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Complexation of Polyelectrolytes with Hydrophobic Drug Molecules in Salt Free Solution: Theory and Simulations

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Abstract

The delivery and dissolution of poorly soluble drugs is challenging in pharmaceutical industry. One way that can significantly improve the delivery efficiency is to incorporate these hydrophobic small-molecules into colloidal polyelectrolytes(PE)-drug complex in their ionized states. Despite its huge application value, the general mechanism of PE collapse and complex formation in this system has not be well understood. In this work, by combining a mean-field theory with extensive molecular simulations, we unveil the phase behaviours of the system in the dilute and salt-free condition. We find that the complexation is a first-order-like phase transition triggered by the hydrophobic attraction between the drug molecules. Importantly, the valence ratio between the drug molecule and PE monomer plays a crucial role in determining the stability and morphology of the complex. Moreover, the sign of zeta potential and net charge of the complex is found inverted as the hydrophobicity of drug molecules increases. Both theory and simulation indicate that the complexation point and complex morphology, as well as the electrostatic properties of the complex, have a weak dependence on chain length. At last, the dynamics aspect of PE-drug complexation is also explored, and it is found that the complex can be trapped into a non-equilibrium glass-like state when the hydrophobicity of drug molecule is too strong. Our work gives a clear physical picture behind the PE-drug complexation phenomenon, and provides guidelines to fabricate colloidal PE-drug complex with desired physical characteristics.

I. INTRODUCTION

Collapse of polyelectrolytes (PE) is a fundamental issue both in biological and chemical areas. In biology, this simple phenomenon can be related to protein fold, DNA and chromatin condensations in nucleus.¹ In chemistry, the collapse of PE is found to be induced by poor solvent², pH³, multivalent ions⁴, macro-ion like proteins⁵, ionic surfactants⁶, or opposite charged PE⁷⁻¹⁰. The resulting product of the three later cases are usually called “polyelectrolytes complex”, which has wide applications in functional nano materials, gene therapy, drug delivery and pharmaceutical industry.¹¹⁻¹⁵

The mechanism of PE collapse and the formation of PE complex have attracted intensive attentions in the theoretical community.¹⁶⁻³² For examples, using molecular simulations as well as theories, Limbach and Holm²⁰ and other theoretical groups²¹⁻²⁵ studied the phase behaviour of a single PE chain in poor solvent, and found that the interplay between short-range hydrophobic interactions and long-range Coulomb interactions can lead to complex with either globular or pearl-necklace-like structures. Brillianov et. al^{26,33} studied the correlation-induced collapse of PE chains in the salt free condition based on a simple mean-field theory and obtained some useful scaling relationships between the size of PE and the electrostatic coupling strength. Relying on a different theory, Solis and de la Cruz²⁷ explained the collapse of flexible PE in multivalent salt solutions, while Hsiao and Luijten²⁸ using molecular simulations further confirmed that multivalent salt can cause collapse as well as re-expansion for highly charged flexible PE. At the same time, many mean-field theories were proposed to explain the complexation between oppositely charged PE.²⁹⁻³² Besides, there are also many simulation works focusing on the formation of polyelectrolytes-macroion complex^{19,34-37} and surfactant-polyelectrolytes complex³⁸⁻⁴¹ in view of their potential application value.

Despite the large amount of previous works on the behaviour of PE chain associating with other components, little theoretical attention have been paid to the polyelectrolytes-drug complex systems, where the collapse of PE chain is intrigued by the condensation of opposite charged hydrophobic drug molecules.⁴²⁻⁴⁵ The resulting product, amorphous colloidal PE-drug complex (or amorphous drug nanoplex) has recently emerged as an ideal formulation to enhance the dissolution of poorly soluble drugs⁴⁶⁻⁵¹, which constitute more than 70% of drugs in test.⁵²

Experimentally, successful preparation of colloidal PE-drug complex has been demonstrated for a wide range of small-molecule drugs (i.e. antibiotics, antifungal, anti-inflammatory, anticancer).^{46,48,53} But there is still a lack of understanding on the influences of different variables, such as (1) drug hydrophobicity, (2) charge ratio of drug to polyelectrolytes, (3) PE chain length etc. on the feasibility of forming complex and the resultant morphology. A better understanding of the drug nanoplex formation mechanism would greatly help us reduce the development efforts of drug nanoplex. Moreover, it would also enable us to conceptually design drug nanoplex with specific functionalities (e.g. controlled drug release, targeted delivery).

In the present work, by combining a mean-field theory with molecular dynamics simulations, we study the formation of PE-drug complex in the dilute and salt-free solution. We first construct a theoretical framework, based on which the free energy landscape of PE-drug complex system and some general physical mechanism about the behaviours of the system are obtained. Then, with the helps from molecular simulations, we verify that the hydrophobicity of drug molecules and the valence ratio between PE monomer and drug molecule are two important factors to determine the stability, morphology and zeta potential of PE-drug complex.

In the following, we will first introduce the PE-drug complex model and the mean-field theory in Section II A. Then the description of simulation technique is briefly given in Section II B. After that, detailed results and discussions are presented in Section III, followed by the conclusion in section IV.

II. EXPERIMENTAL

A. Model and Theory

In our study, we focus on a dilute and salt-free polyelectrolytes solution that contains excess ionized drug molecules. In this condition, the distance between two adjacent PE chains is large enough that each chain can be viewed as that it occupies a single volume cell with size R_0 . In each cell, there are corresponding amount of drug molecules, as well as the associating counterions for both PE and drug molecules (see Figure 1). The drug molecules are intrinsically hydrophobic. Being ionized state, they will not aggregate by themselves

FIG. 1: Coarse-grained model of PE-drug systems. The red chain represents the PE, whereas the blue beads indicate the drug molecules. Green and orange beads are the cations and anions, respectively. The solvents are treated implicitly, regarded as dielectric medium with a relative permittivity ϵ .

due to the strong electrostatic repulsion. But with the helps from the opposite-charged PE, aggregations may occur by the formation of PE-drug complex. Furthermore, we also restrict our study on the electrostatic coupling regime where PE chain will not collapse due to the counterions condensation.^{9,26} This condition is close to that in experiments.⁵³ Therefore, different from previous studies, the formation of complex here requires the help from opposite-charged hydrophobic drug molecules. For simplicity, in our study, we use the same unit length a as the size of PE monomer and drug molecule and set the Bjerrum length of the systems $l_B = \frac{e^2}{4\pi\epsilon k_B T}$ as $l_B/a = 1$. The PE chain length is N . The drug molecules are assumed to be 1 time excess if no further instructions are given.

Our theoretical description of the PE-drug complexation is based on the recent theoretical work³³ on the collapse of strongly charged polyelectrolytes. This simple but powerful theory can describe very well not only separated chains, but also concentrated PE solutions⁵⁴ or PE systems containing dipolar-dipolar interactions⁵⁵. One important assumption that we make is that the free energy of our four-components systems (PE, drug, cation, anion) can be simplified as a function of two variables (or order parameters). One is the expansion factor of the PE, i.e. $\alpha = R_g/R_{g,id}$. Here R_g is the radius of gyration of PE with $R_{g,id} = \sqrt{Na^2/6}$

representing the radius of gyration of an ideal random-walk chain. The another one is the amount of drug molecules condensed on the PE, i.e. N_d . The validity of this simplification is tested later in our molecular simulations, where we find that counterions from either PE or drug molecules are excluded from the complex. In the following, we will briefly introduce different contributions to the free energy of the system.

For polyelectrolytes in dilute solution, the free energy contribution from chain's conformational entropy can be written as⁵⁶

$$\beta F_{conf} = \frac{9}{4} \left(\alpha^2 + \frac{1}{\alpha^2} \right). \quad (1)$$

The non-electrostatic interactions in the complex are described by the Flory-Huggins parameter and high order virial coefficients, namely,

$$\beta F_{ne} = \frac{9}{4} \left[\frac{[B^*(1+\rho_d)^2 + \chi\rho_d^2]N^{1/2}}{\alpha^3} + \frac{C^*(1+\rho_d)^3}{\alpha^6} \right] \quad (2)$$

where $\rho_d = N_d/N$ is the reduced number of drug molecules in the complex. B^* and C^* are the reduced second and third virial coefficients in the complex which are set both to 1 for simplicity. χ is a negative Flory-Huggins parameter⁵⁷, which describes the energy decrease when hydrophobic contacts between drug molecules are formed.

The free energy contribution from electrostatic interactions can be written as²⁶:

$$\frac{\beta F_{elect}}{N} = \frac{3\sqrt{6}l_B N^{1/2}}{5\alpha a} (Z_p - Z_d \rho_d)^2 - \frac{3\sqrt{6}}{2} \left(\frac{2}{\pi^2} \right)^{1/3} \frac{l_B Z_d^2 Z_p^2 \rho_d^{4/3}}{N^{1/6} \alpha a} \quad (3)$$

where the first term is due to the screened Coulombic interactions in the complex and will vanished if the charge on PE is totally neutralized by the absorbed drug molecules. The second term comes from the One-Component-Plasma (OCP) approximation from charge correlation effects. The Z_p and Z_d are the valencies of PE monomers and drug molecules, respectively. Theoretically, the electrostatic coupling regime we focus corresponds to $l_B/a \ll \ln R_0^3$, which makes the charge correlation a non-dominant factor in our systems.²⁶

The free energy contribution from the translational entropy of free drug molecules is

$$\beta F_{trans} = -N(\rho_{d0} - \rho_d) \ln R_0^3 \quad (4)$$

where $\rho_{d0} = N_{d0}/N$ is the reduced number of drug molecules in the cell volume. In our studies, we assume that the drug molecules are 1 time excess i.e. $\rho_{d0} = 2Z_p/Z_d$, which means that about half of drug molecules can still be in free state after fully neutralizing the

PE. It should be mentioned that the translational entropy of the counterions is non-trivial. Nevertheless, since their densities are unchanged during the complexation, their contribution to the free energy is a constant and can be neglected. Moreover, the combination term $N(\rho_{d0} - \rho_d) \ln[N(\rho_{d0} - \rho_d)]$ is also omitted here, since in the dilute limit ($R_0 \gg Na$), this term is negligible compared with the Eq. (4).

Combining all the terms above, the free energy of system per PE monomer can be written as:

$$\begin{aligned} \frac{\beta F}{N} = & \frac{9}{4N} \left[\alpha^2 + \frac{1}{\alpha^2} + \frac{[B^*(1 + \rho_d)^2 + \chi \rho_d^2] N^{1/2}}{\alpha^3} + \frac{C^*(1 + \rho_d)^3}{\alpha^6} \right] \\ & + \frac{3\sqrt{6} l_B N^{1/2}}{5\alpha a} (Z_p - Z_d \rho_d)^2 - \frac{3\sqrt{6}}{2} \left(\frac{2}{\pi^2} \right)^{1/3} \frac{l_B Z_d^2 Z_p^2 \rho_d^{4/3}}{N^{1/6} \alpha a} \\ & - (\rho_{d0} - \rho_d) \ln R_0^3. \end{aligned} \quad (5)$$

Since F is only a function of α and ρ_d , a two dimension free energy landscape could be obtained to help identifying the transition points of PE-drug complexation as shown later.

B. Simulation Details

Apart from the mean-field theory, we further employ molecular dynamics simulations to investigate our systems. We adopt a coarse-grained model where the PE chain is modelled as a spring-bead chain and the drug molecules are simplified as single beads with no-internal structures. The charge neutrality is ensured by adding corresponding amount of monovalent counter-cations and counter-anions that associate with PE and drugs, respectively. The solvent is treated implicitly and regarded as dielectric medium with a permittivity ϵ . Same as in the mean-field theory, we use a single length parameter σ to represent the size of PE monomer, counterions, as well as drug molecules.

The short range interaction between two beads i, j is modeled by truncated and shifted Lennard-Jones potential,

$$u_{i,j}^{LJ} = \begin{cases} 4\epsilon_{LJ} \left[\left(\frac{\sigma}{r_{i,j}} \right)^{12} - \left(\frac{\sigma}{r_{i,j}} \right)^6 - \left(\frac{\sigma}{r_c} \right)^{12} + \left(\frac{\sigma}{r_c} \right)^6 \right] & (r_{i,j} < r_c) \\ 0 & (r_{i,j} > r_c) \end{cases} \quad (6)$$

where $r_{i,j}$ is the distance between two beads, r_c is the cutoff distance, ϵ_{LJ} is the energy parameter. We set $\epsilon_{LJ}/k_B T = 1$ and $r_c = 2^{1/6} \sigma$ for all pairwise interactions except that between drug molecules, for which we choose $r_c = 2.5\sigma$ and let $\epsilon_{LJ}/k_B T$ (denoted as ϵ_d) vary from

0.5 to 5 to mimic different hydrophobicities of drug molecules. ϵ_d can be related with Flory-Huggins parameter in the mean-field theory through a relationship $\chi = n_c \epsilon_d / 2$, where $n_c = 6$ is the coordination number of cubic lattice.⁵⁷ For PE chain, since all monomer-monomer interactions are repulsive, the interaction potential corresponds to the good solvent for the polymer chain.

The long-range electrostatic interactions between charge beads are explicitly accounted for by using Coulomb potential,

$$u_{i,j}^{elect} = \frac{e^2}{4\pi\epsilon} \frac{Z_i Z_j}{r_{i,j}} = \frac{l_B Z_i Z_j}{r_{i,j}} k_B T \quad (7)$$

and are calculated with the particle-particle/ particle-mesh (PPPM) algorithm⁵⁸.

Neighbouring beads in the PE chain are connected by harmonic bond potential,

$$E_{i,i+1}^{bonded} = \frac{1}{2} k_b (r_{i,i+1} - r_0)^2 \quad (8)$$

with $k_b/k_B T = 256$ and equilibrium bond length $r_0/\sigma = 2^{1/6}$.⁵⁹

Our simulations are performed in a NVT ensemble using Langevin thermostat as the temperature controller. The typical PE chain we use has monomer number of $N = 200$. We fix its center of mass in the center of cubic box. For the systems that PE-drug valence ratio is $Z_p : Z_d = 1 : 1$, a box size $L = 200\sigma$ is used, while for other different valence ratio cases, we vary the box size to make sure that drug concentrations are the same. The integral time step size of the simulation is 0.01τ , where $\tau = \sqrt{\sigma^2 m / \epsilon}$ is time unit in the simulation. The total equilibrium time steps is in the order of 10^6 , followed by the same simulation time for sampling. All simulations are conducted by using the LAMMPS package (1 Feb 2016)⁶⁰, while OVITO⁶¹ is used for the visualization.

III. RESULTS AND DISCUSSION

A. Mechanism of PE-drug complexation

In order to explore the general mechanism of PE-drug complex formation, we first do some theoretical analyses of PE-drug system in the long chain and dilute limit based on our mean-field theory. We use the PE expanding factor $R_g/R_{g,id}$ and the net charge of the PE (complex) per monomer to construct the two dimension free-energy landscapes of two systems: one is for *hydrophilic* drug ($\chi = 0$) and the other is for *hydrophobic* one ($\chi = -19$),

FIG. 2: Free energy landscapes of PE-drug systems as functions of expanding factor of PE ($R_g/R_{g,id}$) and the net charge of PE-drug complex per monomer. (a) hydrophilic drug $\chi=0$. (b) hydrophobic drug $\chi=-19$. For both cases, $N=10^3, R_0=10^4$.

as given in Figure 2a and 2b respectively. The $R_g/R_{g,id}$ represents the size of PE(complex), while the net charge of the PE (complex) reflects the amount of drug molecules absorbed on the PE and to what extent the PE are neutralized. A same PE-drug valence ratio $Z_p:Z_d=1:1$ is used for both systems. And the chain length and cell size is chosen large enough ($N=10^3, R_0=10^4$) to make the system approach the long chain and dilute limit.

From Figure 2a, one can find that the free energy landscape of the hydrophilic drug systems has only one local minimum, which corresponds to the expanding state of PE chain and non-absorption of drug molecules. This implies that it is difficult for monovalent hydrophilic drug molecules to form a PE-drug complex. On the contrary, as shown in Figure 2b, the free energy landscape for the hydrophobic drug case clearly shows two local minima, with another one located at small $R_g/R_{g,id}$ and zero PE(complex) net charge area, which corresponds to a compact and highly neutralized PE-drug complex state. The saddle point between these two free energy minima indicates that PE-drug complex formation under this condition would be a sharp first-order like transition.

After revealing that the expanding state and collapsing state are two favourable states in PE-drug systems, it's natural to ask what is the thermodynamic force that stabilizes each states. According to the previous analysis²⁶, the leading terms of free energy for the

expanding state ($\rho_d \rightarrow 0$) are

$$\left. \frac{\beta F}{N} \right|_{\rho_d \rightarrow 0} \simeq \frac{9\alpha^2}{4N} + \frac{3\sqrt{6}l_B N^{1/2}}{5\alpha a} Z_p^2 - \frac{Z_p}{Z_d} \ln R_0^3, \quad (9)$$

from which the equilibrium expand factor $\alpha \sim (l_B/a)^{1/3} N^{1/2}$ can be obtained through the free energy minimization, which corresponds to the stretched chain, i.e., $R_g \sim N$. Re-substituting α into Eq. (9), we find that the first and second terms are negligible compared with the third one, which leads to:

$$\left. \frac{\beta F}{N} \right|_{\rho_d \rightarrow 0} \simeq -\frac{Z_p}{Z_d} \ln R_0^3 \quad (10)$$

This means that the dominant contribution to the free energy for the expanding state comes from the translational entropy of unbound drug molecules.

For the collapsing state ($\rho_d \rightarrow Z_p/Z_d$), the leading terms of the free energy are

$$\left. \frac{\beta F}{N} \right|_{\rho_d \rightarrow Z_p/Z_d} \simeq \frac{9}{4N} \left[\frac{\chi K^2 N^{1/2}}{\alpha^3} + \frac{C^*(K+1)^3}{\alpha^6} \right] \quad (11)$$

where $K = Z_p/Z_d$ and $B^* \ll \chi$ is assumed. After the free energy minimization, an equilibrium $\alpha \simeq \frac{(2C^*)^{1/3}(1+K)}{\chi^{1/3}K^{2/3}N^{1/6}}$ would give a minimal free energy of collapsing state as:

$$\left. \frac{\beta F}{N} \right|_{\rho_d \rightarrow Z_p/Z_d} \simeq -\frac{9\chi^2 K^4}{16C^*(1+K)^3} \quad (12)$$

Eq. (11), (12) show that it is the hydrophobic attraction between drug molecules that stabilizes the collapsing state. This force is balanced by the excluded volume repulsion (third virtual coefficients term), which results in a compact complex whose radius is proportional to $N^{1/3}$.

From the above analysis, one can see clearly that the hydrophobicity of drug molecules plays a crucial role in the formation of PE-drug complex. Without the hydrophobic attraction between drug molecules, the electrostatic attraction from PE alone is not sufficient enough to drive the formation of compact complexes in the electrostatic coupling region we studied. On the other hand, without the electrostatic attraction from PE, hydrophobic drug molecules in their ionized state are supposed to disperse in the solution without aggregation.

Moreover, the PE chain also sets a maximum limit to the number of drug molecules that can be absorbed into the complex, since the total charge of drug molecules in the complex should proximate to that on PE chain in view of the energy penalty of violating the charge neutrality in the complex. This can be seen clearly in Figure 2b, where the free energy

local minimal for the collapsing state is located near the zero charge point. Therefore, the hydrophobicity attractions between the drugs and electrostatic interactions in the system make an elegant cooperation to form the PE-drug complex.

FIG. 3: Simulation results of PEs radius of gyration as functions of the hydrophobic strength ϵ of drug molecules under different PE-drug valence ratios $Z_p:Z_d$. Chain length $N=200$.

B. The influence of PE-drug valence ratio

PE-drug valence ratio $K=Z_p:Z_d$ is an important variable that control the component fractions of PE and drug molecules in the complex. In this section, we want to study the effect of this variable on the formation of PE-drug complex. In fact, the general trend can be roughly predicted based on our mean-field theory in the long chain and dilute limit.

The basic idea is that at the complexation point, the free energies of expanding state and collapsing state should be equal. Based on Eq. (10), (12), the minimal hydrophobic strength of drug that need to form PE-drug complex can be obtained as

$$\chi_m \sim - \left[\frac{(1+K)^3}{K^3} \cdot C^* \cdot \ln R_0 \right]^{1/2}. \quad (13)$$

The decrease of χ_m as a function of K implies that the higher the PE-drug valence ratio, the easier for the hydrophobic drug molecules to form the complex, and most likely, the formed complex would be more stable. The mechanism behind this phenomenon can be obtained from the derivation of Eq. (13). Basically speaking, different K would result in different component fractions of drug in the complex due to charge neutrality. According to the free energy expression of collapsing state, i.e. Eq. (11), only the drug molecules contribute to the

hydrophobic attraction term, which is proportional to the square of drug molecules number in the complex i.e. K^2 . While for excluded volume repulsion, it exists between drug-drug, PE-drug and PE-PE, resulting in a third virtual coefficient term containing $(1+K)^3$ instead of pure drug case, K^3 . Therefore, decreasing K would result in unbalanced drops in the attraction and repulsion in the complex. The latter drops slower than the former, thus requiring a smaller χ to maintain the balance.

Our molecular dynamics simulations confirm this general trend. In Figure 3, we plot the radius of gyration of PE(complex) as a function of the hydrophobic attraction ϵ_d between drug molecules under different PE-drug valence ratios. As can be seen, increasing ϵ_d induces a sudden collapse of PE (formation of complex) for $Z_p:Z_d \geq 1:2$. The threshold value of ϵ_d for the collapsing decreases as $Z_p:Z_d$ increases from 1:2 to 3:1. One exception is the $Z_p:Z_d=1:3$ case, where the size of the PE is found to be close to that in θ condition⁵⁷, namely, $R_g/\sigma \sim N^{1/2}$, and less insensitive to the change of hydrophobic attraction. This is because the drug molecules that associate with PE are not likely to have contacts with each other in this situation in view of a low density of drug molecules in the complex and a stronger local electrostatic repulsion between themselves (proportional to the square of Z_d). However, due to their multivalent nature, the drug molecules under this condition can bridge multiple PE monomers from different chain sections, making the PE chain shrink. This is a pure electrostatic correlation effects²⁸, which explains the relatively small R_g for the cases of $Z_p:Z_d=1:3$ and 1:2.

It is also worth to mention that a recent theoretical work⁶² has shown that not only the valence but also the size of counterion can strongly influence collapsing behavior of PE chain. In our PE-drug system, the size effect of drug molecule would be more subtle, as the decrease of drug molecule's size not only strengthens the electrostatic interaction, but also weakens the hydrophobic attraction. Although detailed study of this effect is out of the scope of our present work, this issue is worth to be clarified in the future.

C. Phase diagram of PE-drug complexation

In this section, we focus on the morphologies of the PE-drug complexes under different valence ratios. Based on Figure 3, we identify five different morphologies, namely, the expanding state ($R_g/\sigma \gg N^{1/2}$), the θ condition state ($R_g/\sigma \sim N^{1/2}$), the necklace state, the

FIG. 4: Morphological phase diagram of the PE-drug systems in dimensions of drug's hydrophobicity ϵ and PE-drug valence ratio $Z_p:Z_d$. $N=200$; $L=200\sigma$.

sausage state and the compact globular state. We plot the morphological phase diagram of PE-drug complex in Figure 4.

Of particular interest is the last three phases. In the necklace state ($Z_p:Z_d=1:2$), small droplets of drug molecules are wrapped and connected by PE chain. At some conditions, it looks like a pearl-necklace chain. In the sausage phase ($Z_p:Z_d=1:1$), drug molecules condense into a liquid column with PE chain wrapping outside, making the complex behave like a flexible super rod. Both the necklace state and sausage state have been observed in system of the polyelectrolytes in a poor solvent^{20,63}. But the ones found in our system have more stable core-shield structures. For compact globule phase ($Z_p:Z_d=2:1, 3:1$), when the hydrophobicity of drug molecule is moderate, complexes are spherical with layer-by-layer distributions of PE and drugs from the center to the outside (see Figure 5d). However, if the hydrophobicity of drug is too strong ($\epsilon_d > 4$), the formation of globular complex can be hindered by the dynamic arresting, resulting in glass-like structures, as discussed later.

Although the morphologies of PE-drug systems share some similarity with that of poly-

electrolytes chain in poor solvent²⁰, the mechanism that gives rise to these morphologies is very different for these two cases. For PE chain in poor solvent, it is the competition between short-range hydrophobic attractions and long-range electrostatic repulsions between the PE monomers determines the morphology of PE. Thus, changing either the electrostatic or hydrophobic strength can effectively modify the morphology. While in our case, hydrophobicity of drug molecules only has a weak influence on the morphology of complex, if the non-equilibrium effect (see the last section) is not taken into account. Instead, component fraction ratio between PE and drug in the complex plays a more important role.

The reason for the distinct features between these two cases is that, compared with PE in poor solvent, the global electrostatic penalty is largely reduced for the PE-drug complex due to the condensation of hydrophobic drug molecules. While, locally it would be more energetically favourable if more drug-drug contacts can form. Meanwhile, segments of PE chain tend to locate apart and outside to reduce local electrostatic repulsions. Different $Z_p:Z_d$ would result in different component fractions of drug molecules in the complex, which makes the system use different morphological strategies to maximize the hydrophobic contacts and minimize the electrostatic repulsions between PE, similar to what happens in block copolymer self-assembling systems.⁶⁴ This mechanism explains the core-shield structures of necklace phase and sausage phase, as well as the layer-by-layer structure of globule phase. On the contrary, these internal structures are not observed in systems of polyelectrolytes in poor solvent.

D. Structural and electrostatic analyses of globular complex

In this section, we investigate the internal structure and electrostatic properties of globular complex under different drug hydrophobicities. The results are summarized in Figure 5. From the inserted snapshots, one can see that when the hydrophobic attraction between the drug molecules is weak, the surface tension of the complex globule is small, which makes the complex less compact and a little non-spherical. While for relatively strong hydrophobic drug case, the globule has a smoother surface and a more compact shape. We plot the radial distributions of PE and drug molecules in Figure 5a and 5d. We identify a characteristic layer-by-layer distributions of PE and drug in the strong hydrophobicity case, while in the weak hydrophobicity one, similar distribution becomes weak or disappears.

FIG. 5: Radial distributions of PE chain and drug molecules (a), (d), and counterions (b), (e), as well as the electrostatic potentials (c), (f), as functions of the distance from the center of complex for $Z_p:Z_d=3:1$. Upper: $\epsilon_d=1.6$, below: $\epsilon_d=4$.

Detail electrostatic analyses reveal that the difference between these two cases is more profound, as the net charge of the complex is found inverted as the hydrophobic strength of drug becomes strong. This can be inferred from the inverted counterion distributions near the complex surface as shown in Figure 5b and 5e. Calculations of charge accumulation from the center confirm this inversion (data not shown). The charge inversion would give rise to an opposite zeta potential of the complex, as clearly demonstrated in Figure 5c and 5f.

The charge inversion of PE-drug complex can be predicted using our mean-field theory, which gives a simple explanation of the mechanism behind this novel phenomenon. Recalling that to obtain the free energy of collapsing state, we assumed that the complex are fully neutralize, i.e. $\rho_d \rightarrow Z_p/Z_d$. Generally speaking, this assumption is right, since drug molecules will not aggregated by themselves to violate the charge neutrality above the ionization point. However, a weak violation of charge neutrality of complex can be allowed if other thermodynamic forces are strong as well. To investigate this effect, we can do a weak perturbation of ρ_d near the neutrality point by introducing negligible $\Delta\rho_d$ that represents the excess charge of the complex. The leading terms in the free energy of collapsing state

thus can be rewritten as a function of $\Delta\rho_d$,

$$\left. \frac{\beta F}{N} \right|_{\rho_d \rightarrow Z_p/Z_d} \simeq -\frac{9\chi^2 K^3 (K+4)}{16C^*(1+K)^4} \Delta\rho_d + \frac{3\sqrt{6}l_B N^{2/3} \chi^{1/3} K^{2/3}}{5(2C^*)^{1/3}(1+K)} Z_d^2 \Delta\rho_d^2 + \Delta\rho_d \ln R_0^3. \quad (14)$$

The equilibrium excess charge $\Delta\rho_d$ can be easily obtained as:

$$\Delta\rho_d = \frac{\mu_{id} - \mu_{hyd}}{u_{elect}} \quad (15)$$

where $\mu_{id} = -\ln R_0^3$ is the chemical potential of idea gas for drug molecules in free state and $\mu_{hyd} = -\frac{9\chi^2 K^3 (K+4)}{16C^*(1+K)^4}$ is the excess chemical potential of drug molecules arising from the hydrophobic attraction and excluded volume repulsion in the complex state. $u_{elect} = \frac{6\sqrt{6}l_B N^{2/3} \chi^{1/3} K^{2/3}}{5(2C^*)^{1/3}(1+K)} Z_d^2$ can be regarded as the energy penalty of excess charge per monomer for the violation of charge neutrality in the complex.

The expression of excess charge itself (Eq. (15)) gives the origin of charge inversion in the PE-drug system: for drug molecules, there is a competition between gaining translation entropy in the free state and lowering the enthalpy by enlarging the hydrophobic attraction in the condensed state. This competition is characterized by the chemical potentials μ_{id} and μ_{hyd} , respectively. If the hydrophobicity of drug molecule is strong, complex still tends to absorb excess drug molecule to minimize the enthalpy at the neutrality point, making the complex positively charged. While if the hydrophobicity of drug is weak, drug molecules on the complex surface are prone to escape to maximize the translation entropy, leaving a slightly negatively charged complex. The amplitude of this excess charge variation is strictly controlled by electrostatic energy penalty u_{elect} for the violation of charge neutrality. Nevertheless, if an intermediate hydrophobicity of drug molecule is chosen, a zero zeta potential on PE-drug complex surface can be achieved, as demonstrated by Eq. (15) and proven by the simulations in the next section.

Controlling the zeta potential of nano-particle is a very important issue in nanotechnology, since the zeta potential can effectively determine the stability of colloidal dispersions and the absorption of other molecules, e.g. proteins.⁶⁵ Charge inversion is also an interesting and fundamental phenomenon in both biology and chemistry.⁶⁶ As for pharmaceutical applications, high zeta potential means that small PE-drug complexes are less likely to merge to form bigger cluster and coagulate during the preparation process, making the final solid drug products contain much larger surface area. This would promote the dissolution of drug and result in a high supersaturated drug concentration in vivo.⁴⁸ Therefore, to fabricate stable

and compact colloidal PE-drug complex, a designing principle obtained from our theory and simulations is that, in the preparing process, one should choose PE chain with high charge density, and the solvent, as well as the concentration of drug molecule, should be carefully chosen to avoid the zero zeta potential point of complex, where the coagulation is expected to occur more easily.

FIG. 6: Radius of gyration of PE chains as a function of the hydrophobic strength ϵ_d of drug molecules under different $Z_p:Z_d$ (left) and the morphological phase diagrams (right). (a) and (b) is for $N=400$; (c) and (d) is for $N=800$.

E. Effects of chain length

According to our mean-field theory, the complexation point represented by Eq. (13) is independent of chain length, provided that chain is long enough. This prediction is worth a test in the simulation. In all above simulations, the length of PE is fixed at $N=200$. To explore how the chain length affects the formation, as well as the morphology of PE-drug complex, we also perform simulations for two long-chain cases i.e. $N=400$, and $N=800$ under

the same drug excess ratio and the same drug concentration.

For both long chain cases, we found that both the collapsing behaviours of PE and the phase diagram of PE-drug complex is very similar to that of $N=200$ (see Figure 6). This insensitivity of the phase behaviour of PE-drug system to the variation of PE chain length indicates that the mechanism of PE-drug complex formation is rather general under the same dilute and salt free condition. Moreover, the phenomenon of zeta potential inversion on globular complex with the increase of hydrophobicity of drug is also unchanged in both long chain cases. The layer-by-layer structure in the liquid globular complex preserves as well. In Figure 7, we show the radial distributions of PE and drug molecules for $N=200, 400, 800$ cases. The electrostatic potentials are shown in the inset as well. For a better comparison, we choose a intermediate hydrophobicity ϵ_d to make sure that the zeta potential in each case approaches zero. As can be seen, a longer chain length will increase the layer number in the complex, but the ϵ_d that gives each complex zero zeta potential are surprisingly proximate. This finding accords well with the theoretical prediction of Eq. (15), which implies that zero zeta potential point is also independent of PE chain length.

F. Dynamics of PE-drug complexation and the dynamically arrested structures

In the last section, we shift our attention to the dynamic aspect of PE-drug complex formation. To mimic the real complexation process, in all our simulations, we first turn off the electrostatic and hydrophobic interactions related to drug molecules and their counterions i.e. setting the charge of both drug and their counterions to zero and set the cutoff of LJ interactions between drug molecules to $2^{1/6}\sigma$. Then, we simulate long enough time to make sure that the PE chain is in its equilibrated and expanding state. After that, we turn on all the interactions, to mimic the mixing process between PE and drug molecules and observe the process of complex formation. For $Z_p:Z_d \geq 1:1$ we find that drug molecules are first absorbed on the PE chain to replace its counterions (Figure 8a). Then the absorbed drug molecules condense into small drops, making the PE chain looks like a stretched pearl-necklace (Figure 8b). These small drops further merge into large ones and final form a connected structure, either sausage (Figure 8c, 8e) or globular complex (Figure 8d). If the hydrophobicity of drug molecules is not very strong ($\epsilon_d < 4$), the final complex is liquid-like and inner structure would be well equilibrated. For liquid sausage phase, this is reflected by

FIG. 7: Radial distributions of PE chain and drug molecules, as well as electrostatic potentials (inset) from the center of complex at the zero-zeta potential condition for three chain length cases: (a) $N=200$; (b) $N=400$; (c) $N=800$. For $N=200, 400$ cases, the hydrophobic strength is found to be $\epsilon_d=3.5$, while for $N=800$, $\epsilon_d=3.4$.

the flexibility of the super rod, while for globular complex, the layer-by-layer structure and the change of the PE pattern on the complex surface with time are the indicators. However, if the hydrophobicity of drug molecule is too strong ($\epsilon_d > 4$), the equilibrium structure may not be reached, since the complex will be easily trapped in an intermediate dynamically

arrested state, especially for long chain systems. Complex in this state has a sausage shape, as shown in Figure 8d. This non-equilibrium rod-like structure is more rigid than the equilibrium one observed in the $Z_p:Z_d=1:1$ case, and it is surrounded by a negative counterion cloud, an evidence of a highly positive zeta potential. This non-equilibrium and non-spherical complex caused by the sudden “quench” (i.e. adding the PE) may not have the lowest free energy, but the surface areas of this morphology is obvious larger than the well equilibrated globular complex, and the zeta potential is also higher. It’s still needed to be determined both in experiments and simulations that which strategy (equilibrium or non-equilibrium) is better to obtain the ultra-stable amorphous drug nano-complex with the best dissolving ability.

FIG. 8: Dynamic pathways of compact PE-drug complex formation ($N=800$): complexation begins from the initial expanded state of PE (a). After transiting through a intermediate necklace state (b), PE and drug molecules can condense into three final morphologies based on different conditions: i.e. (c) equilibrium sausage complex for $Z_p:Z_d=1:1$; (d) equilibrium globular complex at $Z_p:Z_d=2:1/3:1$ under moderate drug hydrophobicity; (e) dynamically arrested sausage state at $Z_p:Z_d=2:1/3:1$ under high drug hydrophobicity.

IV. CONCLUSION

In conclusion, through theoretical analyses and molecular dynamics simulations, we unveil the complexation mechanism of PE and hydrophobic drug in the dilute and salt-free solution. Generally, the formation of compact PE-drug complex is driven cooperatively by both the hydrophobic attraction between drug molecules and electrostatic adhesion between drugs and PE. During the complexation process, there is a competition between maximizing hydrophobic attractions between drugs and minimizing electrostatic repulsions in the complex. This competition gives rise to different morphologies of the complex under different PE-drug valence ratios. Moreover, both theory and simulation indicate that increasing the hydrophobicity of drug can induce charge inversion and sign change of the zeta potential for globular complexes, which is very important to control the stabilization of the colloidal PE-drug complex. We also confirm that the complexation point and complex morphologies, as well as the electrostatic properties of complex, remain almost the same in long PE length cases. This implies that our finding could be generally applicable to experimental systems. At last, we also explore the dynamics of PE-drug complexation, and find that the complex can be trapped into a non-equilibrium glass-like state when the hydrophobicity of drug is too strong. Based on our findings, we suggest that one should choose PE chain with high charge density in the preparation process. And the zero zeta potential of complex that can cause coagulation should be avoided by changing the solvent quality or the concentration of drug molecule, in order to make the complexes stay in suspended and finite-size colloidal state.

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TOC: Morphological phase diagram of the PE-drug systems in dimensions of drug's hydrophobicity and valence ratio between PE monomer and drug molecule.