

# Supporting Information (SI)

## Overcharging of Polyelectrolyte Complexes: An Entropic Phenomenon

Mohsen Ghasemi,<sup>1</sup> Sean Friedowitz,<sup>2</sup> and Ronald G. Larson<sup>1\*</sup>

<sup>1</sup>*Department of Chemical Engineering, University of Michigan, Ann Arbor, Michigan 48109, USA*

<sup>2</sup>*Department of Chemical Engineering, Stanford University, Stanford, California 94305, USA*

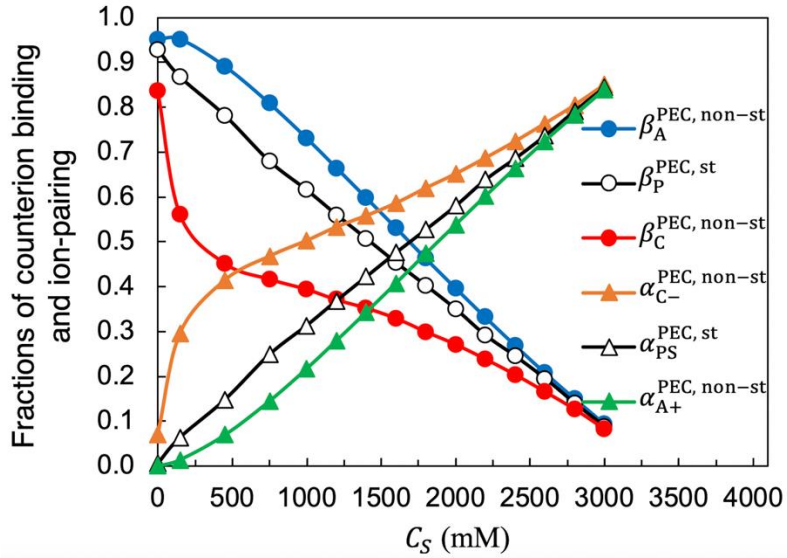


Figure S1. Fractions of ion-pairing and counterion binding for each type of polyelectrolyte in the stoichiometric and overcharged complexes as a function of salt concentration. Filled and open symbols correspond to non-stoichiometric and stoichiometric cases, respectively. The parameters have the standard values listed in the main text.

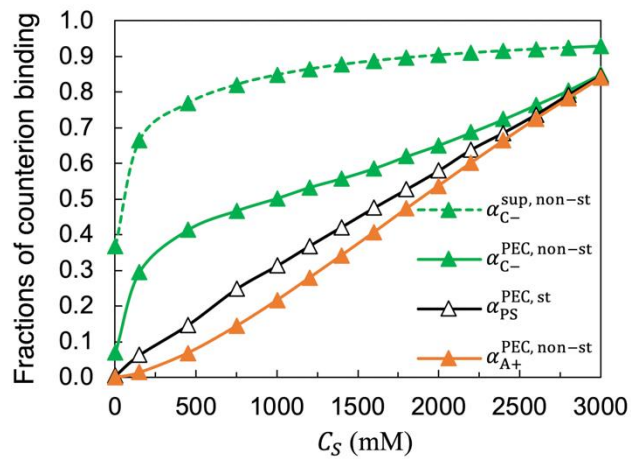


Figure S2. Fractions of counterion binding for polyelectrolytes during overcharging as functions of added salt concentration. Dashed line corresponds to the supernatant phase. The parameters are assigned their standard values.

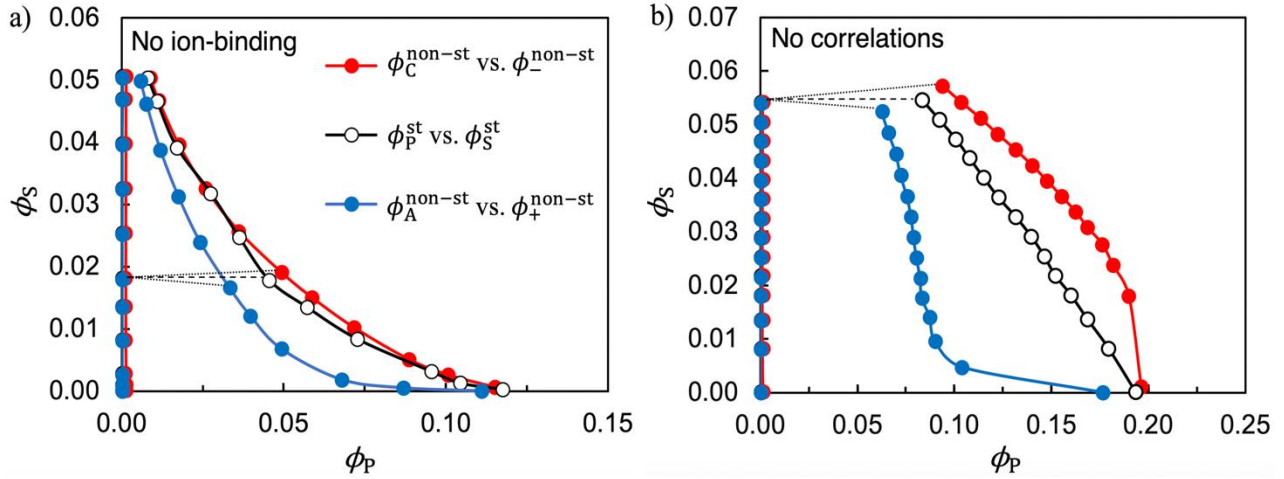


Figure S3. Binodal diagrams for the stoichiometric (open symbols) and non-stoichiometric (filled symbols) solutions for the two cases of a) no ion-bindings with  $f = f^T + f^\chi + f^{\text{corr}}$  and b) no electrostatic correlations with  $f = f^T + f^\chi + f^{\text{rxn}} + f^{\text{comb}}$ . The parameters are assigned their standard values.

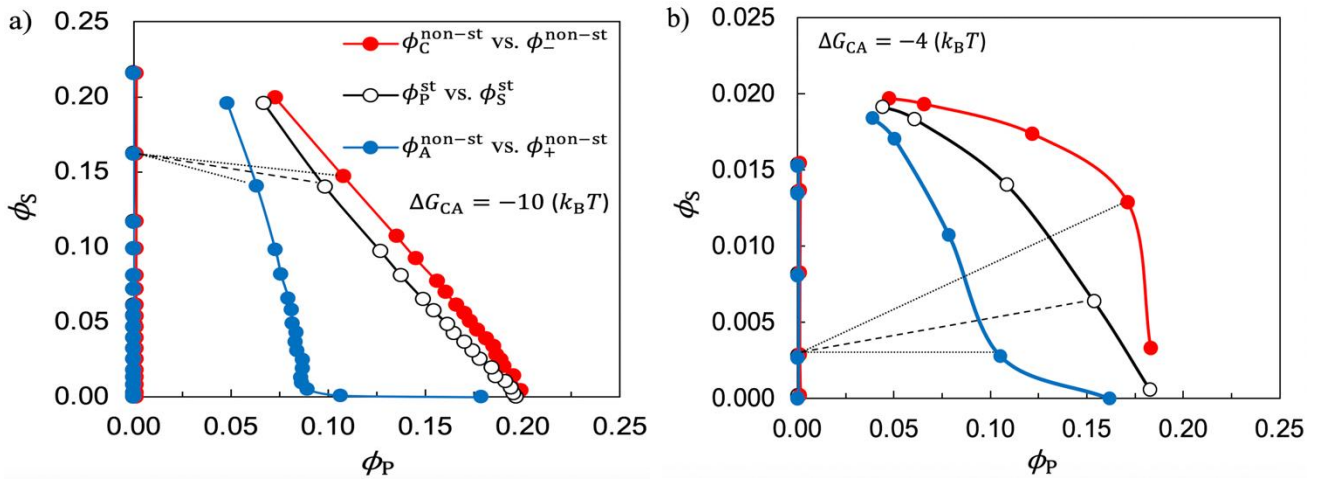


Figure S4. Binodal diagrams for the stoichiometric (open symbols) and non-stoichiometric (filled symbols) solutions for the strengths of ion-pairing of a)  $\Delta G_{CA} = -10 (k_B T)$  and b)  $\Delta G_{CA} = -4 (k_B T)$ . All other parameters are assigned their standard values.

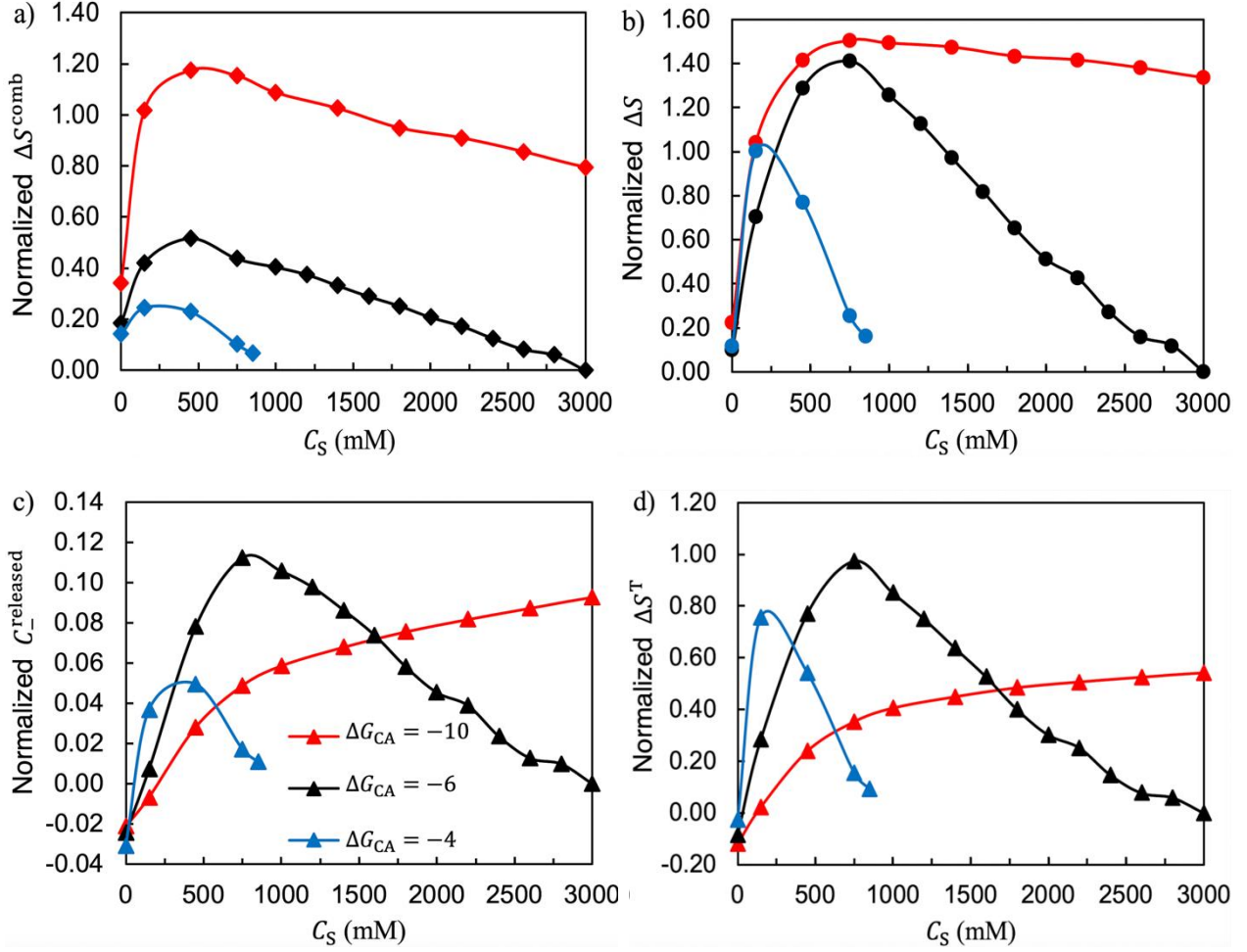


Figure S5. (a) The combinatorial entropy contribution ( $\frac{\Delta S^{\text{comb}}}{N_{\text{Avo}} k_B V C_A}$ ) to (b) the net entropy gain ( $\frac{\Delta S}{N_{\text{Avo}} k_B V C_A}$ ) against added salt concentration for various values of ion-pairing strength  $\Delta G_{CA}$  (in units of  $k_B T$ ). (c) and (d) show the normalized concentration of released anions from polycations  $C_-^{\text{released-n}}/C_A$  and the translational entropy contribution ( $\frac{\Delta S^T}{N_{\text{Avo}} k_B V C_A}$ ), respectively. In the normalization factors above, we use the overall concentration of polyanion,  $C_A = 0.1$  mM. All other parameters are assigned their standard values.

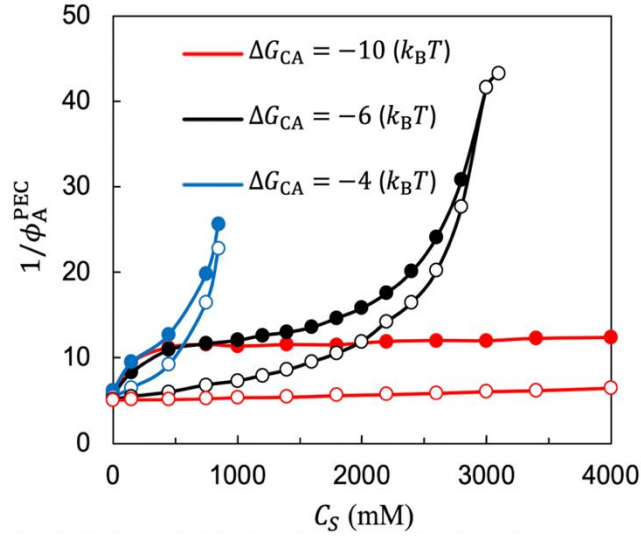


Figure S6. The swelling ratio  $1/\phi_A^{\text{PEC}}$  for the stoichiometric (open circles) and overcharged (filled circles) complexes against added salt for various values of the ion-pairing strength  $\Delta G_{CA}$ . All other parameters are assigned their standard values.

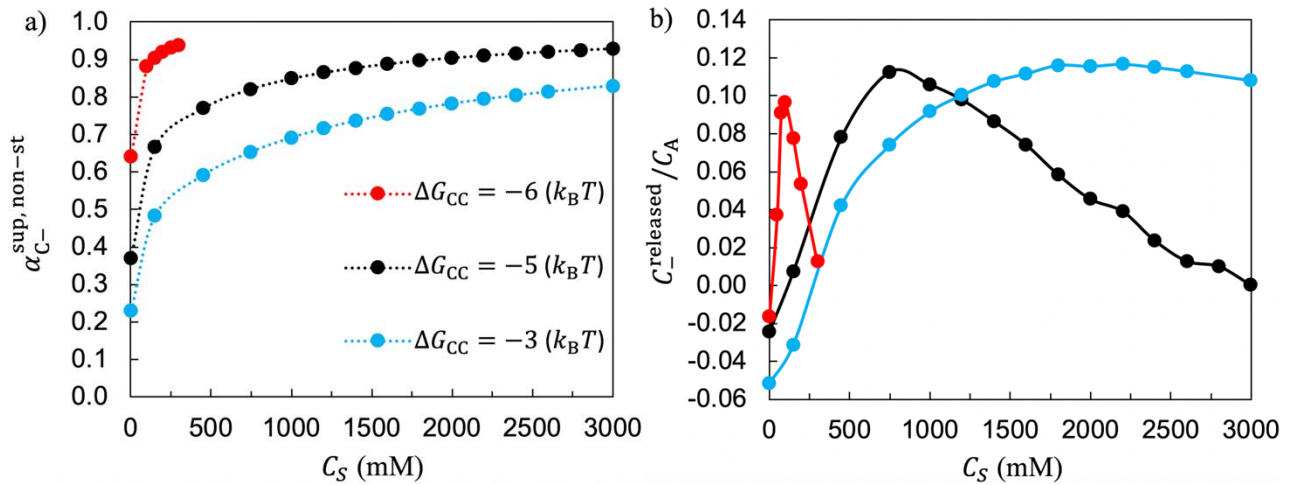


Figure S7. (a) Fraction of counterion binding along excess polycations in the supernatant phase of the non-stoichiometric mixture, and (b) the molar concentrations of released anions as a result of overcharging for various values of  $\Delta G_{CC}$ . All other parameters are assigned their standard values.

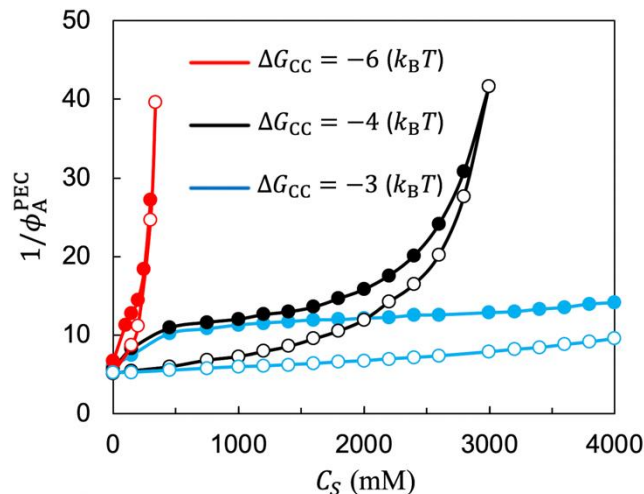


Figure S8. The swelling ratio  $1/\phi_A^{\text{PEC}}$  for the stoichiometric (open circles) and overcharged (filled circles) complexes against added salt concentration for various values of the strength of salt binding to the polyelectrolytes  $\Delta G_{\text{CC}}$ . All other parameters are assigned their standard values.

### Overcharging at zero added salt

Here, we investigate the role of combinatorial binding entropic in adsorption of excess polycation by a stoichiometric complex at zero salt concentration. A similar analysis can be made to rationalize the higher adsorption of excess polycation for higher ion-pairing strengths at a fixed salt concentration. Assume there are  $j_{\text{C1}}$  polycation chains in the stoichiometric complex and,  $j_{\text{C2}}$  is the number of polycation chains (if any) adsorbing from the supernatant, amounting to a total of  $j_{\text{Ct}} = j_{\text{C1}} + j_{\text{C2}}$  polycation chains in the overcharged complex.

Since in the stoichiometric complex polyelectrolytes are almost fully ion-paired (see Figure S1,  $\beta_{\text{p}}^{\text{PEC, st}} \approx 90\%$ ), for simplicity, we take them to be fully ion-paired, ( $\beta_{\text{p}}^{\text{PEC, st}} = 1$ ), which is a good assumption at zero salt or for the case of very strong ion-pairing. On the other hand, Figure S2 tells us that 40% of the monomer units of each polycation chain in the supernatant phase are counterion-bound, and the rest are free. Here, we assume that bound counterions along the adsorbing, excess, polycation chains are re-distributed along all polycations in the (overcharged) complex, including those transferred into the complex; i.e. the number of bound counterions remains

un-changed during overcharging at zero salt. Therefore, the fraction of (all) polycation monomers harboring anions in the overcharged complex becomes  $\alpha_{C-}^{\text{PEC, non-st}} = 0.4 j_{C2}/j_{Ct}$ .

We can write the change in the combinatorial entropy of the solution as,

$$\Delta S^{\text{comb}} = [(S_A^{\text{comb, PEC, non-st}} - S_A^{\text{comb, PEC, st}}) + (S_C^{\text{comb, PEC, non-st}} - S_C^{\text{comb, PEC, st}} - S_{\text{excess-C}}^{\text{comb, sup, non-st}})] \quad (\text{S1})$$

Using eqs 7 and 8, the condition of fully ion-paired polyelectrolytes in the stoichiometric complex leads to  $S_A^{\text{comb, PEC, st}} = S_C^{\text{comb, PEC, st}} = 0$ .

Note that adsorption of excess polycation does not break ion-pairs; thus, polyanion monomers remain fully ion-paired after overcharging at zero salt (see  $\beta_A^{\text{PEC, non-st}} \approx 1$  in Figure S1), leading to  $S_A^{\text{comb, PEC, non-st}} = 0$  using eq 7.

So, eq S1 simplifies to,

$$\Delta S^{\text{comb}} = S_C^{\text{comb, PEC, non-st}} - S_{\text{excess-C}}^{\text{comb, sup, non-st}} \quad (\text{S2})$$

with combinatorial entropies from eqs B9 and B11 (in Appendix B),

$$\frac{S_C^{\text{comb, PEC, non-st}}}{k_B V N_{\text{Avo}}} = - \frac{\phi_C^{\text{PEC, non-st}} v^{\text{PEC, non-st}}}{\omega_C v_W N_{\text{Avo}}} \times [\alpha_{C-} \ln \alpha_{C-} + \beta_C \ln \beta_C + (1 - \alpha_{C-} - \beta_C) \ln(1 - \alpha_{C-} - \beta_C)]^{\text{PEC, non-st}} \quad (\text{S3})$$

$$\frac{S_{\text{excess-C}}^{\text{comb, sup, non-st}}}{k_B V N_{\text{Avo}}} = - \frac{(\phi_C^{\text{PEC, non-st}} v^{\text{PEC, non-st}} - \phi_P^{\text{PEC, st}} v^{\text{PEC, st}})}{\omega_C v_W N_{\text{Avo}}} \times [\alpha_{C-} \ln \alpha_{C-} + \beta_C \ln \beta_C + (1 - \alpha_{C-} - \beta_C) \ln(1 - \alpha_{C-} - \beta_C)]^{\text{sup, non-st}} \quad (\text{S4})$$

Here, we note that the polycation volume fractions can be converted into the corresponding chain numbers using,

$$j_{C1}/V = \phi_C^{\text{PEC, st}} v^{\text{PEC, st}} / \omega_C v_W N_C \quad (\text{S5})$$

$$j_{C2}/V = (\phi_C^{\text{PEC, non-st}} v^{\text{PEC, non-st}} - \phi_P^{\text{PEC, st}} v^{\text{PEC, st}}) / \omega_C v_W N_C \quad (\text{S6})$$

$$j_{Ct}/V = \phi_C^{\text{PEC, non-st}} v^{\text{PEC, non-st}} / \omega_C v_W N_C \quad (\text{S7})$$

We also note that  $\beta_C^{\text{sup, non-st}} = 0$  and  $\alpha_{C-}^{\text{sup, non-st}} = 0.4$  at zero added salt. Using these values and eqs S4 and S6, the combinatorial entropy of excess polycations adsorbing from the supernatant phase can be written as,

$$\frac{S_{\text{excess-C}}^{\text{comb, sup, non-st}}}{k_B V N_{\text{Avo}}} = \frac{j_{C2} N_C}{V N_{\text{Avo}}} \times 0.67 \quad (\text{S8})$$

where the factor 0.67 arises from computing the bracketed term in eq S4 (using the values  $\beta_C^{\text{sup, non-st}} = 0$  and  $\alpha_{C-}^{\text{sup, non-st}} = 0.4$ ). After adsorption, as obtained above, the fraction of counterion-bound polycation monomers is  $\alpha_{C-}^{\text{PEC, non-st}} = 0.4 j_{C2}/j_{Ct}$ . On the other hand, since all polyanion monomers remain paired, one can obtain a relationship between the total number of polycations and fraction of ion-pairing,  $\beta_C^{\text{PEC, non-st}}$ , in the (overcharged) complex as,

$$j_{Ct} = j_{C1} \frac{1}{\beta_C^{\text{PEC, non-st}}} \quad (\text{S9})$$

or

$$j_{C2} = j_{Ct} - j_{C1} = j_{C1} \left( \frac{1}{\beta_C^{\text{PEC, non-st}}} - 1 \right) \quad (\text{S10})$$

Therefore, we can re-write eq S3 for the combinatorial entropy of all polycations in the overcharged complex using eqs S7 and S9 as,

$$\begin{aligned} \frac{S_C^{\text{comb, PEC, non-st}}}{k_B V N_{\text{Avo}}} &= - \frac{j_{C1} N_C}{\beta_C^{\text{PEC, non-st}} V N_{\text{Avo}}} \\ &\times [\alpha_{C-} \ln \alpha_{C-} + \beta_C \ln \beta_C + (1 - \alpha_{C-} - \beta_C) \ln(1 - \alpha_{C-} - \beta_C)]^{\text{PEC, non-st}} \end{aligned} \quad (\text{S11})$$

Substituting eqs S8 and S11 into eq S2, we get the change in combinatorial entropy due to adsorption of excess polycations, which is a function of only the number of polycation chains in the stoichiometric complex,  $j_{C1}$ , and the fraction of ion-pairing for polycations  $\beta_C^{\text{PEC, non-st}}$  after adsorption (note  $\alpha_{C-}^{\text{PEC, non-st}} = 0.4 j_{C2}/j_{Ct}$ ),



$$\frac{\Delta S^{\text{comb}}}{k_B V N_{\text{Avo}}} = -\frac{j_{C1} N_C}{\beta_C^{\text{PEC, non-st}} V N_{\text{Avo}}} \times [\alpha_{C-} \ln \alpha_{C-} + \beta_C \ln \beta_C + (1 - \alpha_{C-} - \beta_C) \ln(1 - \alpha_{C-} - \beta_C)]^{\text{PEC, non-st}} + \frac{j_{C2} N_C}{V N_{\text{Avo}}} \times 0.67 \quad (\text{S12})$$

One can readily see that if  $\beta_C^{\text{PEC, non-st}} = 1$ , i.e. the fraction of polycation monomers bound to polyanion monomers in the (overcharged) complex is unity and polycations already present in the stoichiometric complex do not move off some of the polyanion monomers and open space for the adsorbing, excess polycations, then  $\Delta S^{\text{comb}} = 0$  and no excess polycation is adsorbed ( $j_{C2} = 0$  from eq S10).

To analyze the adsorption when  $\beta_C^{\text{PEC, non-st}} < 1$ , we normalize the entropy in eq S12 by the solution concentration of polyanions  $C_A = 0.1$  mM. Note that in the stoichiometric complex the number of polyanion and polycation chains are equal to each other ( $= j_{C1}$ ), and this normalization therefore removes the dependency of the combinatorial entropy on  $j_{C1}$ .

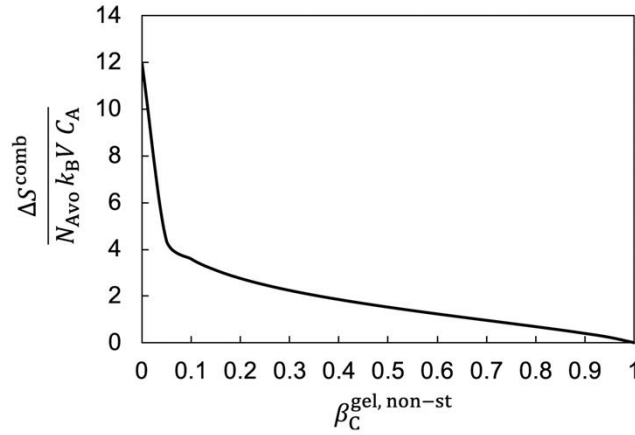


Figure S9. Combinatorial entropy change upon adsorption of excess polycations at zero salt concentration as a function of ion-pairing fraction of polycations in the (overcharged) complex, from eq S12. The parameters are assigned their standard values.

Figure S9 shows the change in combinatorial binding entropy upon overcharging at zero salt as a function of  $\beta_C^{\text{PEC, non-st}}$ . Note that if  $\beta_C^{\text{PEC, non-st}} < 1$  and substituting  $\alpha_{C-}^{\text{PEC, non-st}} = 0.4 j_{C2}/j_{Ct}$ , we get  $\Delta S^{\text{comb}} > 0$  and  $j_{C2} > 0$ , indicating that a gain in combinatorial binding entropy due to transfer of excess polycations into the complex. The stoichiometry of ion-pairing dictates that the lower the extent of ion-pairing per polycation chain in

the final complex, the more polycations have been transferred to the complex to maintain polyanions fully ion-paired, as shown by eq S10. This result is valid when all polyanions charges remain ion paired, which is approximately valid at zero salt concentration or when the strength of ion-pairing between the charged groups of oppositely polyelectrolytes is strong. However, note that we do not expect  $\beta_C^{\text{PEC, non-st}}$  to be small at equilibrium at zero salt because this would correspond to an increase in the number of free (or unpaired) polycation monomers per chain and therefore, to strong electrostatic repulsions in the PEC. This would be unfavorable, and we actually find that  $\beta_C^{\text{PEC, non-st}} = 0.84$  (in Fig. S1) for the parameter values of our qualitative study. Although the release of counterions is highly favorable at low salt concentrations, we note that, instead, a small number of additional counterions actually bind to the polycations as polycations enter the complex upon overcharging at zero salt (see the small negative values for  $C_-^{\text{released}}$  in Figure 2 or  $\frac{\Delta S^T}{N_{\text{Avo}} k_B V C_A}$  in Figure 3). We conjecture that this binding of additional counterions at zero salt is driven by a further gain in combinatorial entropy and/or to relieve the electrostatic repulsion between the polycation monomers.

## Effect of hydrophobicity

Here, we explore how the hydrophobicity of polyelectrolytes affects the adsorption of excess polycation. One experimental approach to fine-tuning the degree of hydrophobicity of polyelectrolytes is to introduce co-solutes, such as ethanol, into the solution.<sup>1</sup> To model the backbone hydrophobicity, we vary the Flory-Huggins  $\chi_{PW}$  parameter between polymer and water, while keeping all other parameters untouched. The backbone hydrophobicity of the polycations and polyanions are treated identically, i.e.  $\chi_{PW} \equiv \chi_{AW} = \chi_{CW}$ .

As can be seen from Figure S10b, small changes in  $\chi_{PW}$  lead to large differences in the extent of adsorbed polycation. For instance, for  $\chi_{PW} = 0.6$ , the amount of adsorbed polycation can even reach up to almost 180% of the stoichiometric amount. For lower values of  $\chi_{PW}$ , both the binodal envelop and the overcharging window shrink, suggesting that more hydrophilic polyelectrolytes can more easily leave the complex upon increasing salt concentration. Complexes formed from hydrophobic polymers, on the other hand, are more salt resistant (Figure S10a) and absorb (hydrophobic) polymers more effectively (Figure S10b).

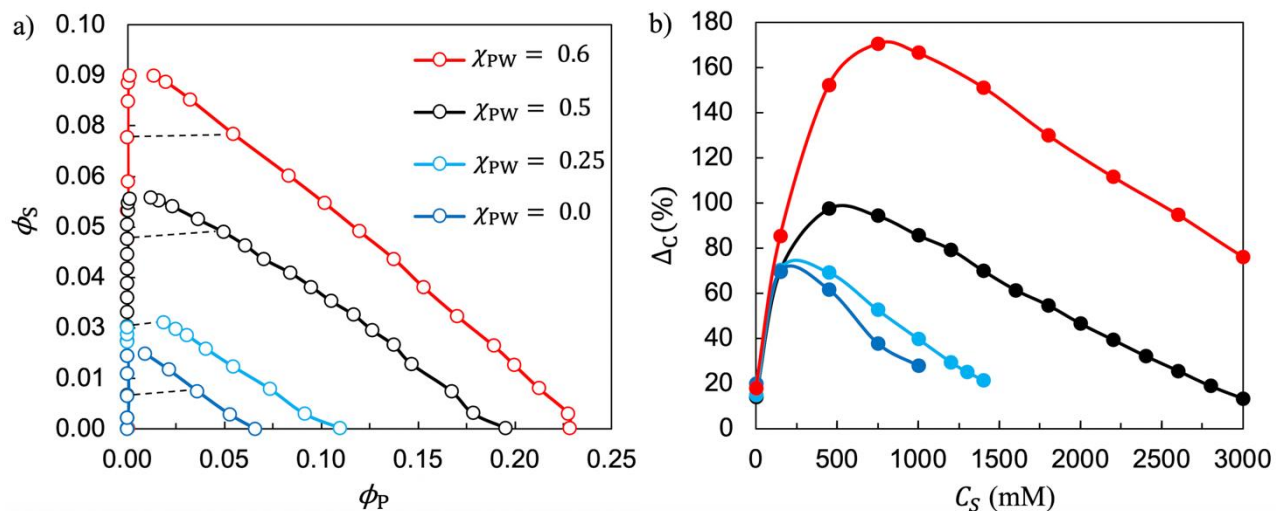


Figure S10. (a) Stoichiometric binodal phase diagrams, and (b) extents of polycation overcharging against added salt concentration (for the non-stoichiometric mixture) for different values of the Flory-Huggins  $\chi_{PW}$  parameter. All other parameters are assigned their standard values.

Interestingly, for the most hydrophobic polymers studied ( $\chi_{PW} = 0.6$ ), the volume fraction of the PEC phase becomes very small and varies linearly with the added salt (see Figure S11), similar to the case of polyelectrolytes with strong ion-pairs ( $\Delta G_{CA} = -10 k_B T$ ) studied in the main text. Thus, we observe that the effect of hydrophobicity on complexation of polyelectrolytes is qualitatively similar to that of specific ion-pairing between oppositely charged polyelectrolytes, in that the PEC becomes smaller, more salt resistant, and more overcharged in the non-stoichiometric mixture, as either  $\chi_{PW}$  or  $\Delta G_{CA}$  increase. To highlight this analogy, the term “hydrophobic ion-pairing” has been introduced in the literature.<sup>1,2</sup>

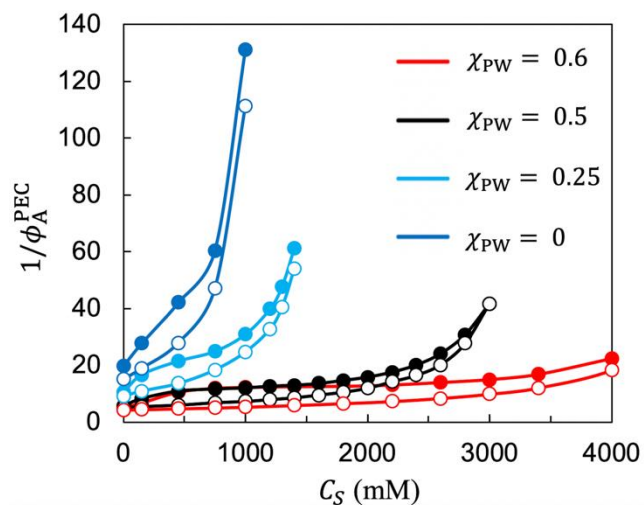


Figure S11. The swelling ratio  $1/\phi_A^{PEC}$  for the stoichiometric (open circles) and overcharged (filled circles) complexes against added salt for various values of water-polymer chi parameter  $\chi_{PW}$ . All other parameters are assigned their standard values.

## References

1. S. T. Dubas and J. B. Schlenoff, *Macromolecules*, 1999, **32**, 8153-8160.
2. H. D. Lu, P. Rummanethorn, K. D. Ristorph and R. K. Prud'homme, *Molecular Pharmaceutics*, 2018, **15**, 216-225.