Received: 3 December 2021 | Revised: 7 February 2022 | Accepted: 10 February 2022

DOI: 10.1002/aic.17646

AIChE JOURNAL

PERSPECTIVE

Thermodynamics and Molecular-Scale Phenomena

Future directions in physiochemical modeling of the thermodynamics of polyelectrolyte coacervates

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Funding information
The National Science Foundation, Grant/Award Number: 2100513

Abstract

We review theories of polyelectrolyte (PE) coacervation, which is the spontaneous association of oppositely charged units of PEs and phase separation into a polymer-dense phase in aqueous solution. The simplest theories can be divided into "physics-based" and "chemistry-based" approaches. In the former, PEs are treated as charged, long-chain, molecules, defined by charge level, chain length, and chain flexibility, but otherwise lacking chemical identity, with electrostatic interactions driving coacervation. The "chemistry-based" approaches focus on the local interactions between the species for which chemical identity is critical, and describe coacervation as the result of competitive local binding interactions of monomers and salts. In this article, we show how these approaches complement each other by presenting recent approaches that take both physical and chemical effects into account. Finally, we suggest future directions toward producing theories that are made quantitatively predictive by accounting for both long range electrostatic and local chemically specific interactions.

KEYWORDS
materials, phase equilibrium, thermodynamics/classical

1 | INTRODUCTION

Polyelectrolyte coacervates (PECs) are dense polymeric phases obtained from spontaneous association of positively and negatively charged units on polyelectrolytes (PEs) in aqueous solutions. Properties of PECs vary widely depending on the conditions of the solution, such as types[1–4] and concentrations of PEs and salt, charge patterns along PEs,[5–11] mixing stoichiometry,[12–15] pH,[16] porosity,[17] and temperature.[18,19] In addition to macroscopic phases, which will be the main focus of this article, PECs can be produced in a variety of micro- or nano-structures, including colloidal suspensions,[15,20] films,[21–24] lamellae,[25,26] and so on.[26] Novel technological applications of PECs include encapsulation and delivery of therapeutics,[27–30] stabilization of vaccines,[31] microfiltration membranes,[32] water treatment,[33–36] and food processing.[37] Owing to their importance in technology and biology, significant efforts in recent years have been devoted to fill in the many gaps in our understanding of PECs. In addition, there has been a resurgence of activity in this area due to the recent realization that coacervation, which is referred to as liquid–liquid phase separation (LLPS) in the context of cell biology, can drive cellular compartmentalization in biology.[38–42] For instance, coacervation has been used to rationalize the formation of dense droplets, or organelles, containing intrinsically disordered proteins (IDPs) inside cells.[6,9,11,43]

In this perspective, we focus on the phase behavior of coacervates made from (synthetic) PEs, complemented by a brief discussion of single PE solutions. Many theoretical,[44–52] simulation,[53–55] and experimental[16,38,55–60] studies have been performed to identify the driving forces for PE coacervation and to seek a comprehensive description of the phenomenon. Sophisticated theories along with experiments using materials produced by modern polymer synthesis techniques have provided significant insights into the behavior of coacervates in recent years. However, given their rich physiochemistry spanning multiple length-scales, an accurate, quantitative, theory has remained elusive. Broadly speaking, some researchers have adopted an approach in which PEs are

defined by charge level, chain length, and chain flexibility, but otherwise lacking chemical identity, with *long-range* electrostatic interactions driving coacervation.[45,47,61] Others have focused on the *local* interactions between the monomer and salt species for which chemical identity is critical, and describe PEC formation as the result of competitive *local* binding interactions of monomers and salts. As a short-hand, we label the former approach "physics-based" and the latter "chemistry-based" from here on, for lack of better labels. Some "chemistry-based" approaches completely neglect the long-range electrostatics that provide the sole driving force for coacervation in some purely "physics-based" theories.[58,62] While each route ("physics-based" and "chemistry-based") has shed light on the behavior of PEs and coacervates, for the field to progress further, the two approaches must be merged so that the contributions to coacervation of both long-range nonspecific, and short-range specific, interactions can be delineated. In this article, we will highlight recent advances in the description of PE coacervation from both schools of thought. Then, we discuss how these approaches can complement each other by presenting recent approaches that take both physical and chemical effects into account. Finally, we present remaining challenges as well as forward-looking ideas for combining both approaches more intimately to develop more complete, and system-specific, understanding and modeling of coacervation.

2 | EARLIER REVIEWS

This article seeks to present a brief perspective on recent progress and future directions in modeling of coacervates obtained from linear PEs. While we will highlight key coacervate models, this article is not intended to be an exhaustive review of all previous works. Readers are encouraged to explore other important reviews to get a more comprehensive view of complexation of oppositely charged macromolecules. These include works by Rumyantsev et al.[62] on thermodynamics and rheology of PECs, by Sing and Perry[63] on a diverse range of coacervation theories, simulations and experimental approaches, by Srivastava and Tirrell[64] on thermodynamics, structure, and interfacial properties of PECs, by Cohen Stuart and co-workers[65] on PE colloids and their distinction from bulk PECs, and by Muthukumar[66] on kinetics, thermodynamics and structure of PE solutions.

3 | "PHYSICS-BASED" APPROACH

The first model of PE coacervation, known as the Voorn–Overbeek (VO) theory,[61] combines the Debye–Hückel (DH) free energy with a mixing entropy for the species. Coacervation is then the result of competition between mixing entropy and long-range electrostatic attractions of polyanions and polycations in the presence of salt ions, which can screen these attractions. Major deficiencies of the DH theory are its failures to account for electrostatic correlations at high ionic strengths (even for monovalent salt ions) and PE chain connectivity.[63–65] Efforts to correct this limitation have included the application of the random phase approximation (RPA),[45,46,66,67] field theoretic simulations (FTS),[9,47,68] liquid-state (LS)-based theories,[64,69] and scaling theories.[50,70,71]

The RPA method, developed for mass density fluctuations in polymers by de Gennes,[72] and then for charge density fluctuations in PEs by Borue and Erukhimovich,[66] provides a closed-form expression for the (mass or) charge fluctuation free energy up to the pair-correlation level.[66] This method was later applied to coacervates by Castelnovo and Joanny;[45,46] however, the PE conformation, or form factor (which reflects monomer connectivity and must be supplied to the RPA formalism), was taken to be fixed and not responsive to the concentrations or binding states of species.[63,65] Recently, a variational method developed by Shen and Wang rectifies this limitation by allowing the PE conformation and the electrostatic free energy to be self-consistently determined.[63]

The Chan group applied the RPA formulism to model coacervation of sequence-defined polyampholytes.[5,6,73] These are analogs of IDPs, in which the precise amino-acid sequence along the protein controls its structure–function properties. Hence, coacervates made from these PEs are analogous to organelles assembled from IDPs in biology. In line with several *in vitro* and *in vivo* experiments on organelles,[74] the Chan group found that PEs with longer like-charge blocks, that is, "blockier" sequences, yield a larger coacervation composition window than do PEs with a random distribution of charges along the chain. Coacervates formed from the latter dissolve more readily in the solution with increased temperature or salt concentration, or in some cases do not even form coacervates at all.

The method of FTS developed by Fredrickson and co-workers includes two forces between charged species: long-range electrostatic, and short-range excluded volume interactions, the strengths of which are controlled, respectively, by the Bjerrum length l_B and an excluded-volume parameter ν.[47] The advantage of FTS is its ability to move beyond the limitations of RPA by capturing higher order electrostatic correlations (i.e., beyond pair-correlation level) in PE solutions. In addition to homo-PEs,[47,60] this approach has been applied to coacervation of sequence-defined polyampholytes.[9,10,68]

Such advancements in "physics-based" theories of coacervation open up unprecedented opportunities for progress not only in understanding cellular organization in biology, but also in materials science more generally. Nevertheless, the coacervation mechanism in the aforementioned approaches is mainly limited to the electrostatic attractions between oppositely charged monomers of PEs in a dielectric continuum. In addition to the electrostatic interactions, however, the behaviors of synthetic as well as naturally occurring PEs are significantly affected by the chemical identities of the species involved, which control local interactions on the nanoscale and are considered to lie in the domain of physical chemistry.

4 | "CHEMISTRY-BASED" APPROACH

For a fixed concentration, charge pattern, and temperature, a coacervate can have rheological properties ranging from that of a viscous liquid to a glassy solid,[1] depending on the chemical identity of PE monomers and salts. In addition to charge regulation,[16] which will be discussed later, the effects of the specificity of charged groups

(i.e., ions and PE monomers) are largely reflected in their hydration,[1,75,76] which in turn influences how the charged groups interact at short distances. Due to their strong polarity, water molecules are attracted to bare charged groups in solution and form hydration shell around them,[77,78] which reduces their mixing entropy. The number of waters drawn into a hydration shell of a charged group in aqueous solutions depends on a number of factors (such as size of the charged group, dispersion interactions, etc.), and can be thought of as the solvent affinity or, "hydrophilicity," of the charged group.[1,75]

A lower solvent affinity generally leads to enhanced association of oppositely charged groups in aqueous solutions.[1,75] For instance, Douglas, de Pablo and co-workers found that cations with lower solvent affinities, such as Cs^+, form stronger "pairs" with Cl^- ions in water than do highly hydrated Li^+ ions.[75] This finding is in accord with the experiments of Hofmeister more than 100 years ago, who observed that the more hydrophobic anions denature proteins more easily (due to stronger binding to proteins) than do highly hydrated ones.[79] Similar interactions drive strong association of hydrophobic PEs with each other, and thus induce their phase separation in solutions (as discussed later), or their localization at the water-air interface.[80] The mean-field Flory-Huggins χ_{PW} parameter is traditionally employed to describe, in a crude way, these hydrophobic interactions of PEs with water.

In addition to hydrophobicity, binding of oppositely charged groups at short distances can also be driven by release of overlapping hydration waters, hydrogen bonding, $\pi-\pi$ interactions, and of course, electrostatic attractions.[81–86] In an interesting study, Sinn et al. showed that K^+ and Cl^- ions even bind to a neutral polymer by liberating hydration waters,[87] indicating that electrostatic attractions are not required for binding between two species.

Highly hydrated PECs generally display a lower critical solution temperature (LCST),[10,60,88] as was recently observed for ε-poly-L-lysine (εPL)/hyaluronic acid (HA)[60] and tau protein/RNA coacervates.[10] LCST behavior indicates a major role of entropy gain(s) in driving coacervation, and aligns with calorimetric experiments which show nearly athermal ($\Delta H \approx 0$) coacervation.[59,89] Based on this, Han, Shea, Fredrickson and co-workers pointed to the release of overlapping hydration waters from PEs as a driving force for coacervation,[10,60] which is enhanced at high temperatures due to the weakening of the hydration shells around PEs. The release of hydration waters leads polymers to become more hydrophobic, and so to enhanced coacervation. This effect is usually captured by a temperature-dependent expression for χ_{PW}.[10,19] Despite this chemically-specific influence, the most popular explanation for coacervation is entropy gain by counterion release.[48,49,53,54,56–58,90–92] Regardless, noting the above discussion, since water binding, like ion binding, to a PE-monomer is local and chemically specific,[78] both release mechanisms highlight the need to incorporate local interactions into coacervation theory. We note that upper critical solution temperature behavior, on the other hand, has also been observed in some PECs, but PEs forming these PECs are highly hydrophobic, i.e., have weak hydraion shells, and have abundant directly contacting ion-pairs (i.e., with no mediation waters of hydration) between polyanion and polycation.[88,93,94]

A relatively simple "chemistry-based" approach, which focuses on the local interactions, is to consider coacervation to be the product of a reaction between polyanions and polycations, as envisioned first by Veis and Aranyi.[95] Along this line, the Schlenoff group developed an ion-exchange coacervation model, which is based on a local charge-binding picture of PEC formation.[62] This model views the PEC as the product of a competition between polyanion and polycation pairing against ion-PE pairings:

$$(\mathrm{A}+)+(\mathrm{C}-) \overset{\Delta G_f^0}{\Leftrightarrow} (\mathrm{AC})+(+)^+ +(-)^- \tag{1}$$

Here, (AC) represents an ion-pair between charged polyanion (A) and polycation (C) monomers, $(\mathrm{A}+)$ denotes a charged polyanion monomer paired with a small cation, and $(\mathrm{C}-)$ is a charged polycation monomer-anion pair. ΔG_f^0 denotes the standard free energy of PEC formation (where the subscript "*f*" stands for "formation"), and $(+)^+$ and $(-)^-$ designate the salt cation and anion, respectively.[62]

Based on the above reaction, a PEC forms if ion-pairing between PEs along with any associated release of salt ions (initially bound to PEs) is energetically and entropically more favorable than the reverse. The overall strength of the PEC formation, accounting for all these factors, is then given by an equilibrium constant $\mathcal{K}_f = \exp\left(-\Delta G_f^0\right)$.[1,2,62] The inverse of this, termed "doping" constant $\mathcal{K}_{dop} = 1/\mathcal{K}_f$, describes the efficacy of salt ions in breaking ion-pairs and dissociating PECs (i.e., the reverse of the Reaction (1)) in salt solutions.[2] Recently, Schlenoff and co-workers revealed, by varying salt anions along a Hofmeister series, that more hydrophobic salt anions (with lower hydration numbers) are more effective at breaking ion-pairs (i.e., have a higher doping constant $\mathcal{K}_{dop}$) and partition more into the PEC phase than into the dilute phase (see Figure 1).[2,96]

The ability of anions to break ion-pairs and dope PECs reflects their relative hydrophobicity, as is also seen in their ability to denature proteins, as observed by Hofmeister.[79,97] In parallel with the above studies on the salt type, the Schlenoff group found that the more hydrophobic PEs yield glassy, solid, PECs with stronger ion-pairs, which are especially hard to dissociate by salt ions.[1] Similarly, Tirrell, de Pablo and co-workers showed that more hydrophobic PEs tend to produce PECs which require high salt concentrations to dissolve, with the phase diagrams often left "open" at the top, where in one case the coacervate did not dissolve even at a very high [NaCl] of 6 M.[4] The Lutkenhaus and Shull groups, among others, have shown that hydration level acts as a kind of "master" control parameter in the phase behavior and rheology of coacervates, with differing PEs and salt ions influencing behavior through their ability to attract waters of hydration into the coacervate.[98,99]

Taking advantage of chemical specificity, in a recent work, Spruijt and co-workers mixed different coacervate droplets, each formed from a distinct polyanion/polycation pair, to form multiphase droplets with hierarchical structures due to the immiscibility of the original coacervates, mimicking the structure of cell nuclei.[39]

These novel experiments clearly show the importance of chemical identity effects of salt ions and PE monomers in the phase behavior of

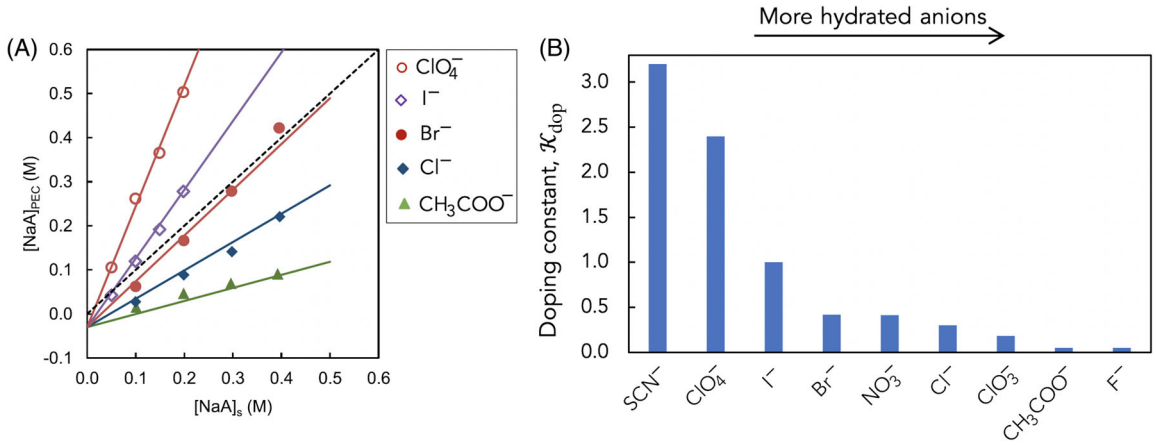

FIGURE 1 (A) Salt partitioning and (B) doping constant of various sodium salts from References 2 and 96, with salt anions, from left to right, in order of increasing hydration number. Figure 1 is adapted or reprinted with permission from References 2 and 96

coacervates. Nevertheless, we note that, as will be discussed shortly, the importance of chemical effects does not imply that the electrostatic correlations are negligible in PE solutions. Thus, while qualitative predictions of coacervation can be made using either the "physics-based" or "chemistry-based" models, quantitative predictions need to draw on ideas from both realms.

5 | NEXUS OF PHYSICS- AND CHEMISTRY-BASED APPROACHES

In this section, we explore recent PE theories that incorporate both short-range associations (reflecting chemical specificity) and longer-ranged electrostatic interactions. In particular, Lytle and Sing developed a novel transfer matrix (TM) framework to determine the free energy of binding of oppositely charged species.[49] Within this theory, PE chains and salt ions with chemical potentials of μ_P and μ_S, respectively, can bind to the monomers of a (test) chain from the surrounding environment (or reservoir). Given the binding state of monomer $i-1$ of the (test) chain, the TM determines that the binding of monomer i to an oppositely charged species makes a contribution to the grand canonical partition function of the chain defined by the matrix:[49]

$$M(s_i,s_{i-1}) = \begin{bmatrix} SS & SP & SP' & S0 \\ PS & PP & PP' & P0 \\ P'S & P'P & P'P' & P'0 \\ 0S & 0P & 0P' & 00 \end{bmatrix} = \begin{bmatrix} e^{\mu_S} & e^{\mu_S} & e^{\mu_S} & e^{\mu_S} \\ 0 & 1 & 2 & 0 \\ e^{\mu_P} & e^{\mu_P} & e^{\mu_P} & e^{\mu_P} \\ e^{-\varepsilon} & e^{-\varepsilon} & e^{-\varepsilon} & e^{-\varepsilon} \end{bmatrix} \quad (2)$$

Here, s_i denotes the binding state of monomer i, which can be free (0), bound with salt (S), or bound with a monomer of an oppositely charged PE (P' or P). The designation (P') applies if the monomer is the first monomer of the oppositely charged chain to bind and is P if it is a subsequent neighboring monomer of that chain in a consecutive sequence or "run" of monomers to bind. The TM elegantly accounts for the fact that binding of one monomer of an oppositely charged PE to the test chain biases the binding of the adjacent monomer to the test chain. The chemical potentials are prescribed as $\mu_S = \ln A_0\phi_s$ and $\mu_P = \ln B_0\phi_P$, where A_0 and B_0 determine the deviations of salt and PE species from ideal solution behavior and implicitly reflect the strengths of binding of the respective species onto the chain.[3,49] To account for the electrostatic energy for each unpaired monomer along the chain, an energy penalty ε is assigned, which is approximated from single-chain molecular simulations, thus indirectly accounting for long-range electrostatic correlations.[49]

The free energy of the binding of oppositely charged species to the chain, F_{int}, is obtained from the partition function of the test chain.[49] The total free energy of the system is then written as $F = F_{mixing} + F_\Lambda + F_{FH} + F_{int}$, where F_{mixing}, F_Λ, and F_{FH} denote the free energies due to mixing entropy, excluded volume between charged species, and Flory–Huggins dispersion interactions, respectively.[3]

This approach was applied to coacervates from sequence-defined PEs, where, in parallel to the findings of the Chan and Fredrickson groups, it was found that PEs with longer (or blockier) sequences of like charges on the chain tend to form more salt-resistant PECs (see Figure 2).[7] Sing and co-workers suggested that blockier sequences result in higher electrostatic repulsions between like charges in each sequence, which are relieved by more extensive salt binding to the chain (prior to coacervation).[7] Hence, the coacervation of PEs with blockier sequences results in stronger PE associations due to more entropy gain from release of more (initially bound) salt ions.[7]

In addition to charge patterns on PEs, Perry, Sing and co-workers recently performed a systematic study of the effects of PE chemistry and chain length on coacervation using experiment and theory.[3] In line with Schlenoff, Tirrell, de Pablo and their co-workers,[1,4] for the same chain length it was found that increasing the hydrophobicity of the PE (by incorporating methacryloyl instead of acryloyl in monomers) yields more salt-resistant coacervates, which was modeled using the χ_{PW} parameters in the FH free energy (Figure 3). Similarly, longer PEs (i.e., PEs with higher degrees of polymerization) produced a larger coacervate window.[3]

Within the TM approach, the strengths of binding for both types of salt ions to their opposite PEs are assumed to be identical (captured

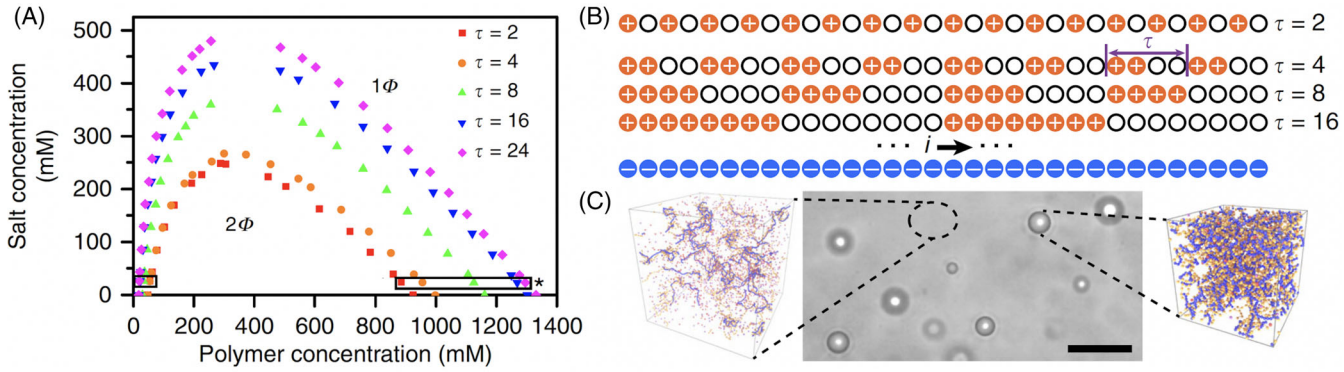

FIGURE 2 (A) Binodal phase diagrams for the complexation between (B) polyanion and polycation with different periodicity (τ) of charged monomers, where τ gives the periodicity length (in polycation monomers) of the charged and neutral monomer blocks. (C) Representative dense coacervate and dilute phases. All three panels are adapted from Reference 7

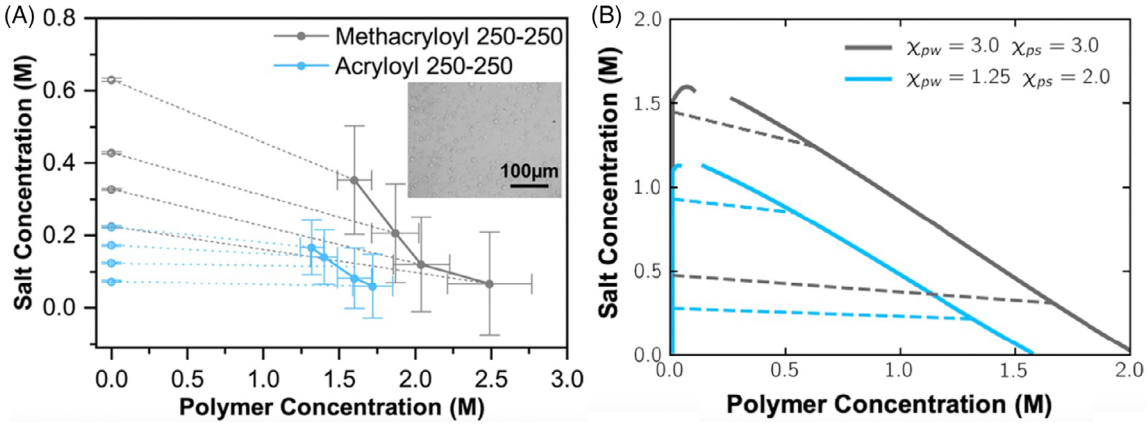

FIGURE 3 (A) Experimental and (B) theoretical binodal diagrams of coacervation between pairs of PEs of two different hydrophobicities. The chain lengths of both polyanions and polycations are kept fixed at 250. In (A), the less hydrophobic polycation and polyanion both contain an acryloyl backbone, while the more hydrophobic ones have methacryloyl backbones. The charge is controlled by the side group, which is identical in the acryloyl and methacryloyl polyelectrolytes. Figure is reprinted from Reference 3

by the parameter A_0), which could be extended to assign different strengths for each type of salt ion-PE pairing. Thus, while the parameters are empirical, they can be adjusted to account for chemical specificity, local architecture, and correlations between opposite species.

Olvera de la Cruz and co-workers developed one of the first theories for PE coacervation that combined long-range electrostatics, captured by the RPA, with ion-pairing between opposite PEs.[44] Later, Salehi and Larson incorporated all ion-binding effects, including ion-pairing between oppositely charged PEs, salt ion binding, and protonation/deprotonation reactions, while using DH theory for long-range electrostatics.[48] Noting the aforementioned deficiencies of the DH, Friedowitz et al. recently improved this model by replacing the DH theory with the RPA, yielding a contribution from the electrostatic correlations to ion-binding, that accounts for charge connectivity in the PEs.[100] Within this model, the binding of oppositely charged groups is driven by electrostatic correlations μ^{corr} and intrinsic binding free energies, ΔG's, that capture chemical specificity effects, such as ion-specific release of hydration waters, etc. The binding reactions are described as,[101]

$$A^- + (+)^+ \overset{\Delta G^{eff,0}_{A+}}{\Leftrightarrow} (A+) \tag{3}$$

$$C^+ + (-)^- \overset{\Delta G^{eff,0}_{C-}}{\Leftrightarrow} (C-) \tag{4}$$

$$A^- + C^+ \overset{\Delta G^{eff,0}_{CA}}{\Leftrightarrow} (CA) \tag{5}$$

where $\Delta G^{eff,0}_{ij} = \Delta G^0_{ij} + \mu^{corr}_{ij} - 1$ denotes the effective free energy of binding between the groups of i and j, taking account both intrinsic and longer-ranged electrostatic contributions.

Within this model, the total free energy of the solution receives contributions from the mixing entropy f_{mixing}, Flory–Huggins dispersion interactions f_{FH}, combinatorial entropy f_{comb} of the ways of

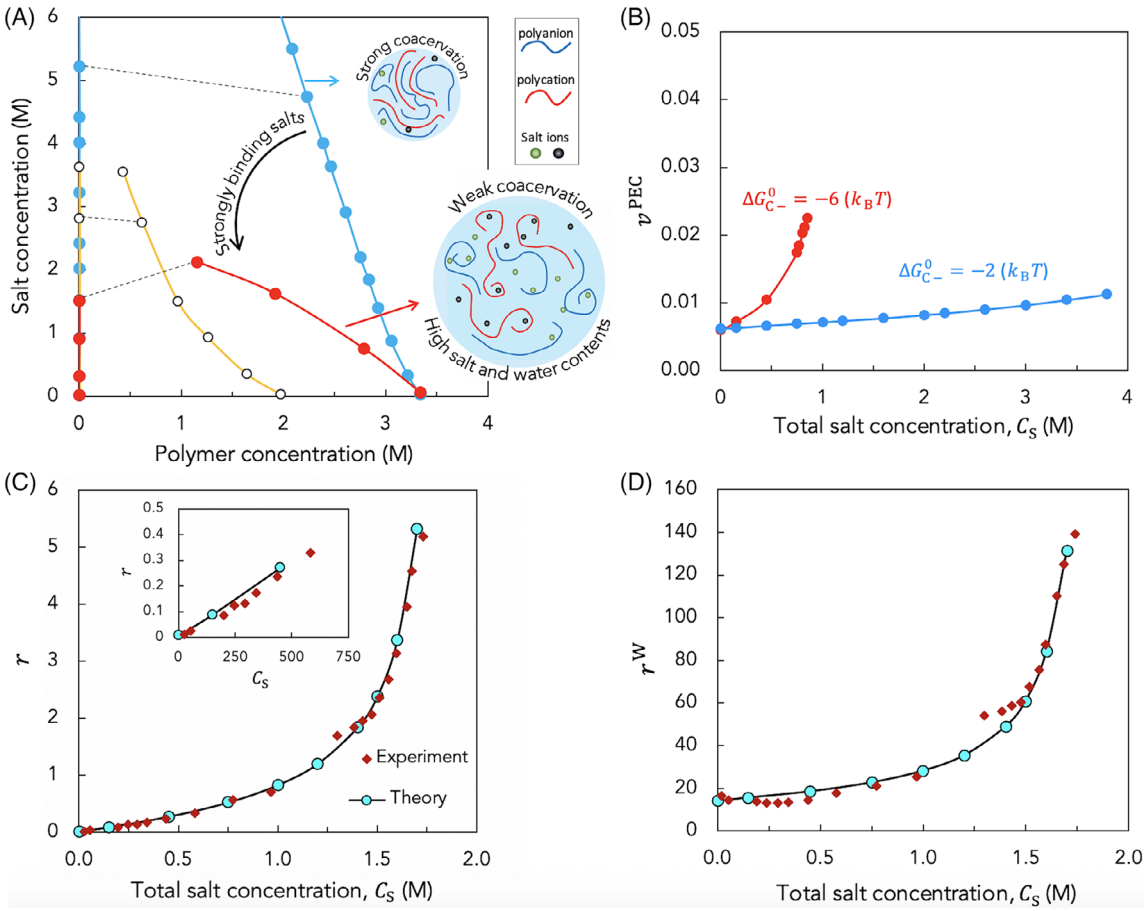

FIGURE 4 (A) Theoretical binodal diagrams for various strengths ΔG^0_{C-} of salt anion binding to polycations including absence of ion-binding (yellow line with open symbols). (B) variation of the PEC volume fraction, defined as the PEC volume over the entire solution volume as a function of total salt concentration, C_S. (C) and (D) show, both theoretically and experimentally, the variation of the salt and water content in the PEC, respectively defined as $r = [\text{salt}]^{PEC}/[\text{PE}]^{PEC}$ and $r^W = [\text{water}]^{PEC}/[\text{PE}]^{PEC}$ for PDADMA/PSS in KBr (reprinted or adapted with permission from Reference 101)

arranging different binding pairs along the chain, electrostatic correlations f_{corr}, and of course binding reactions f_{rxn}, leading to $f = f_{mixing} + f_{FH} + f_{comb} + f_{rxn} + f_{corr}$. Minimization of the total free energy with respect to the extent of the reactions yields three mass action equations, each with an equilibrium constant written in the general form $K_{ij} = \frac{C_{ij}C_W}{C_iC_j} = \exp\left(-\Delta G^{eff,0}_{ij}\right)$, with C_k being the concentration of species $k\,(=i,\ j,\ ij, W$ (the latter denoting water)).[101]

Dissociation of PEC ion pairs through salt binding is controlled by a "doping" equilibrium constant obtained from the model of Friedowitz et al. by multiplying the two equilibrium constants for salt binding to the oppositely charged PE, and dividing by the ion-pairing equilibrium constant, that is, $K_{dop} = K_{A+}K_{C-}K^{-1}_{CA}$.[101] This doping constant interestingly boils down to an expression similar to that employed by Schlenoff and co-workers $\left(K_{dop} \propto \mathcal{K}_{dop}\right)$.[101] Ghasemi and Larson modeled the experiments of Schlenoff and co-workers on doping of PECs from poly(diallyldimethylammonium), PDADMA, and poly(styrene-sulfonate), PSS, with different sodium salts, by tuning the intrinsic strength, ΔG^0_{C-}, of salt anions binding to polycations within the model.[101] In accord with the experiments, they found that strongly binding salt ions with more negative ΔG^0_{C-} (corresponding to more hydrophobic anions), more effectively localize on PEs and break ion-pairs between them and hence, are more concentrated in the PEC than in the dilute phase (Figure 4A).[101] However, we note that the hydrophobic interaction of salt ions with water could also affect their partitioning behavior, with more hydrophobic salt ions expected to partition more into the coacervate, which has a lower water content (than does the supernatant phase).

Interestingly, in the absence of binding reactions (yellow line with open symbols in Figure 4A), so that only the RPA and mixing entropy terms are present in the free energy, salt ions behave similarly to ideal solutions and are almost equipartitioned throughout the solution,[101] mimicking field theoretic predictions in which ion and PE specificity are absent and the mixing entropy constitutes the major part of the chemical potential of ions.[102] Since experiments have shown that salt partitioning is sensitive to salt identity,[2,96] the above results highlight the importance of including the effects of chemical specificity, for example, through the value of ΔG^0_{C-}, in the phase behavior of PECs.

Note that the variations of salt content, water content, and PEC volume within the above model can, by fitting ΔG^0_{C-} and other parameters, match the experimental data of PDADMA/PSS in KBr (see

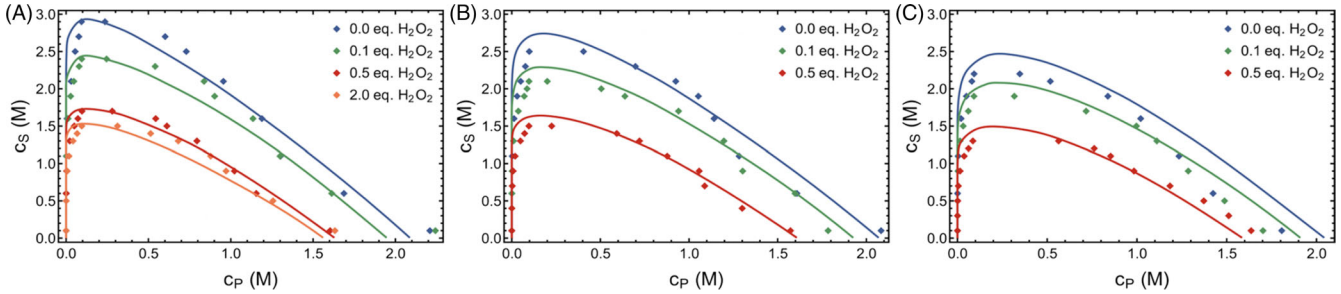

FIGURE 5 Theoretical (lines) and experimental (symbols) binodal diagrams for complexation of PEs with various chain lengths and monomer polarities tuned by peroxide reaction at equivalence levels given in the legends. Fitted χ_{PW} parameter at equivalence levels of 0.0, 0.1, 0.5, and 2.0 are respectively, 0.556, 0.531, 0.472, and 0.445. The degree of polymerization of the polycation ion equals that of the polyanion in each case and are set at (A) 180, (B) 100, and (C) 50. Figure is reprinted from Reference 106

Figure 4C,D) as functions of total salt concentration, C_S.[101,103] Similar to data in Figure 1A, the salt (and water) content of the PEC exhibits a linear change with C_S at low C_S, which is termed the "doping" regime.[103] At high salt concentrations, polyanions and polycations in the PEC are extensively bound by the salt ions and only loosely associate with each other. The incompressibility condition, which includes excluded volume interactions at the mean-field level, ensures a steep increase of water (and free salt) content in the PEC upon weakening of ion pairing at high C_S. The same mechanism leads to a high water content in the PEC when the salt ions bind strongly to PEs for a given C_S (see Figure 4B). This also supports the experimental observation that less hydrated salt ions swell PECs more effectively than do more hydrated ones.[83,104] Further, this corroborates the notion that increasing the binding strength of salt ions acts similarly to increasing the concentration of weakly binding salt. For instance, recently it was experimentally observed that switching to less hydrated salts (which bind strongly to PEs) influences the rheology of PECs similarly to increasing the concentration of more hydrated (or, weakly binding) salt.[99] A recent review discusses hydration effects and relaxation mechanisms in the rheology of PECs.[105]

Lou, Friedowitz, Qin, and Xia explored the effect of water-affinity of PE monomers on coacervation of well-defined PEs of identical backbones with different side groups each containing a sulfide group with controllable degree of oxidation, leading to various mixtures of sulfoxide and sulfone side groups.[106] The level of oxidation was varied from one pair of PEs to the next but kept the same for each PE in the pair, leading to variable but controlled monomer polarities and water affinities. Higher oxidation levels corresponded to stronger monomer hydrations and hence, lower χ_{PW}. Treating χ_{PW} as an adjustable parameter at each oxidation level of PEs, Lou et al. produced excellent fits to the experimental data for different degrees of polymerization.[106]

Figures 3–5 clearly show how the chemical structures of the charged groups, captured in the theory through ΔG^0_{ij} and/or χ_{PW}, control the coacervate phase behavior. Electrostatic correlations also significantly affect the phase behavior, as inferred from the dependence on monomer sequence in Figure 2 and also from the coacervation studies of Chan,[6,73] Fredrickson,[9,10] and their co-workers. The significance of both generic electrostatic correlations and specificity effects can also be revealed through investigation of single-PE solutions.

Single PE solutions have been much more extensively studied than PECs, and it is not our purpose here to review this extensive literature. Interested readers can find excellent discussions in References 107–109 as well as the references given therein. Here, we provide a few illustrative examples of how coacervate models that include both of local chemical effects and long-range electrostatics, can be tested by applying them to single PE solutions, which can therefore aid in development of PE coacervate models.

Sammalkorpi and co-workers recently used fully atomistic molecular dynamics (MD) simulations to study the neutralization of a single PE chain, stretched across the simulation box, by various salt ions.[110] They showed that localization of salt ions around fully charged PEs (in particular polyglutamic acid (PGA)) is generally dependent on the type of ion, especially in the vicinity of the chain (Figure 6A).[110] Upon increase of charge fraction of poly(acrylic acid) or PAA, they found strong localization of sodium ions near the chain (Figure 6B), thus, highlighting the effects of electrostatic correlations. Molecular-level information, such as salt-PE radial distribution functions (RDFs) and/or diffusivity of salt ions near PE, can be employed to separate the effects of chemical specificity from electrostatic correlations in PE solutions.

Tian, Ghasemi, and Larson recently performed full atomistic MD simulations of poly(acrylic acid) or PAA, chains, and K^+ potassium ions to obtain a priori the intrinsic free energies of salt-PE binding, ΔG^0_{ij}, and probe the contributions of ΔG^0_{ij} and electrostatic correlations (μ^{corr}_{ij}) to potassium binding to PAA.[111] Free energies of salt-PE binding were extracted using the equilibrium constant given by $\frac{C_{A+}C_W}{C_A C_+} = \exp\left(-\Delta G^{eff,0}_{A+}\right)$, where the concentrations of "bound" cations, C_{A+}, were defined as ones within the first two peaks of potassium-(charged)monomer RDFs representing directly bound cations and cations separated by a single water of hydration from the monomer; see Figure 7A.[111] (The presence of two near-neighbor peaks in Figure 7A while only one appears in Figure 6B seems result from the details of the simulation and definitions used).

As the charge fraction of the chain decreases, the RDF approaches that of short, singly-charged chains (see Reference 111), suggesting little effect of electrostatic correlations along the chain. In this limit, one can obtain an estimate for the intrinsic binding free energy (ΔG^0_{A+}).[111]

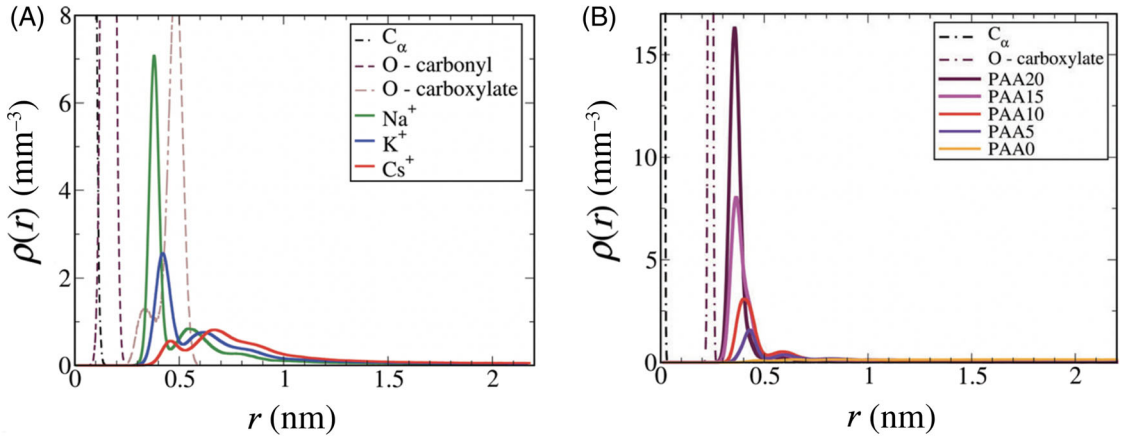

FIGURE 6 Cylindrical radial distribution functions $\rho(r)$ of ions around polyelectrolyte chains, stretched across the simulation box. Results for (A) a polyglutamic acid chain (PGA), with sodium, potassium, or cesium counterions, and (B) a polyacrylic acid chain of various charge fractions and sodium ions. PGA and PAA are 20 monomer long chains, and the number next to "PAA" in the legend in B denotes the number of charged monomers in the PAA chain. Figure is reproduced from Reference 110

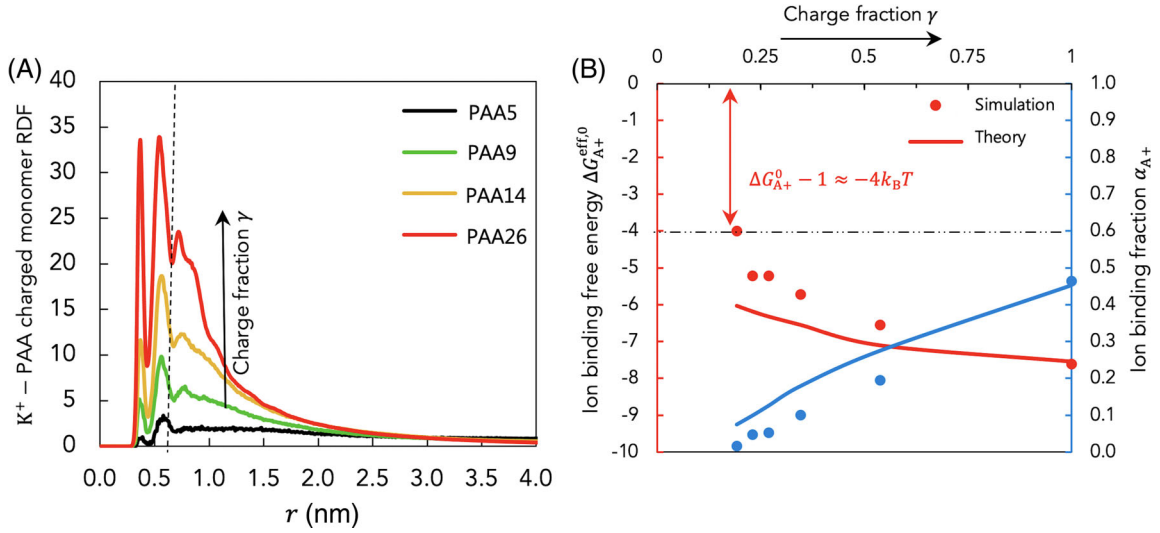

FIGURE 7 Potassium-charged monomer radial distribution function (RDF) for aqueous solutions containing potassium ions and a 30-monomer PAA chain with varying numbers of charged monomers on the chain. The number next to "PAA" in the legends in a denotes the number of charged monomers in PAA chain. Note that, in this figure the terminal two monomers at each end of the chain are kept neutral and these monomers are not included in definitions of α_{A+} and γ. Within the theory, the contributions to the effective binding free energy $\Delta G^{\mathrm{eff},0}_{A+} = \Delta G^0_{A+} + \mu^{\mathrm{corr}}_{A+} - 1$ beyond $\Delta G^0_{A+} - 1 = -4\,k_BT$ are due to the electrostatic correlations, given by μ^{corr}_{A+}. In B, $\Delta G^0_{A+} - 1 = -4\,k_BT$ is obtained from MD simulations of weakly-charged PAA chains and is used an input into the theory (lines are theoretical predictions). Figure is reprinted from Reference 111

By increasing the charge fraction γ on the other hand, the chain takes on more extended configurations, while simultaneously, the ion binding extent and binding strength increases, which similar to Figure 6B, highlights the effects of electrostatic correlations; see the increasingly sharp peaks of the RDF in Figure 6B, and higher ion binding fraction α_{A+} and more negative $\Delta G^{\mathrm{eff},0}_{A+}$ with increase of γ in Figure 7.[111]

These results corroborate a number of experiments and simulations. First, it has long been known that "counterion condensation," which relieves electrostatic repulsion, is prominent for PEs with high charge densities.[112,113] This effect is observed, for example, in the Langevin simulations of Muthukumar and co-workers.[53] Second, it has been experimentally shown that the degree of counterion binding to a PE chain is higher in the middle of chain than near the chain ends,[114] since the middle of chain is more concentrated in PE charged groups than the ends. In addition, these results explain the stronger association of oppositely charged PEs with blockier sequences, which leads to larger coacervation windows in the studies of Sing and Perry,[7] Chan,[5,6] de Pablo,[115] and Fredrickson[9] and their co-workers. The proximity of like charges in the blocky sequences creates strong electrostatic repulsions within each block, which are relieved by

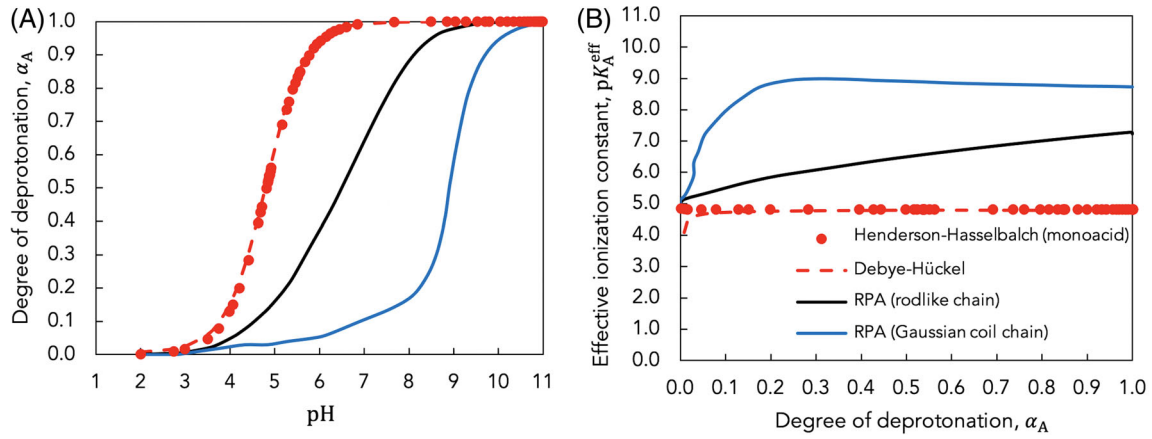

FIGURE 8 (A) Predicted degree of deprotonation as a function of pH for polyacids with charge interactions modeled in four ways: (1) using the Henderson-Hasselbalch expression (red symbols, which is valid for monoacids), (2) using the Debye-Hückel theory with no charge connectivity (dashed line), (3) using the RPA theory with a rodlike chain (black solid line), or (4) using the RPA with a Gaussian chain (blue solid line). (B) Corresponding effective ionization constants as functions of degree of deprotonation. Figure is adapted from Reference 117

higher levels of binding between the oppositely charged chains (and by release of more initially bound counterions).

The interplay of physical and chemical effects in the equilibrium behavior of PE solutions is also revealed in the phenomenon of charge regulation.[116,117] Ghasemi and Larson modeled charge regulation of weakly dissociating polyacids in aqueous solutions by combining acid–base equilibria and ion binding to polyacids with an electrostatics theory.[117] Similar to the above discussion of the ion binding to PE chains, one finds that polyacid monomers deprotonate with an effective (or apparent) ionization constant pK_A^{eff} that has two contributions: (1) the "intrinsic" constant, $pK_A = \Delta G_A^0/2.3$, which solely depends on the chemical structure of the polyacid monomer and (2) the electrostatic correlations between ionized monomers along the chain, quantified by $\mu_A^{corr}/2.3$.[117]

Figure 8 depicts the predicted titration data of monoacids (red symbols) and polyacids with different assumed chain structures (lines). According to the Henderson–Hasselbalch (HH) theory, monoacids deprotonate with their intrinsic ionization constant, that is, $pK_A^{eff} = pK_A$ (see Figure 8B). Using the DH theory to account for electrostatics of polyacid solution yields the same response as the HH prediction for monoacids, with no change in the strength of deprotonation of monomers as they deprotonate that is, $pK_A^{eff} = pK_A$ (see Figure 8B). This result can be attributed to the neglect of polyacid chain connectivity within the DH theory.[117]

Employing the RPA to account for electrostatics, which incorporates chain connectivity, causes polyacid monomers to "feel" increases in electrostatic repulsions on the chain as they deprotonate. To minimize these repulsions, monomers resist further deprotonation (or ionization) through an increase (or shift) of the ionization constant, pK_A^{eff}, from its intrinsic value (pK_A) (see Figure 8B).[117] This shift in the ionization constant is accounted for by the contribution of μ_A^{corr} to pK_A^{eff}. Using a rodlike chain within the RPA theory yields the weakest repulsions along the chain, whose titration response mimics that of the hydrophilic PAA.[117]

The effect of μ_A^{corr} on ionization reflects the influence of long-range physics on weak PEs while the effect of physical chemistry is captured by the intrinsic ionization constant pK_A, chain hydrophobicity, and other local physical chemical effects. The hydrophobic effects are implicitly accounted for through choice of a PE structure more compact than the rodlike chain. Assuming a Gaussian polyacid configuration in solution, we obtain a very strong resistance of the polyacid to deprotonation, as shown by the lower degree of deprotonation α_A at a given pH in Figure 8A and sharp increase in pK_A^{eff} in Figure 8B.[117] This resistance to deprotonation, which is captured by μ_A^{corr}, arises from the proximity of charges to each other. Thus, when a polyacid chain becomes more (intrinsically) hydrophobic, it takes a more compact structure to avoid interaction with water, making it resist deprotonation so that it behaves as a weaker polyacid. Such behavior is observed in the titration curve of poly(acrylamido-2-methyl-1-propanesulfonic acid), or PAMPs, due to its compactness derived from its hydrophobic methyl and amide groups.[117]

This approach is in essence similar to a new theory by Gallegos et al. for charge regulation of single PE solutions, which decomposes a monomer apparent ionization constant into contributions from its chemical reactions and nonbound (electrostatic) interactions.[116]

The above studies show an interplay between short-range chemical interactions (salt binding and protonation) and long-range physical interactions (electrostatics), in governing equilibrium behavior of PE solutions. Coacervate theories should thus incorporate both effects in a self-consistent fashion to produce quantitative predictions.[118,119]

6 | OUTLOOK

Recent developments in the description of PE coacervates have provided remarkable insights into their complex behavior, opening up exciting future research avenues in this field. Accounting for electrostatic correlations in sequence-defined PEs, Chan[6,73] and

Fredrickson[9,10] and their co-workers have advanced the current understanding of coacervate formation from such PEs. Such studies suggest intriguing parallels between such coacervates and liquid organelles formed from IDPs, which may be correlated with neurodegenerative diseases.[120] In addition to electrostatic correlations, interactions of biopolymers are affected by local binding between charged groups, which are governed by the chemical identity of charged groups, and are reflected in charge regulation and hydrophobicity.[121–125] For instance, the effective pK_A of a protein and its dependence on charge can influence the structure–function properties of the protein in solution.[126–129] Hence, an exciting prospect in this area could be to include the local binding and chemical effects into the RPA or FTS and apply these to coacervation of sequence-defined (protein-like) PEs. One possible approach in this direction might be to merge RPA theories for far-field electrostatics with the TM theory[34,72] for nearest-neighbor correlations in binding free energies.

Another interesting avenue for exploration of binding of charged groups is to scrutinize the role of hydration water during binding:[130] How big a role does the release of waters from hydration shells play in binding?[131] While such interactions are ubiquitous, they remain incompletely understood. Recent theoretical and experimental studies by Abbott and coworkers on hydrophobic effects in peptide binding may provide useful new ways of addressing these issues.[132,133]

Although coacervation of PEs requires electrostatic interactions, the primary driving force has been recognized to be entropic.[53,54,57,60] While counterion release has been taken as the obvious source of increased entropy driving coacervation,[53,57] additional work has also identified the release of hydration waters,[10,60] as well as the combinatorial entropy of the arrangements of oppositely charged salt ions and monomers along the chain.[49,51,134] The combinatorial entropy can be calculated simply if ion pairing is uncorrelated along the chain, but is likely strongly influenced by binding correlations, considered at the nearest-neighbor level in the work of Lytle and Sing.[48] Thus, the significance of each of these mechanisms is still only partly resolved;[135] studies of coacervation in a dielectric medium by Muthukumar[53] and Whitmer[54] and their co-workers suggest that counterion release is the primary driver of coacervation, while Han, Shea, Fredrickson and co-workers[60] point to water release as a major coacervation driving force. These two mechanisms are chemically specific and distinguishing the significance of each in specific systems under various solvent conditions (e.g., salt, pH) would be an attractive target for future research.

To date, in coacervate theories with closed-form expressions for free energy, the chain structure, used for example in the RPA theory, is taken as given and not allowed to respond to the concentration of species or the charge or ion-binding state of the PE. This oversimplification was recently addressed for a single-component PE by Shen and Wang through self-consistent adjustment of chain structure in response to concentrations of species in the solution.[63] An interesting future effort would be to incorporate this formulism into coacervate theories involving oppositely charged PEs, and to allow charge regulation as well as species concentrations to affect the PE conformation.

Lastly, although the current PE theories are limited to coacervates with uniform distribution of PEs, PEs form a wide variety of soft materials in aqueous solutions, including layer-by-layer films,[28,136] hydrogels containing lamellae, or hexagonally packed cylinders,[26] complexes of PEs and oppositely charged surfactant micelles,[137,138] and nano- or micro-particle complexes.[20,29,139,140] These morphologies continue to attract strong interest due to their novel applications, especially in the area of biotechnology. For instance, overcharged nanocomplexes containing DNA or RNA are being used to transport genetic materials into cells, for gene therapy or vaccine delivery. Improved theoretical understanding of these new morphologies would help these applications to realize their full potential.

ACKNOWLEDGMENT

This work was supported by the National Science Foundation under Grant No. 2100513. Any opinions, findings, and conclusions or recommendations expressed in this material are those of the authors and do not necessarily reflect the views of NSF.

CONFLICT OF INTEREST

The authors declare no potential conflict of interest.

DATA AVAILABILITY STATEMENT

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

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How to cite this article: Ghasemi M, Larson RG. Future directions in physiochemical modeling of the thermodynamics of polyelectrolyte coacervates. *AIChE J*. 2022;68(5):e17646. doi:10.1002/aic.17646