overall decrease of volume upon ion association. As an illustration of the ramifications of our theoretical framework, several polyampholyte concentration vs ion concentration phase diagrams under constant temperature conditions are presented to elucidate the dependence of phase separation behavior on the polyampholyte sequence charge pattern as well as ion pair dissociation constant, volumetric effects on ion association, solvent quality, and temperature.

I. INTRODUCTION
Numerous studies in the past decade have indicated that liquid–liquid phase separation (LLPS) of intrinsically disordered proteins (IDPs) or intrinsically disordered regions (IDRs) of proteins, often in the presence of nucleic acids and folded protein domains, is a critical physical mechanism behind the formation of biologically functional membraneless organelles, such as the nucleolus, Cajal bodies, and stress granules.1–12 Electrostatics, among other multivalent interactions, is one of the major drivers of the biological LLPS because IDP/IDRs generally contain charged amino acid residues in their composition and are often polyampholytic in nature.13–16 In addition to a polyampholyte sequence’s charge pattern—or more generally its charge composition—and concentration,17–20 the phase separation propensity of a specific polyampholyte depends on the condition of the solution determined by such factors as temperature,21,22 pressure,21,23,24 pH,25 as well as the concentration and type of salts present.24,26 Because of the charged nature of the polyampholytes, electrostatic interactions from the ion pairs in the solution affect its LLPS24,26,27 and could be a controlling factor in its bio-engineering.28 In general, an oppositely charged pair of ions can stay as solvated ions or they can form a chemically distinct complex, e.g., solvent-shared ion pair and contact ion pair, through association reactions depending on the solution condition. Although the effects of ion-strength on polyampholyte LLPS have been addressed by several analytical and computational studies, the consequences of ion association have largely been unexplored.

In general, chemical reactions in biomolecular condensates are of much interest because the regulation of biochemical reactions is one of several major biologically relevant functions of biomolecular condensates.29 Indeed, several recent computational studies have addressed phase separation in chemically reactive environments. For instance, the pH dependence of LLPS was studied by Adame-Arana et al.25 in a setup where the net charge on the phase separating macromolecules is chemically coupled to the self-ionization of water. An investigation by Lin et al. elucidated the role of complex formation between the SynGAP and PSD-95 molecules in the LLPS of their mixture.30 The study by Bartolucci et al. considered both equilibrium and fuel-driven phase separation of a polymeric...
component undergoing an internal molecular transition. These studies provided valuable insights. However, they treated relevant interactions only up to mean field theory (MFT) and did not incorporate the amino acid sequence of the polyampholytes explicitly. Sequence specificity and generally phase separation driven by electrostatic interactions are inaccessible to the aforementioned MFT approaches because of the non-negligible contributions from fluctuations; however, these effects are physically and biochemically important. One of the goals of our present work is to develop theoretical approaches that allow these effects to be tackled.

Pinpointing the exact roles played by all the physical interactions affecting in vivo LLPS, or even its simplified in vitro counterpart, could be immensely difficult. For analytical and computational tractability, we consider a simple model where a polyampholyte species is phase-separating in the presence of two oppositely charged chemically reacting ion species $A^+$ and $B^-$. We assume that the concentrations of $A^+$ and $B^-$ are in thermal equilibrium with the concentration of their charge-neutral product $AB$, following the balance equation

$$A^+ + B^- \underset{K_{d}}{\overset{K_{a}}{\rightleftharpoons}} AB.$$  (1)

The dissociation/association constants $K_d/K_a$ in Eq. (1) are defined by

$$K_d = \frac{1}{K_a} = \frac{[A^+][B^-]}{[AB]}.$$  (2)

where $[X]$ is the equilibrium concentration of the species $X (= A^+, B^-, AB)$. Reaction (1) could be used to describe several chemical processes including self-ionization of water, dissociation of weak organic acids (e.g., formic acid, acetic acid, or carbonic acid) in the solution, and ion association in concentrated solutions of electrolytes at physical temperatures, in non-polarity solvents, or at low temperatures. The dissociation constant $K_d$ of a chemical reaction is an experimentally measurable observable whose value can indicate the chemical state of the solution. If the initial reactant concentrations are lower than $K_d$, most of the ion pairs are expected to be in the dissolved state, which will result in screening of the polyampholyte’s electrostatic interactions. On the other hand, when the reactant concentrations are considerably higher than $K_d$, most of the ion pairs are expected to be in the charge-neutral complex state, which will affect the configuration entropy of the polyampholyte by modulating the effective excluded volume through steric repulsion. In addition, ion pair association is often accompanied by a change in volume. Any such volume change might have an important effect—in addition to the electrostatic screening effects of the ions—on polyampholyte conformation at high reactant concentrations. Thus, the chemical state of the solution determined by Eq. (1) has the potential to dictate LLPS behavior of the polyampholyte species.

At high salt concentrations, non-electrostatic interactions are expected to play a major role in determining LLPS behavior. Indeed, in a recent explicit chain molecular dynamics (MD) study, phase separation behavior of several proteins at high salt concentrations was attributed to non-electrostatic interactions, such as hydrophobic interactions. In a different study, the importance of the excluded volume interaction from polyethylene glycol (PEG) crowding agents was highlighted in a system with a phase separating protein that lacks hydrophobic amino acids in its sequence. A model that includes both ion association along with any volume change upon ion association and explicit residue level electrostatics of the phase separating polyampholyte thus offers a unique possibility of capturing many of the diverse results mentioned above in a unified setup, at least qualitatively.

With that in mind, here, we adopt a trade-off between complexity and analytical/computational tractability by introducing a simple field theory model where molecular species in the model interact via excluded volume and explicit sequence dependent electrostatic interactions. Specifically, we introduce a bare dissociation constant (corresponding to the dissociation constant in a solution consisting only of $A^+$, $B^-$, and $AB$) as a control parameter for the non-electrostatic energy gain associated with ion pairing. To account for the volume change upon association, we introduce further a relative excluded volume factor γ of the product AB in Eq. (1) with respect to the reactants A and B. We study the model using MFT, random phase approximation (RPA)—where relevant Gaussian level fluctuations are included above the MFT, and fully fluctuating field theoretic simulation (FTS). We expect the model to be useful as a base for studying specific systems with suitable modifications.

The structure of this article is as follows. In Sec. II, we introduce our model and derive its corresponding field theory representation. The model is studied analytically in Sec. III using MFT and RPA, and then using FTS in Sec. IV. Numerical results obtained from the approximate analytical calculations and from FTS are shown and compared in Sec. V, and concluding remarks are given in Sec. VI.

II. MODEL DEFINITION

Our system of consideration contains $n_p$ linear polymers of identical composition, each consisting of $N$ residues with electric charges $\sigma_\alpha$, $\alpha = 1, \ldots, N$. Effects from solvent molecules are assumed to be implicitly encoded in the microscopic interaction parameters. All electric charges in this work are given in units of the elementary protonic charge, and we restrict our calculations to polymers with zero net electric charge, $\sum_{\alpha=1}^{N} \sigma_\alpha = 0$. The “K”–“E” sequences [Fig. 1(b)] considered here are representative of the “sv” sequences used extensively to study LLPS in computational models. An analytically derived single chain property, the sequence charge decoration (SCD) parameter (defined as $\text{SCD} = \sum_{\beta=1}^{N} \sum_{\alpha=1}^{N} \sigma_\beta \sigma_\alpha \sqrt{(\beta - \alpha)}/N$), could be used to discriminate between otherwise charge neutral “sv” sequences. The phase separation propensity trends of the “sv” sequences are known to correlate well with their SCD parameter values. A “K”–“E” sequence $((K_\alpha E_\alpha))$ with a SCD value of $-4.290$ has recently been seen to be phase separating in an in vitro experiment. Compared to that, the SCD values of the sequences studied here are $-2.098$, $-4.349$, and $-7.374$, respectively, for sv10, sv15, and sv20. However, we emphasize that the model presented here is not intended to be quantitatively accurate given that we ignore some effects that are present in experimental systems, such as the possibility of ion condensation onto the polymer residues due to attractive interactions beyond the simple Coulomb forces. Consequently, while the
trend predicted by our theory is expected to hold for corresponding experimental results, there are uncertainties in mapping the theoretical variables, such as temperature, to their experimental counterparts for individual systems.

The system further contains equal amounts of positive and negative model ions (denoted A⁺ and B⁻, respectively) that can undergo a pairwise chemical reaction forming a neutral complex AB according to Eq. (1). The number of A/B pairs in the dissociated and bound state, n_A (= n_B) and n_{AB}, respectively, is thus constrained according to

\[ n_A + n_{AB} = n, \]  

(3)

where \( n \) denotes the total number of A and B units in the system.

The position of bead \( a \) on polymer \( i = 1, \ldots, n \) is denoted \( r_{ia} \), while the positions of the A⁺, B⁻, and AB particles are represented by \( \{r_{A,i}\}_{i=1}^{n_A}, \{r_{B,i}\}_{i=1}^{n_B}, \) and \( \{r_{AB,i}\}_{i=1}^{n_{AB}} \), respectively. Quantities that depend explicitly on molecule positions are indicated by a hat (e.g., \( \hat{H} \)). All energies in this work are given in units of the thermal energy \( k_B T \). The total Hamiltonian is \( \hat{H} = \hat{H}_0 + \hat{H}_1 + \hat{H}_2 + \hat{H}_3 \), where

\[
\begin{align*}
\hat{H}_0 &= \frac{3}{2b^2} \sum_{i=1}^{n} \frac{1}{a_i} \left( R_{ia+1} - R_{ia} \right)^2, \\
\hat{H}_1 &= \frac{1}{2} \int d\rho(r)^2, \\
\hat{H}_2 &= \frac{l_0}{2} \int d\rho \left( \frac{\partial}{\partial r} \cdot \frac{\partial}{\partial r} \right) \rho(r) \rho(r) \\
\hat{H}_3 &= (n - n_{AB}) \epsilon_{\text{bound}},
\end{align*}
\]

(4)

These terms provide, respectively, the energies associated with harmonic chain connectivity, excluded volume repulsion, and electrostatic interactions. Here, \( b \) is the polymer segment length, \( v \) is the excluded volume parameter, and \( l_0 = e^2/4\pi\varepsilon_0 k_B T \) is the Bjerrum length (\( v \) and \( l_0 \) modulate the strengths of the excluded volume and electrostatic interactions, respectively). We use the segment length \( b \) as our unit of length, and thus, values of all dimensionful quantities (those that carry a physical unit) are given in powers of \( b \). We have expressed \( \hat{H}_{1,2} \) in terms of the microscopic particle densities and charge densities, defined respectively as

\[ \hat{\rho}(r) = \hat{\rho}_A(r) + \hat{\rho}_B(r) + \hat{\rho}_{AB}(r), \]  

(5)

\[ \hat{\epsilon}(r) = \hat{\epsilon}_A(r) + \hat{\epsilon}_B(r) - \hat{\epsilon}_{AB}(r), \]  

(6)

where \( \hat{\rho}_A(r) = \sum_{a} \Gamma(r - r_{ia}), \hat{\epsilon}_A(r) = \sum_{a} \alpha_a \Gamma(r - r_{ia}), \hat{\rho}_B(r) = \sum_{b} \Gamma(r - r_{ib}), \) and \( \hat{\epsilon}_B(r) = \sum_{b} \gamma_a \Gamma(r - r_{ib}) \). The resulting AB–AB and AB–[other monomer] excluded volume interaction strengths are scaled by the same powers of \( \alpha \) and \( \gamma \), respectively, to their dissociated counterparts. The Gaussian distributions \( \Gamma(r) = e^{-r^2/2a^2}/(2\pi a^2)^{1/2} \) with smearing length \( a = b/\sqrt{6} \) to regulate ultraviolet divergences arising from contact excluded volume and electrostatic contact interactions.\(^{41,52}\) This Gaussian smearing procedure has also been shown to remedy unphysical binding behavior in models of highly concentrated polyelectrolyte solutions that combine equilibrium constants with Debye–Hückel treatment of electrostatic interactions.\(^{46}\)

In this work, we view the Gaussian smearing as a part of the model definition, rather than as an approximation. While leaving the long-range behavior of inter-particle forces unaffected, the Gaussian smearing results in short-range interactions that are considerably "softer" than typical hard-sphere or Lennard-Jones particle representations.\(^{53}\) Consequently, the model is not expected to capture microscopic phenomena, such as strongly oscillating radial distribution functions, on short distance scales that typically characterize liquid phases.\(^{54}\)

The parameter \( \gamma \) in Eq. (5) may be interpreted through the effective volume of the AB state relative to its dissociated counterpart, with the sign of \( \gamma - 2 \) corresponding to the sign of \( \text{vol}(\text{AB}) - \text{vol}(\text{A}^+\text{}\!-) - \text{vol}(\text{B}^-\text{}\!-) \) as illustrated schematically in Fig. 1(b). The resulting AB–AB and AB–[other monomer] excluded volume interaction strengths are scaled by \( \gamma \) and \( \gamma_i \), respectively, to their dissociated counterparts. The MFT level of an analogous model that takes the AB bound state as an independent system component, these excluded volume interactions result in effective \( \chi \)-parameters that are scaled by the same powers of \( \gamma \) and \( \gamma_i \), respectively. The framework is, therefore, related to other approaches in the literature,\(^{55-57}\) where effective ion size effects are modeled through appropriate \( \chi \)-parameter variations. Implications of effective ion sizes on counterion condensation have also been considered within the context of polyelectrolyte complex coacervation.\(^{58}\)

The final piece of the Hamiltonian, \( \hat{H}_3 \), models the chemical binding of the AB complex,

\[ \hat{H}_3 = (n - n_{AB}) \epsilon_{\text{bound}}, \]  

(7)

where \( \epsilon_{\text{bound}} \) is the decrement in free energy associated with a single AB binding, in addition to electrostatic and excluded volume contributions coming from \( \hat{H}_{1,2} \). Note that \( \epsilon_{\text{bound}} \) may include both an energetic component as well as an entropic component related, e.g., to changes in the number of configuration states, or the entropy release from reduced electrostriction of nearby solvent molecules.\(^{59}\)

In the remainder of this article, we find it convenient to trade \( \epsilon_{\text{bound}} \) in favor of another parameter \( K_0 \), defined as

\[ K_0 = \frac{e^{-\epsilon_{\text{bound}}}}{l_0^3}, \]  

(8)
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The Journal defined through the proton activity parameter rather than concentration, as shown in Sec. III B. The partition function of the system,

\[ Z = \sum_{n_{AB}=0}^{n_B} \frac{\langle \mathrm{d}R \rangle \langle \mathrm{d}r_A \rangle \langle \mathrm{d}r_B \rangle \langle \mathrm{d}r_{AB} \rangle}{V^{-n_{AB}}(n_{AB})^2!} e^{-\beta H}, \tag{9} \]

is expressed as a sum over the number of paired ions \( n_{AB} \), where \( n_{AB} \) is constrained to \( n_T - n_{AB} \) according to Eq. (3) and \( V = V/\lambda_0^3 \). In Eq. (9), we use a short-hand notation \( \langle \mathrm{d}R \rangle \equiv \prod_{i=1}^{n_T} \langle \mathrm{d}R_i \rangle \) and \( \langle \mathrm{d}r_i \rangle \equiv \prod_{i=1}^{n_T} \langle \mathrm{d}r_i \rangle \) (for \( j = A, B, AB \)) to indicate that the integrals are performed over the positions of all polymer beads, solvated ions, and bound ion pairs. The factorials in the denominator follow from the fact that the polymers and the A* - B* and AB solute are separately indistinguishable. Note that the particular combination \( n_A!^3 \langle n_{AB} \rangle \) implies that a bound state AB is distinguishable from any configuration of a free A* - B* pair.

The logarithm of the partition function in Eq. (9) can be used to define a free energy density \( f(\rho_A, \rho_T; K_0, n_T, \ldots) = -(\ln Z)/V \), where \( V \) is the system volume, \( \rho_A = n_A/N^3 \) is the bulk polymer bead number density, and \( \rho_T = n_T/V \) is the total bulk number density of A and B ions. We can compute the average number density of solvated ions \( [A^+] = [B^-] = \langle n_{AB} \rangle / V \) through the first derivative of \( f \) with respect to \( K_0 \),

\[ [A^+] = -K_0 \frac{\partial f}{\partial K_0} \tag{10} \]

The constraint (3) then gives the average number density of bound ion pairs as \( [AB] = \rho_T - [A^+] \). Knowledge of \( [A^+] \) and \( [AB] \) can subsequently be used to compute the dissociation constant \( K_d \) according to Eq. (2). Other thermodynamic quantities of interest to this work are the polymer chain and ion pair chemical potentials, \( \mu_A = \partial f/\partial \rho_A \) and \( \mu_T = \partial f/\partial \rho_T \), respectively, and the osmotic pressure \( \Pi = \rho_A \mu_A - f \).

Note that \( K_0 \) in Eq. (2), in principle, depends on how the bound state concentration \( [AB] \) is defined. In this work, we consider the contributing AB states as chemically distinct (e.g., through the appearance of a covalent bond between A and B), an ionic bond between A* and B* with strength beyond that of simple electrostatic attraction, or some other mechanisms) from any configuration of free A* and B* molecules, as follows from the relation in Eq. (10) combined with the factorials in the denominator in Eq. (9). If \( [AB] \) is measured by exploiting the electrostatic properties of the A* - B* solution, rather than the chemical nature of the AB bond, a different definition of \( [AB] \) might be more appropriate where nearby A* and B* molecules are counted as effectively bound. This would be better captured by defining \( [A^+] \) and \( [B^-] \) through their corresponding activity parameters, similarly to how pH is formally defined through the proton activity parameter rather than concentration. For simplicity, this alternative approach is not pursued further in this work.

### A. Deriving the field theory

The basis of the analytical calculations and lattice simulations is a field representation of the partition function in Eq. (9). To derive its corresponding field theory, we first decouple the interaction terms in \( H_1 \) and \( H_2 \) through standard Hubbard-Stratonovich transformations. This introduces two fields \( w(r) \) and \( \psi(r) \), conjugate to \( \partial w/\partial K_0 \) and \( \partial \psi/\partial K_0 \), respectively, and leads to

\[ Z = \int \mathcal{D}w \mathcal{D}\psi Q_p \left\{ Q_p, Q_w \right\} e^{-W[w,\psi]} S[w, \psi] \tag{11} \]

up to an inconsequential multiplicative constant, where \( H_0[w, \psi] = \int \mathcal{D}w \mathcal{D}\psi \left( \frac{w^2}{2V} + (\nabla \psi)^2/8\pi l_B^3 \right) \). The factor \( S[w, \psi] \) contains the sum over \( n_{AB} \),

\[ S = \sum_{n_{AB}=0}^{n_R} \frac{1}{n_{AB}!} \frac{1}{(n_T - n_{AB})!} \left( \frac{V^{-1}Q_{AB}^\dagger}{K_0Q_p} \right)^{n_{AB}}. \tag{12} \]

In the above expressions, \( Q_i = Q_i[w(r), \psi(r)] \) for \( i = p, A, B, AB \) is the partition function for a type \( i \) molecule in the presence of chemical and electrostatic potential fields \( w(r) \equiv \Gamma \ast w(r) \) and \( \psi(r) \equiv \Gamma \ast \psi(r) \) (where * denotes spatial convolution, i.e., \( \Gamma \ast \phi(r) \equiv \int \mathcal{D}r \Gamma(r - r') \phi(r') \) for any function \( \phi(r) \)). For our model components, we have

\[ Q_p = \int \mathcal{D}r_1 \ldots \mathcal{D}r_n \left\{ e^{-\frac{\lambda}{2\pi} \sum_{i=1}^n \Delta R_{i+1}^2 + \lambda w(r_i)} \right\} \left( \frac{V}{(2\pi b^2/3)^{3/2}} \right)^{1/2}, \tag{13} \]

where \( \Delta R_{i+1} \equiv R_{i+1} - R_i \), \( \tilde{W}_i \equiv \tilde{w}(R_i) + \sigma \tilde{w}(R_i) \), and

\[ Q_{AB} = \frac{1}{V} \int \mathcal{D}r e^{-\lambda w(r)} \psi(r) \], \[ Q_A = \frac{1}{V} \int \mathcal{D}r e^{-\lambda w(r)} \psi(r), \] \[ Q_B = \frac{1}{V} \int \mathcal{D}r e^{-\lambda w(r)} \psi(r). \tag{14} \]

Note that all \( Q_i \) are normalized to \( Q_i[0,0] = 1 \). The factors \( Z_w \equiv \int \mathcal{D}w \exp \left( -\int \mathcal{D}w \mathcal{D}\psi \mathcal{D}\psi \mathcal{D}w \mathcal{D}\psi \mathcal{D}w \mathcal{D}\psi \right) \) and \( Z_p \equiv \int \mathcal{D}w \exp \left( -\int \mathcal{D}w \mathcal{D}\psi \right) \) have been inserted to provide additive density-independent contributions to the osmotic pressure that cancels the divergent contributions of the \( |k| > a^{-1} \) modes of the functional integrals in Eq. (11).\[52,61\]

In the thermodynamic limit, the sum over \( n_{AB} \) in \( S \) may be replaced by an integral that can be solved in the saddle-point approximation (this step is shown in Appendix A). The resulting field picture representation of the partition function becomes

\[ Z = \left[ \frac{\int \mathcal{D}w \mathcal{D}\psi e^{-\beta H[w,\psi]} Z_w Z_p}{Z_w Z_p} \right], \tag{15} \]

where the field Hamiltonian is

\[ H[w, \psi] = H_0 - n_T \ln Q_p - n_T \ln Q_{AB} + n_T h_0(x). \tag{16} \]
Here, we have introduced a field operator \( x[w, \psi] \),
\[
x[w, \psi] \equiv \frac{2\phi}{K_0} \frac{Q_{AB}}{Q_{A} Q_{B}},
\]
and the function
\[
h_0(x) = \ln \left( 1 - \frac{\sqrt{1 + 2x} - 1}{x} \right) - \sqrt{1 + 2x} - 1.
\]
In the following, we find it useful to define two additional functions, \( h_1(x) \equiv x h_0(x) \) and \( h_2(x) \equiv x h_1'(x) \), where, in particular,
\[
h_1(x) = \sqrt{1 + 2x} - 1
\]
satisfies \( 0 \leq h_1(x) \leq 1 \) for any real \( x \geq 0 \) [similarly, it may be shown that \( h_2(x) \leq 0 \)]. The physical interpretation of the field operator \( h_1(x[\bar{w}, \psi]) \) becomes clear when applying the relation in Eq. (10) to the field partition function in Eq. (15), leading to
\[
\left[ A^* \right] = \left\langle h_1(x) \right\rangle_{\bar{\gamma}},
\]
i.e., \( h_1(x[\bar{w}, \psi]) \) is a field operator that averages to the fraction of dissociated A/B pairs. Correspondingly, \([AB] = \left\langle \bar{\gamma}_T [1 - \left\langle h_1(x) \right\rangle] \right\rangle \) gives the number density of the bound ion species. The field averaged \( h_1(x[\bar{w}, \psi]) \) can, therefore, be used to evaluate \( K_d \) in Eq. (2),
\[
K_d = \left\langle h_1 \right\rangle_T^2 \left( 1 - \left\langle h_1 \right\rangle \right).
\]

### III. ANALYTICAL THEORY

To understand the physical implications of our model in Eq. (9), we now proceed to approximately evaluate the functional integrals in the field representation of the partition function in Eq. (15). The mean field theory (MFT) solution, which only accounts for the spatially homogeneous field configurations, captures the dominant effects on ion dissociation from excluded volume interactions and, in particular, showcases the crucial role of the parameter \( \gamma \). However, effects from electrostatic interactions are entirely given by fluctuations of the charge-conjugate field \( \psi(r) \), which are not accounted for in MFT. To capture the leading-order electrostatic effects, we next evaluate Eq. (15) in the random phase approximation (RPA). This accounts for Gaussian field fluctuations described by the expansion of \( H[w, \psi] \) truncated beyond quadratic order in \( w(r) \) and \( \psi(r) \) (corresponding to fluctuations in density and charge, respectively).

### A. Mean field theory

A spatially homogeneous \( \psi(r) = \bar{\psi} \) does not contribute in the field Hamiltonian in Eq. (16) due to the overall charge neutrality of the system and hence does not contribute in MFT. The MFT solution for \( w(r) = \bar{w} \), given by the vanishing first derivative of \( H[w, 0] \), satisfies
\[
\bar{w} = \psi \bar{\rho}_{\text{tot}},
\]
where
\[
\bar{\rho}_{\text{tot}} = \bar{\rho}_B + \left[ 2\bar{h}_1 + \gamma(1 - \bar{h}_1) \right] \bar{\rho}_T.
\]
is the total concentration in MFT (counting each AB complex as \( \gamma \) units), and \( \bar{h}_1 \equiv h_1(\bar{w}[0]) \) with \( \bar{w}[0] = 2\pi e^{(2-\gamma)\bar{w}}/K_0 \). The exponential dependence of \( \bar{h}_1 \) on \( \bar{w} \) means that Eq. (22) generally lacks a closed-form solution, except for in the special case when \( \gamma = 2 \), and therefore has to be solved numerically for each given set of parameter values.

Setting \( \left\langle h_1 \right\rangle = \bar{h}_1 \) in Eq. (21) gives the MFT expression for the dissociation constant, which may be simplified to
\[
\bar{K}_d = K_0 \exp \left( (y - 2) v \bar{\rho}_{\text{tot}} \right).
\]
In the dilute limit, \( \bar{\rho}_B, \bar{\rho}_T \rightarrow 0 \), this expression reduces to \( \bar{K}_d = K_0 \), verifying the claim in Sec. II that the parameter \( K_0 \) is the dilute-limit MFT dissociation constant. In the opposite limit, where either \( \bar{\rho}_B \) or \( \bar{\rho}_T \) is large, the dissociation constant instead becomes either exponentially suppressed (for \( \gamma < 2 \)) or exponentially enhanced (for \( \gamma > 2 \)) at densities \( \geq (\gamma - 2)^{-1} \). Physically, this may be interpreted as the bound AB state being either favored or disfavored in a dense system depending on if it yields favorable excluded volume interactions compared to the dissociated state \( A^* / B^{-} \). At \( y = 2 \), the MFT dissociation constant is density independent.

The MFT evaluation of the functional integrals \( Z_w^{-1} Z_{\bar{w}}^{-1} \int \mathcal{D}w \int \mathcal{D}\bar{w} \exp(-H[w, \psi]) = \exp(-H[\bar{w}, 0]) \) leads to the following expression for the free energy density:
\[
\hat{f} = -s_0 + \bar{\mu}_T \bar{h}_0 + \frac{v}{2} \bar{\rho}_{\text{tot}} \left[ \bar{\rho}_{\text{tot}} - 2(2 - y) \bar{h}_1 \bar{\rho}_T \right],
\]
where \( \bar{h}_0 \equiv h_0(\bar{w}[0]) \) and
\[
s_0 = \frac{1}{V} \ln \frac{V^{n_{+} n_{-}}}{n_{+}! n_{-}!},
\]
is the configuration entropy density for a system with fully associated ions. The MFT chemical potentials and osmotic pressure that follow are
\[
\bar{\mu}_p = \ln \frac{\bar{\rho}_B}{N} + v N \bar{\rho}_{\text{tot}},
\]
\[
\bar{\mu}_T = \ln \left[ \bar{\rho}_T \left( 1 - \bar{h}_1 \right) \right] + v \bar{\rho}_{\text{tot}},
\]
\[
\bar{\Pi} = \frac{\bar{\rho}_B}{N} \left( 1 + \bar{h}_1 \right) \bar{\rho}_T + \frac{v}{2} \bar{\rho}_{\text{tot}}.
\]

### B. Gaussian fluctuations: Random phase approximation

The Gaussian field fluctuations can be accounted for by expanding the field Hamiltonian \( H[w, \psi] \) in Eq. (16) to quadratic order about the MFT solution and then performing the resulting Gaussian functional integrals in the partition function. This leads to the following correction term to the free energy density:
\[
f = \hat{f} + \frac{1}{2} \int \frac{dk}{(2\pi)^2} \ln \left[ \frac{4\pi h_{\psi}^2}{k^2} \right] \det G(k),
\]
where \( G(k) \) is the \( 2 \times 2 \) matrix with the Fourier representation of the coefficients of the quadratic terms in the expansion of \( H[w, \psi] \) and the factor \( 4\pi h_{\psi}^2/k^2 \) comes from the product \( Z_w Z_{\bar{w}} \) in the denominator of Eq. (15). Using the field basis \( (w, \psi) \), we may write
\[
G(k) = C_0(k) + \bar{\rho}_B C_0(k) + \bar{\rho}_T C_T(k),
\]
where \( C_{0}(k) = \text{diag}(1/v, k^2/4\pi b) \), \( C_{r}(0) = \text{diag}(-(2 - \gamma)^2h_2, 0) \), \( C_{r}(k \neq 0) = \hat{I}(k)^2 \text{diag}(y^2 + (2 - \gamma)h_2, 2h_1) \), \( C_{p}(0) = \text{diag}(0, 0) \), and
\[
C_{p}(k \neq 0) = \hat{I}(k) \begin{pmatrix} g_{mm}(k) & g_{mc}(k) \\ g_{mc}(k) & g_{cc}(k) \end{pmatrix}.
\]

The entries of \( C_{p}(k \neq 0) \) are the standard single chain monomer density-monomer density, monomer density-charge and charge-charge correlations following from the expansion of \( Q_{g} \), i.e.,
\[
\bar{g}_{mm}(k) = \sum_{n=0}^{\infty} \sum_{j;a} \bar{g}_{mm}(\gamma - a - \beta)k^{a} e^{-\beta k^{2}}/N, \quad \bar{g}_{mc}(k) = \sum_{n=0}^{\infty} \sum_{j;a} \bar{g}_{mc}(\gamma - a - \beta)k^{a} e^{-\beta k^{2}}/N, \quad \text{and} \quad \bar{g}_{cc}(k) = \sum_{n=0}^{\infty} \sum_{j;a} \bar{g}_{cc}(\gamma - a - \beta)k^{a} e^{-\beta k^{2}}/N.
\]

In the above expressions, \( \hat{I}(k) = e^{-\beta k^{2}/2} \) is the Fourier transformation of the Gaussian smearing function \( I(r) \).

The derivatives of Eq. (25) with respect to species numbers, volume, and \( K_{0} \) yield corrections from potential field fluctuations to the chemical potentials, osmotic pressure, and fraction of dissociated ion pairs \( \mu_{p} = \mu_{B} + \mu_{B}^{(0)} \), \( \mu_{B} = \mu_{B} + \mu_{B}^{(0)} \), \( \mu_{T} = \mu_{T} + \mu_{T}^{(0)} \), \( \Pi = \Pi + \Pi^{(0)} \), \( \langle h_{i}(x) \rangle = h_{i} + h_{i}^{(0)} \), respectively. The full expressions for the RPA corrections \( x^{(d)} \) are given in Appendix B.

The RPA contributions to our thermodynamic observables of interest all involve integrals over wave numbers \( k \) that generally need to be computed numerically. A special case that can be treated fully analytically is the dilute limit of \( h_{i}^{(d)} \), leading to the following RPA expression for the dilute limit dissociation constant:
\[
\lim_{\rho_{B} \rho_{A} \to 0} K_{0} = K_{0} \left( \frac{1 + (2 - \gamma)w}{16\pi^{3/2}a^3} + \frac{h_{B}}{\sqrt{\pi a}} \right)^{-1}.
\]

This shows that the bare parameter \( K_{0} \) can no longer be interpreted as the dilute limit dissociation constant when fluctuations are included in the calculation.

**IV. FIELD THEORY SIMULATIONS**

We complement our findings from RPA calculations with field theoretical simulations (FTSs) that fully capture the fluctuations of the fields \( w(r) \) and \( \psi(r) \). Other alternative simulation approaches to biomolecular LLPS include explicit chain MD simulations \cite{42,43,44,45} and finite-size scaling theory \cite{64,65}. In FTS, equilibrium evolution of the system dictated by the Hamiltonian Eq. (16) is studied by following a complex Langevin (CL) prescription, \cite{66,67,68,69} where the real-valued continuous fields \( w \) and \( \psi \) are analytically continued to their respective complex planes and evolved in the fictitious CL time through the equations given by
\[
\begin{align*}
\frac{\partial w(r, t)}{\partial t} & = - i \bar{\rho}(r, t) w(r, t) + \eta_{w}(r, t), \\
\frac{\partial \psi(r, t)}{\partial t} & = - i \bar{\psi}(r, t) \bar{\psi}(r, t) + \eta_{\psi}(r, t).
\end{align*}
\]

This allows ensemble averages to be computed as asymptotic CL time averages. In Eq. (27), \( \eta_{w} \) and \( \eta_{\psi} \) are real valued random numbers with zero mean and variance \( 2\delta(r - r')\delta(t - t') \). The field operators for the number and charge densities, \( \rho \equiv \rho_{B} + \rho_{A} + \rho_{B} + \rho_{AB} \) and \( \xi \equiv \xi_{B} + \xi_{A} - \rho_{B} \), respectively, are obtained from
\[
\frac{\partial \rho_{B}(r, t)}{\partial t} = i\rho_{B} \frac{\delta \ln Q_{R}}{\delta w(r, t)} \quad \text{and} \quad \frac{\partial \xi_{B}(r, t)}{\partial t} = i\xi_{B} \frac{\delta \ln Q_{R}}{\delta \psi(r, t)}
\]
for polymers and
\[
\frac{\partial \rho_{B}(r, t)}{\partial t} = h_{1}(x) i\rho_{B} \frac{\delta \ln Q_{R}}{\delta w(r, t)} + i \cdot A, B,
\]
\[
\frac{\partial \rho_{AB}(r, t)}{\partial t} = [1 - h_{1}(x)] i\rho_{B} \frac{\delta \ln Q_{R}}{\delta w(r, t)}
\]
for ions, where \( x[w, \psi] \) is defined in Eq. (17). Detailed expressions for the above density operators can be found in Appendix C. Note that contributions from field fluctuations up to all orders are kept in Eqs. (28) and (29). We numerically solve Eq. (27) on a cubic lattice with periodic boundary conditions and lattice spacing \( \Delta \) using a semi-implicit first-order time stepping method \cite{37} with a CL time step \( \Delta t = 10^{-3} \). Use of the semi-implicit time stepping method results in significantly better numerical stability compared to the Euler–Maruyama type explicit time-discretization methods. As with the CL-time discretization, the spatial discretization of the continuous fields \( w \) and \( \psi \) is an approximation, and, formally, FTS reproduces exactly the continuum field theory in the limit of \( \Delta x \to 0 \). However, the Gaussian smearing already provides a strong exponential suppression of contributions from field fluctuations on distance scales smaller than the smearing length \( a \) such that these modes may be omitted with a negligible numerical effect on physical observables. In this work, we, therefore, set \( \Delta x = a \) in all FTS computations.

Thermally averaged bulk densities of solvated and bound ion concentrations can be obtained from the field averaged value of \( h_{i}(x) \). Information about how the components are spatially distributed in the system can be gleaned from potential of mean forces (PMFs). The PMF \( U_{i,j}(r) \) between two components \( i \) and \( j \) describes the free-energy landscape for the separation \( r \) between two units of \( i \) and \( j \) and is related to the corresponding radial distribution function (RDF) \( g_{ij}(r) \) through the relation
\[
U_{i,j}(r) = -\ln g_{ij}(r).
\]

Here, \( U_{i,j}(r) \) is given in units of the thermal energy \( k_{B}T \). Since explicit particle coordinates have been traded for the field degrees of freedom in the field picture, RDFs have to be computed through their field operators defined by
\[
g_{ij}(r') = \frac{1}{\rho_{B} \rho_{B}^{(0)}} \left[ \sum_{r} \left( \frac{\rho_{B}(r)[\rho_{B}(r')]}{4\pi r^{3}} \right) \right] - 1 e^{(-r')^{2}/a^{2}} \quad i = A, B, AB,
\]
\[
g_{ij}(r') = \frac{1}{2\rho_{B} \rho_{B}^{(0)}} \left[ \rho_{B}(r) \rho_{B}(r') \right] \quad i = A, B, AB,
\]
and
\[
g_{ij}(r') = \frac{1}{\rho_{B} \rho_{B}^{(0)}} \left[ \rho_{B}(r) \rho_{B}(r') \right] \quad \rho_{B} \rho_{B}^{(0)}(1 - \langle h_{1}(x) \rangle),
\]

156, 194903-6
where the subscripts p, S, and B on the RDFs stand for polymer bead, solvated, and bound, respectively. The last term in the expression for $g_{pp}(r)$ subtracts the contribution from the polymer bead with itself. The RDFs in Eq. (31) have been normalized to $g_{ij} = 1$ when units of $i$ and $j$ are uncorrelated, which is expected, e.g., at large $r$ if the system contains a single liquid phase. In Eq. (31), the factor $\rho_b = \rho_0 - N/V$ provides the correct finite-volume correction to the polyampholyte–polyampholyte RDF normalization and approaches $\rho_b$ at large $V$.

If the system is in a globally inhomogeneous state, e.g., by containing several co-existing macro-phases, certain PMFs may approach non-zero values at large separations $r$. In particular, the large $r$ behavior of the polyampholyte–polyampholyte PMF $U_{pp}(r)$ indicates if the polymers are homogeneously distributed on large scales or are concentrated in, e.g., a dense droplet. The information of the influences of the solvated ions and the neutral ion pairs on phase separation, on the other hand, is obtained from the polyampholyte–solvated ions and polyampholyte–ion PMFs, respectively.

V. RESULTS

The effects on ion pairing from the solution condition can be understood from Fig. 2, showing $K_d$ and $[A^+][B^-]$ computed in RPA and FTS as a function of $\rho_T$ at $\gamma = 1.5, 2.0$, and 2.5 in polymer empty ($\rho_b = 0$) and dense ($\rho_b = 5b^{-3}$) systems with polyampholyte species sv15. The FTS calculations were done on a 32$^3$ lattice. The phase diagrams in Fig. 3 display strong dependence on $I_b$ and $\gamma$ and, in particular, predict the possibility of re-entrant phase separation with increasing $\rho_T$. A high $\rho_T$ re-entrant phase separation region occurs at $I_b \approx 0.6b$ for $\gamma = 1.5$, but vanishes for $\gamma \geq 2$, which can be understood from the preferred ion pairing state in the dense system. As can be seen from Fig. 2, for $\gamma = 1.5$, a large $\rho_T$ strongly favors the charge neutral bound state AB, which only interacts through excluded volume repulsion. The resulting crowding effects lead to an effective attraction between the chains that further promotes phase separation, which enables the re-entrant phase separation. At $\gamma = 2.5$, the high $\rho_T$ system contains almost exclusively solvated ions that strongly screen out the polymer electrostatic interactions, thus inhibiting phase separation. At the boundary value $\gamma = 2$, although the high $\rho_T$ state still contains a substantial amount of bound ions, the small amount of solvated ions (behaving roughly as $[A^+] \sim \sqrt{I_b}$) is enough to dissolve the condensates. When increasing $I_b$, the two disconnected co-existence regions at $\gamma = 1.5$ merge into one. Note that the considered polyampholyte species phase separates for both the $I_b$ values used in Fig. 3 in the absence of any ions.

All tie-lines in Fig. 3 are roughly horizontal, but tend to have slightly positive and negative slopes (indicated by $t^+$ and $j^-$) when

\begin{equation}
\rho_b = \frac{1}{V} \int \rho_b \, dV
\end{equation}

The phase diagrams in Fig. 3 display strong dependence on $I_b$ and $\gamma$ and, in particular, predict the possibility of re-entrant phase separation with increasing $\rho_T$. A high $\rho_T$ re-entrant phase separation region occurs at $I_b \approx 0.6b$ for $\gamma = 1.5$, but vanishes for $\gamma \geq 2$, which can be understood from the preferred ion pairing state in the dense system. As can be seen from Fig. 2, for $\gamma = 1.5$, a large $\rho_T$ strongly favors the charge neutral bound state AB, which only interacts through excluded volume repulsion. The resulting crowding effects lead to an effective attraction between the chains that further promotes phase separation, which enables the re-entrant phase separation. At $\gamma = 2.5$, the high $\rho_T$ system contains almost exclusively solvated ions that strongly screen out the polymer electrostatic interactions, thus inhibiting phase separation. At the boundary value $\gamma = 2$, although the high $\rho_T$ state still contains a substantial amount of bound ions, the small amount of solvated ions (behaving roughly as $[A^+] \sim \sqrt{I_b}$) is enough to dissolve the condensates. When increasing $I_b$, the two disconnected co-existence regions at $\gamma = 1.5$ merge into one. Note that the considered polyampholyte species phase separates for both the $I_b$ values used in Fig. 3 in the absence of any ions.

All tie-lines in Fig. 3 are roughly horizontal, but tend to have slightly positive and negative slopes (indicated by $t^+$ and $j^-$) when

\begin{equation}
\rho_b = \frac{1}{V} \int \rho_b \, dV
\end{equation}
the ions are dominantly solvated or exist in the bound state, respectively. This is exemplified in Fig. 4, where we show the tie-line slopes for the phase diagram in Fig. 3(d) (i.e., at $l_B = 0.9b$ for $\gamma = 1.5$). The inset of Fig. 4 shows the region where the tie-line slopes change sign, beyond which the tie-line slope becomes increasingly more negative at higher $\rho_T$ values.

In Fig. 5, we focus on the $\gamma = 1.5$ and $l_B = 0.6b$ phase diagram of Fig. 3 and investigate the dependence of the re-entrant phase behavior on (a) model parameters and (b) polyampholyte charge sequence. The black curves in (a) and (b) correspond to the reference sv15 phase diagram for $\gamma = 1.5$ and $l_B = 0.6b$ in Fig. 3 (top left). Other curves in (a) correspond to varying $v$, $l_B$, and $K_0$. Other curves in (b) instead show the charge sequence dependence (sequences used are shown in Fig. 1).
behavior on the model parameters and the polymer charge sequence. The excluded volume parameter \( v \) plays different roles in the upper and lower regions of the re-entrance phase diagrams, as can be seen in Fig. 5(a). While reducing the value of \( l_B \) (which reduces the strength of electrostatic interactions) and increasing \( K_0 \) (giving more solvated ions that provide electrostatic screening) both decrease the phase separation propensity by shrinking the two co-existence regions, a reduced \( v \) simultaneously enlarges the low \( \rho_T \) region and shrinks the high \( \rho_T \) region. The excluded volume interactions with AB molecules, therefore, act as to stabilize the high \( \rho_T \) condensates, while the excluded volume interactions among the polymers in the low \( \rho_T \) region inhibit phase separation. In Fig. 5(b), we instead swap the sv15 charge sequence by either sv10 or sv20, which are characterized by smaller or larger blocks of consecutive same-sign charges, respectively. This degree of “blockiness” can be quantified, e.g., by the \( \kappa \) parameter of Das and Pappu,\(^{40}\) or by the sequence charge decoration parameter of Sawle and Gosh,\(^{43}\) and has been shown to strongly correlate with phase separation propensity.\(^{17,19,20,40,53}\)

We qualitatively verify some of the LLPS properties obtained from RPA calculations using FTS calculations of the PMFs \( U_{pp}(r) \), \( U_{pS}(r) \), and \( U_{pB}(r) \). For the PMF calculations, we now use a larger (48\(^3\)) lattice and focus on the polymer charge sequence sv15. Other fixed parameter values are \( l_B = 0.6b^{-3} \), \( v = 0.05b^3 \), \( K_0 = 0.01b^{-3} \), and \( \rho_b = 0.1b^{-3} \). We simulate the system at various initial reactant concentrations and values of \( \gamma \). The results for different \( \rho_T \) values are shown in Fig. 6. To obtain reliable statistics, we run five independent simulations for each \( \rho_T \). For each independent run, out of a total of \( 1.5 \times 10^5 \) CL time steps, we discard the first \( 5 \times 10^4 \) steps and use the rest to compute field averages of the RDFs using a sampling interval of 500 steps. The equilibration time was taken as the CL time step after which the real part of the single unit partition functions stabilized around constant values and the production phase of the simulations were run until a reasonably small standard deviation of the computed observables, \( K_d \) and PMFs, was reached. In Fig. 6(a), we show representative snapshots of the field picture polymer bead density \( \tilde{\rho}_b(r) \), defined in Eq. (28), at different reactant densities for \( \gamma = 1.5, 2, \) and 2.5. Consistent with the RPA phase diagrams of Fig. 3, we see polymer droplet formation for all three \( \gamma \) values at relatively low reactant concentrations, but at high reactant concentrations, droplet formation occurs only at \( \gamma = 1.5 \). Different LLPS behavior is also evident from the polymer–polymer PMF plots.
shown in Figs. 6(b)–6(d), where, at the highest reactant concentrations shown, the PMFs go to zero for \( \gamma = 2 \) and 2.5 and to a small non-trivial positive value for \( \gamma = 1.5 \) at large separations. The short-range effective attraction between polymers and solvated ions or repulsion between polymers and bound AB molecules, respectively, demonstrated by the corresponding PMFs in Figs. 6(e) and 6(f), is consistent with the tie-line slopes (see Fig. 4), which tend to be positive and negative, respectively, when ions are in their solvated and bound states.

VI. CONCLUSIONS

In this work, we have introduced a model for polyampholytes undergoing LLPS in the presence of monovalent reactants \( A^+ \) and \( B^- \) that can form a chemically distinct bound state AB. The dissociated ions have diametrically different effects on the polymer phase separation from their electrically neutral bound counterpart. While the free ions screen the LLPS driving electrostatic interactions between the chains (thus decreasing the phase separation propensity), the bound pairs AB instead function as a crowding agent that promotes LLPS. Conversely, the crowded and highly charged environments inside the polyelectrolyte condensates have non-trivial effects on the A–B dissociation constant compared to the co-existing dilute phase. This complex interplay between polymer phase separation and ion dissociation is studied using both analytical and simulation approaches, which, in particular, consistently point toward a novel mechanism for re-entrant phase behavior under the circumstances where bound ion pairs yield favorable excluded volume interactions over their dissociated counterpart.

Among the plethora of biological functions of IDP condensates that are currently being uncovered, regulating chemical reactions seems to be one of their major functional roles.\(^{29}\) Additionally, chemical reactions in the cellular environment have been shown to be able to both dissolve condensates and trigger their formation.\(^{3,4,7,8}\) The field theoretic approach presented in this work constitutes a major methodological advancement for modeling such phenomena because of the ability of FTS and RPA to account for fluctuations beyond those treated by existing MFT approaches for polymer phase separation in chemically reactive environments. Accounting for field fluctuations is necessary to capture amino acid sequence dependence\(^{10}\) and is essential for describing phase separation driven by the electrostatic interactions\(^{10,11}\) characterized by many IDP species.

The proposed mechanism for ion-triggered re-entrant phase separation relies on electrostatic screening from solvated ions \( A^+/B^- \) combined with crowding effects from their electrically neutral bound state AB. While Coulombic screening is a long established effect of free ions, LLPS promoted by molecular crowding is a relatively less studied phenomenon.\(^{3,5,7,10}\) Our model connects these two effects through ion association, quantified by the bare dissociation constant \( K_0 \). Although it remains to be seen if this particular mechanism for re-entrance is realized in nature, our general framework for including chemical reactions in RPA and FTS will be applicable to a wide range of systems.

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AUTHOR DECLARATIONS

Conflict of Interest

The authors have no conflicts to disclose.

Author Contributions

J.W. and T.P. contributed equally to this work.

DATA AVAILABILITY

The data that support the findings of this study are available from the corresponding author upon reasonable request.

APPENDIX A: CALCULATION OF THE SUM OVER \( n_{AB} \) IN DERIVING THE FIELD HAMILTONIAN

Here, we show how the sum \( S[w, \psi] \) over bound ion pairs \( n_{AB} \) in Eq. (12) is computed in the thermodynamic limit. We express \( S[w, \psi] \) as

\[
S = \sum_{n_{AB}=0}^{n_{max}} \frac{y^{n_{AB}}}{n_{AB}!((n_{T} - n_{AB})!)^2}, \quad y = \frac{Q_{AB}}{K_0 V_{QA} Q_{B}}
\]

In the thermodynamic limit, we can replace the sum by an integral, which we solve in the saddle-point approximation,

\[
S = \int_0^{n_{max}} d\nu_{AB} e^{-I(\nu_{AB})} = e^{-I(\nu_{AB}^*)},
\]

where

\[
I(n_{AB}) = \ln n_{AB}! + 2 \ln (n_{T} - n_{AB})! - n_{AB} \ln y,
\]

and \( n_{AB}^* \) satisfies the saddle condition \( I'(n_{AB}^*) = 0 \). Using Stirling’s approximation \( \ln n! = n \ln n - n \) on the logarithm of the factorials gives the saddle condition

\[
y = \frac{n_{AB}^*}{(n_{T} - n_{AB}^*)^2},
\]

which has two solutions as follows:

\[
n_{AB}^* = n_{T} \left[ 1 + \frac{1 \pm \sqrt{1 + 2x}}{x} \right], \quad x = 2n_{T}y.
\]

However, only the “−” solution is contained in the integration interval \( 0 \leq n_{AB} \leq n_{T} \) and contributes to \( S[w, \psi] \). Plugging this solution into \( I(n_{AB}^*) \) gives

\[
I(n_{AB}^*) = \ln n_{T}! - n_{T} \ln y + n_{T} h_0(x),
\]
where we have again used Stirling’s approximation in writing $\ln(n!)=\ln n! \approx n \ln n - n$. The function $h_0(x)$ is defined in Eq. (18). The thermodynamic limit of $S[w, \psi]$ then becomes

$$S[w, \psi] = \frac{1}{nT} \left[ \frac{Q_{AB}}{K_0 N Q_a Q_b} \right] n \ln h_0(x).$$

Plugging this expression into Eq. (11) gives the final field representation of the partition function in Eq. (15).

**APPENDIX B: COMPLETE RPA EXPRESSIONS FOR $\mu_p, T, \Pi$, AND $K_d$**

This appendix describes the derivation of the RPA expressions for the chemical potentials, osmotic pressure, and dissociation constant. We start by expanding the field Hamiltonian in Eq. (16) around the mean field solution $\bar{\psi}$ given in Eq. (22). To achieve this, we write

$$w(r) = \bar{\psi} + \phi(r),$$

with $f d\phi(r)$ $= 0$ and $\phi_0$ is the $k=0$ fluctuation mode. In this field basis, the single molecule partition functions defined in Eqs. (13) and (14) become

$$Q_p[\bar{\psi}, \bar{\psi}] = e^{-\mu(x) \bar{\psi}} Q_0[\phi, \phi],$$

$$Q_{AB}[\bar{\psi}, \bar{\psi}] = e^{-\mu(x) \bar{\psi}} Q_{AB}[\phi, \phi].$$

Charge neutrality results in a global shift symmetry $\psi(r) \rightarrow \bar{\psi}(r) + (\text{const.})$ that can be used to eliminate the $k=0$ mode of $\psi(r)$ such that we can assume $f d\psi(r) = 0$ in what follows. The single molecule partition functions $Q_0[\phi, \phi]$ have the quadratic expansions

$$Q_0[\phi, \phi] \approx 1 - \frac{1}{2V} \int d\mathbf{k} 2\pi \Psi^T(-\mathbf{k}) C \Psi(\mathbf{k})$$

for $p = \bar{\psi}, \bar{\psi}$, AB, with $\Psi(\mathbf{k}) = (\bar{\psi}(\mathbf{k}) + \bar{\psi}^*(\mathbf{k}))^T$ containing the Fourier transformed field fluctuations. Here, $C_p$ is defined as in the main text and

$$C_{\bar{\psi}, \bar{\psi}} = \Gamma^2 \begin{pmatrix} 1 & 1 \\ -1 & -1 \end{pmatrix}, \quad C_{AB} = \Gamma^2 \begin{pmatrix} 0 & 0 \\ y^2 & y^2 \end{pmatrix}.$$

To find the RPA expansion of the term $nT h_0(x[w, \psi])$ in the field Hamiltonian, we first express the expansion of the field operator $x[w, \psi]$, defined in Eq. (17), as

$$x[w, \psi] \approx \bar{x} \exp^{(2-\gamma)\phi_0} (1 + e) \approx x_0 (1 + e),$$

with $\bar{x} = 2\pi T e^{(2-\gamma)\phi_0} / K_0$ and $e = \int d\mathbf{k} \Psi^T(-\mathbf{k}) (G_A + C_B - G_{AB}) \Psi(\mathbf{k}) / 2V (2\pi)^2$. The RPA expansion of $h_0(x)$ simplifies to $h_0(x) \approx h_0(x_0) + h_1(x_0) e \approx h_0(x_0) + h_1(x)$. The expansion of $h_0(x_0)$ to the quadratic order in $\phi_0$ becomes $h_0(x_0) \approx h_0 + (2-\gamma)h_1^0 \phi_0 + (2-\gamma)^2 h_2^0 \phi_0^2$. This last step can be obtained using the relation $h_0(x) x^T = h_2(x) x - h_1(x)$, which can be proven by considering $h_1(x) x$ and $h_{1+1} \approx h_1(x) x$.

Combining the above expansion of $h_0(x)$ with the expansion of the other terms in Eq. (16) leads to the RPA expansion of the field Hamiltonian $H[w, \psi] = H_0[\bar{\psi}, 0] + H_{\text{RPA}}$ with

$$H_{\text{RPA}} = -\frac{\eta_0}{2} \int d\mathbf{k} (2\pi)^2 \Psi(-\mathbf{k}) C \Psi(\mathbf{k}),$$

where $C$ is defined as in the main text. One can show that the contribution from $\phi_0$ vanishes in the infinite volume limit $(V \rightarrow \infty)$ and will therefore be omitted in the following RPA calculations. Note, however, that the quadratic expansion of $H[w, \psi]$ is also used for the semi-implicit CL time integration in FTS, which accounts for the $k=0$ modes explicitly.

Performing the Gaussian field integrals over $\phi(r)$ and $\psi(r)$ leads to the RPA free energy density in Eq. (25) $f \approx \tilde{f} + f^{(0)}$ with

$$f^{(0)} = \frac{1}{4\pi^2} \int_0^\infty d\mathbf{k} k^2 \ln \left( \frac{2\pi l^h v}{k^2} \right) \det C.$$

The derivatives required for the RPA chemical potentials are

$$\frac{\partial f^{(0)}}{\partial \rho_i} = \frac{1}{4\pi^2} \int_0^\infty d\mathbf{k} k^2 \operatorname{Tr} \left[ C^{-1} \frac{\partial C}{\partial \rho_i} \right], \quad i = b, T.$$

The density derivatives of the matrix $C$ include contributions from the derivatives of the MFT bound fraction $h_1$, $p^{(\theta)} = N \partial f^{(\theta)}/\partial \rho_b$ and $\mu^{(\theta)} = \partial f^{(\theta)}/\partial \rho_T$, become

$$\mu^{(\theta)} = \frac{N}{4\pi^2} \int_0^\infty d\mathbf{k} k^2 \operatorname{Tr} \left[ C^{-1} \left( \frac{(2-\gamma)h_2^0 \rho_T}{1 - (2-\gamma)^2 h_2^0 \rho_T} \right) \right],$$

$$\mu^{(\theta)} = \frac{1}{4\pi^2} \int_0^\infty d\mathbf{k} k^2 \operatorname{Tr} \left[ C^{-1} \left( \frac{(2-\gamma)h_2^0 \rho_T}{1 - (2-\gamma)^2 h_2^0 \rho_T} \right) \right].$$

The fluctuation contribution to the osmotic pressure can then be obtained as $\Pi^{(\theta)} = p^{(\theta)} / N + \mu^{(\theta)} T^{(\theta)} - f^{(\theta)}$.

The RPA expression for the density of dissociated ions $[A^+]$ follows from Eq. (10),

$$[A^+] = h_1 \rho_T - \frac{1}{4\pi^2} \int_0^\infty d\mathbf{k} \operatorname{Tr} \left[ C^{-1} K_0 \frac{\partial C}{\partial K_0} \right],$$

with

$$K_0 \frac{\partial C}{\partial K_0} = -\rho_T h_2 (C + C_B - C_{AB}).$$
The resulting RPA formula for the fraction of dissociated ions becomes \( \langle h_1(x) \rangle = h_1 + h_1^{(0)} \) with

\[
 h_1^{(0)} = \frac{\hat{h}_2 f_0^G \delta(k^d) \text{Tr}[G^{-1}(G_A + G_B - G_{AB})]}{4\pi^2 \left(1 - (2 - \gamma)^2 r_1^2 \hat{r}_2^2\right)},
\]

which can be plugged into Eq. (21) to obtain the RPA dissociation constant.

**APPENDIX C: DETAILED EXPRESSIONS OF THE DENSITY OPERATORS USED IN FTS**

For a given field configuration \( \{w(x), \psi(x)\} \), the forward and backward propagators \( q_0^+ (r, \alpha) \) and \( q_0^-(r, \alpha) \), respectively, associated with the discrete bead–spring polymer chain model in this work can be used to calculate the field operators \( \tilde{p}_F (r), \tilde{c}_F (r) \), and \( Q_0 \{ \tilde{u}, \tilde{\psi} \} \). The chain propagators are constructed iteratively through Kolmogorov–Chapman equations as

\[
 q_0^+ (r, \alpha + 1) = e^{-iW(r, \alpha + 1)} \Phi \ast q_0^+ (r, \alpha), \\
 q_0^- (r, \alpha - 1) = e^{-iW(r, \alpha - 1)} \Phi \ast q_0^- (r, \alpha),
\]

where \( W(r, \alpha) \equiv \tilde{w}(r) + \sigma_\alpha \tilde{\psi}(r) \), \( \Phi(r) \equiv (3/2\pi e^2 2^{3/2}) \exp[-3r^2/2\beta^2] \), and starting from \( q_0(1) = \exp[-iW(r, 1)] \) and \( q_0(N) = \exp[-iW(r, N)] \). Here, \( \ast \) denotes a spatial convolution. One can then show that

\[
 Q_0 \{ \tilde{u}, \tilde{\psi} \} = \frac{1}{V} \int \text{d}r q_0^+(r, N), \\
 \tilde{p}_F (r) = \Gamma \ast \frac{\rho_n}{N Q_0 \{ \tilde{u}, \tilde{\psi} \}} \sum_{\alpha=1}^N e^{-iW(r, \alpha)} q_0^+(r, \alpha) q_0^-(r, \alpha), \\
 \tilde{c}_F (r) = \Gamma \ast \frac{\rho_n}{N Q_0 \{ \tilde{u}, \tilde{\psi} \}} \sum_{\alpha=1}^N e^{-iW(r, \alpha)} q_0^+(r, \alpha) q_0^-(r, \alpha) \sigma_\alpha.
\]

Bead density operators for \( A^-, B^-, \) and \( AB \) are given by

\[
 \tilde{p}_A = h_1(x) PT \frac{Q_{AB} \{ \tilde{u}, \tilde{\psi} \}}{Q_{AB} \} e^{i \tilde{w} + i \tilde{\psi}}, \\
 \tilde{p}_B = h_1(x) PT \frac{Q_{AB} \{ \tilde{u}, \tilde{\psi} \}}{Q_{AB} \} e^{i \tilde{w} + i \tilde{\psi}}, \\
 \tilde{p}_{AB} = h_1(x) PT \frac{Q_{AB} \{ \tilde{u}, \tilde{\psi} \}}{Q_{AB} \} e^{i \tilde{w} - i \tilde{\psi}}.
\]

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