Modeling of polymer-enzyme conjugates formation: Thermodynamic perturbation theory and computer simulations

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A simple model for the formation of the polymer-enzyme conjugates has been proposed and described using corresponding extension of the Wertheim’s first-order thermodynamic perturbation theory (TPT1) for the system of associating chain molecules. A set of computer simulation data for different number of functional groups along polymer chains has been obtained and used to access the accuracy of the theoretical results. Predictions of the present theoretical approach are more accurate than that of the conventional TPT1 and are in a very good agreement with the computer simulation data. In particular the theory is able to account for the difference in position of the polymer functional groups along its backbone.

1 Introduction

The application of enzymes in chemical industry is of great interest and it is constantly developing. This is primarily because of unique catalytic properties of enzymes, which allow them to be considered as an environmentally friendly alternative to many traditional toxic and hazardous technologies. One of the areas of such application is converting cellulose biomass into biofuel. The enzymatic biocatalysis is pivotal in the production of first and second generation bioethanol from cellulose using a mixture of different types of enzymes, so-called cellulases (endocellulases, cellobiohydrolases, beta-glucosidases). Such kind of enzymes are involved in the process of cellulose hydrolysis, where they synergistically break polysaccharide chains into monosaccharide and oligosaccharide molecules, from which ethanol is subsequently produced by fermentation. Despite the attractiveness of this approach, there is still the problem of efficiency and commercial viability of this technology. There are a number of strategies to increase a catalytic efficiency of enzymes. One of the most promising approaches is immobilization of enzymes on scaffolds, like polymers, nanoparticles and microgels. For instance, it was found the synthetic enzyme-polymer conjugates mimicking natural cellulosomes can exhibit a sufficiently higher catalytic activity in cellulose hydrolysis if to compare with dispersions of totally free enzymes. This obviously indicates a higher synergism level of enzymes constrained into groups. Moreover, the enzymes captured on scaffolds can be more easily collected for further reutilization. It was also observed that enzymes in complexes with brush-like polymers have a higher thermal stability.

We report a theoretical study of conjugation process in a mixture of enzymes with brush block copolymers. We propose the very basic coarse-grained model to describe a mixture of enzyme globules and multifunctional polymer chains. Brush copolymer is mimicked by linear chain of spherical beads with an implicit side-chains representation. It is assumed that some of polymer blocks can bear functional groups specific to active sites located at enzyme molecule, which can bind to polymer by affinity interactions. The simplicity of considered model is motivated by two reasons. The first is to distinguish the role of functional groups arrangement at polymer chains playing in the polymer-enzyme conjugation. Secondly, we are aimed to develop a theoretical approach to describe this process. In fact, it still remains a challenge to provide a fast and sufficiently accurate theoretical prediction for properties of polymer-protein mixtures made of beads bearing attractive sites even for simple models. For this purpose, some approaches of the associative liquids theory seems to be the most appropriate ones, however they need an essential improvement.

In this study we focus on the development of the model and its theoretical description of the polymer-enzyme conjugation process. With this goal in mind we extend and apply the first order multi-density thermodynamic perturbation theory (TPT1) of Wertheim and perform detailed computer simulation study of the model. Our theoretical and computer simulation results are compared and the accuracy of the theoretical predictions are assessed. The models similar to that we suggested have been used earlier to describe effects of aggregation and association in the surfactant systems, alkanols, organic acids, aminoacids, etc (see and references therein). In these studies the particles of the system were modeled by the associating totally flexible chains of spherical Lennard-Jones (LJ) monomers (beads). Similar as in our model association occurs due to the off-center short-ranged square-well bonding sites placed on the surface of certain monomers. For the theoretical description the authors have been using TPT1 with the reference system represented by the fluid of the LJ monomers. The theory, being relatively successful in describing the models with bonding sites placed on the terminal beads of the chains, appears much less accurate if bonding sites were located on the intermediate beads. This drawback of the theory is due to the “single bond” approximation.
utilized in the TPT1, i.e. bonding abilities of each of the sites of the monomer do not depend on the bonding state of the rest of the sites. There are two possibilities to improve performance of the theory: to apply the higher-order approximations in the framework of the TPT or to use the better choice for the reference system. Unfortunately, the former one is not much feasible, since it requires the application of the higher-order reference system correlation functions, which restrict practical application of this scheme for the second order case. To achieve the higher accuracy using low-order version of the TPT in many cases it is more profitable to stick with the former option. This route was undertaken in to account for the effects of changes in the system excluded volume upon association. In this study we propose to use the reference system represented by the fluid of nonassociating chain molecules. Somewhat similar approach was used to correct TPT1 predictions for the low-density behavior of the equation of state for the chain fluid. However, to the best of our knowledge this idea has not been used for the description of the associating chains, in particular when association occurs due to the intermediate monomers of the chain. In addition, in the majority of these studies the properties of the reference fluid were calculated using the fit of the corresponding computer simulation results. Here description of the reference system is carried out analytically, using solution of the multi-density Ornstein-Zernike (OZ) equation supplemented by the associative Percus-Yevick (APY) approximation.

The paper is organized as follows. First, in Section 2 we introduce the general model, which can be described by the developed theory and applied in particular to the problem stated above. Then we present the theory in Section 3. Technical details of computer simulation performed in this study can be found in Section 4. The obtained results are discussed in Section 5 and concluding remarks are presented in the last Section 6.

2 The model

We start with the two-component mixture of flexible chain molecules consisting of tangentially bonded hard-sphere monomers of different size. Some of the monomers in the chains of one type and one monomer in the chains of the other type bear single off-center attractive square-well site (functional group), located on the surface. This site-site attractive interaction is valid only between the sites, which belong to the chains of different type. In the limiting case of the latter component represented by only one functional monomer this model reduces to the model of polymer-enzyme mixture. The pair potential acting between the monomers is

\[ U_{a_i b_j}^{(sw)}(z) = \begin{cases} - (1 - \delta_{ab}) \epsilon_{ij}, & \text{for } z \leq k, \\ 0, & \text{otherwise} \end{cases} \]  

(3)

\[ z = \sqrt{\sigma_{a_i b_j} - r}, \]

where \( z \) is the distance between the square-well sites, \( \sigma_{a_i b_j} \) is the hard-sphere diameter, \( \epsilon_{ij} \) and \( k \) are the square-well depth and width, respectively. The indices \( a, b \) each take the values 1 and 2, and the indices \( i \) and \( j \) take the values \( 1, \ldots, n_a \) and \( 1, \ldots, n_b \), respectively. Here \( n_a(n_b) \) is the number of the monomers in the chain of the type \( a(b) \). The system number density is \( \rho = \rho_1 + \rho_2 \), where \( \rho_1 \) and \( \rho_2 \) are the number density of the chains of the type 1 and 2, respectively.

3 Theory

Following Wertheim we assume that Helmholtz free energy \( A \) of the mixture at hand can be written as a sum of Helmholtz free energy of the reference system \( A_{ref} \) and corresponding contribution due to bonding \( \Delta A_{bond} \), i.e.

\[ A = A_{ref} + \Delta A_{bond}, \]

where the reference system is represented by the original two-component mixture of hard-sphere chain molecules with \( \epsilon_{ij} = 0 \) and for \( \Delta A_{bond} \) we have

\[ \beta \frac{\Delta A_{bond}}{V} = \sum_{a=1}^{2} \rho_a \left[ \ln \chi_a - \frac{1}{2} \chi_a + \frac{1}{2} n_a \right]. \]

(5)

Here \( n_a \) is the number of the square-well sites located on the chain molecule of the type \( a \) and \( X_{ai} \) is the fraction of the molecules of the type \( a \) with nonbonded square-well site on the monomer of the type \( i \). These fractions satisfy the mass action law type of the relation

\[ X_{ai} = \frac{1}{\beta} \left( \frac{\rho_b}{\rho_a} \right)^{n_b} \exp \left( - \beta \sum_{b=1}^{n_b} \chi_{ab} \right), \]

(6)

where

\[ \chi_{ab} = \frac{1}{2 \sigma_{a_i b_j}} \left( k + \sigma_{a_i b_j} - r \right)^2 \left( 2k - \sigma_{a_i b_j} + r \right). \]

(8)

3.1 Description of the reference system

Thermodynamic properties of the reference system can be calculated using first-order thermodynamic perturbation theory (TPT1) of Wertheim. For Helmholtz free energy of the reference system we have

\[ \beta \frac{A_{ref}}{V} = \sum_{a=1}^{2} \rho_a \sum_{i=1}^{n_a-1} \ln \frac{\rho_{a_i+1}}{\rho_a} \chi_{a_i a_{i+1}}. \]

(9)
where \( r_{a,b}^{(hb)}(r) \) is the hard-sphere cavity correlation function. The fractions \( X_{a,b} \) follow from the solution of equation (refmass) provided that the value of the integral \( K_{a,b} \) is known. To simplify calculation of this integral we assume that the Mayer function \( f_{a,b}(r) \) can be approximated by the Dirac delta-function, i.e.

\[
\hat{f}_{a,b}^{(sw)}(r) = T_{a,b} \delta(r - \sigma_{a,b}),
\]

where

\[
T_{a,b} = \sigma_{a,b}^{-2} \int_{\sigma_{a,b}}^{\infty} r^2 \hat{f}_{a,b}^{(sw)}(r) dr.
\]

Now for \( K_{a,b} \) we have

\[
K_{a,b} = 4\pi \sigma_{a,b}^2 T_{a,b} \hat{g}_{a,b}^{(ref)}(\sigma_{a,b}),
\]

where the contact values of the radial distribution functions \( \hat{g}_{a,b}^{(ref)}(\sigma_{a,b}) \) can be calculated using analytical solution of the polymer Percus-Yevick ideal chain approximation for heteronuclear hard-sphere chain fluids.

\[
\sigma_{a,b} \hat{g}_{a,b}^{(ref)}(\sigma_{a,b}) = \sigma_{a,b}^{-1} + \frac{\rho_s}{4(\eta - 1)} \left[ \left( 1 - \delta_{j,1} \right) \frac{\sigma_{b,j-1}}{\sigma_{a,b,j-1}} + \left( 1 - \delta_{j,n_b} \right) \frac{\sigma_{b,j+1}}{\sigma_{a,b,j+1}} \right]
\]

\[
+ \frac{1}{4(\eta - 1)} \sigma_{a_i} \left[ \left( 1 - \delta_{i,1} \right) \frac{\sigma_{a_i,j-1}}{\sigma_{a_i,a_i,j-1}} + \left( 1 - \delta_{i,n_a} \right) \frac{\sigma_{a_i,j+1}}{\sigma_{a_i,a_i,j+1}} \right]
\]

\[
+ \frac{\delta_{ab}}{8\pi \rho_a} \left[ (1 - \delta_{i,j})(1 - \delta_{i,j+1}) \delta_{i,j+2} + \left( 1 - \delta_{i,n_a} \right) \frac{\sigma_{a_i,j+1}}{\sigma_{a_i,a_i,j+1}} \delta_{i,j+2} \right].
\]

Here \( \eta = \pi/6 \sum \rho_a \Sigma_i \sigma_{a_i}^3 \) and \( s_n = \pi/\rho \sum \rho_a \Sigma_i \sigma_{a_i}^2 \).

### 4 Computer simulation details

Computer simulations were performed of the models of polymer-enzyme mixtures (see Fig. 2) using the method of Langevin dynamics (LD) in the NVT ensemble with the LAMMPS software package (https://www.lammps.org, version 30Nov2020). Since force fields in LD simulations require continuous pair potentials, the square-well and hard-sphere potentials used in the theory should be substituted with their continuous analogs. Therefore, for the square-well interaction acting between functional sites of polymer and enzyme the original code of LAMMPS was modified by implementing the force field, which according to describe the continuous square well (CSW) pair potential:

\[
u_{csw}(r) = -\frac{1}{2} \epsilon_{csw} \left[ 1 - \tanh\left( \frac{r - r_{w}}{\alpha} \right) \right].
\]

In contrast to we use more steep square well shape of \( \epsilon_{csw} \) by taking \( \alpha = 0.001 \sigma \). The radius of attractive well was chosen \( r_w = 0.12 \sigma \), the cutoff radius was \( 0.17 \sigma \) and the attractive well depth \( \epsilon_{csw} \) was defined as the energy unit. With these parameters the shape of \( \epsilon_{csw}(r) \) very closely fit the conventional square well potential. All functional sites are fixed on the corresponding enzyme and polymer beads at the distance \( \sigma/2 \) to their centers using the SHAKE algorithm with the tolerance 0.0001. Sizes of all beads in the polymer and size of enzyme molecules were taken equivalent and set to \( \sigma \), which was used as the unit of distances in our simulations.

The hard-sphere pair interaction between particles was substituted with the pseudo-hard sphere pair potential (PHS) using the repulsive part of the cut and shifted (50,49)-Mie potential as it was suggested
the corresponding systems type as the Models C2, C3, C4, C5, C7 and C13 (see Fig. 2). The chains representing polymer scaffolds consist of 13 hard-sphere beads of the size $\sigma$ and the number in our notation for the type of the model corresponds to the number of the polymer beads with functional groups. These beads are placed along the chain backbone uniformly and symmetrically. Enzyme molecules are represented by the hard spheres of the same size $\sigma$ and one functional site which can conjugate to the polymer functional group due to the square-well attractive potential. The width and depth of the square-well site-site interaction was chosen to be $\kappa = 0.119\sigma$ and $\epsilon_{ij} = \epsilon$ and we consider two values of the temperature: $T^* = T/\epsilon = 0.09$ and 0.12.

Next we have studied the system at fixed the densities of the chain molecules $\rho_1 = \rho_p$ and different densities of the enzymes $\rho_2 = \rho_e$ in the range of 0 – 0.18 and two values of the temperature, $T^* = 0.09, 0.12$. The density of the polymer chains were chosen to be different for each model: (see Table 1). Our theoretical (mTPT1) and computer simulation (symbols) results are shown in Fig. 4-8. They are presented in reduced units: the distance is measured in the units of $\sigma$ and the energy (temperature) in the units of the square-well depth $\epsilon$. In addition, we include results of the conventional TPT1 approach (TPT1), which is based on the application of the hard-sphere reference system. One can observe a very good agreement of our theoretical predictions with the data of computer simulations. On the other hand, the theoretical results of the unmodified version of the TPT1 is much less accurate.

6 Conclusions

We propose a theoretical description for the formation of polymer-enzyme conjugates using a simple model of two-component mixture consisting of flexible hard-sphere chains of polymer bearing a number of uniformly arranged functional groups and hard-sphere enzyme molecules with a single specific site able to conjugate to these groups. Depending on the grafting density of functional groups on the polymers and the polymer-enzymes composition, different number of enzymes can bind to the polymer scaffolds at different places. Theoretical description of the model was carried out using a corresponding extension of the Wertheim’s first-order thermodynamic perturbation theory (TPT). To assess the accuracy of the theory we have performed a set of computer simulation data. Our analyses is focused on the abilities of the theory to correctly reproduce the number of the bonds created between enzyme molecules and polymer functional groups differently located along the chain. We have shown that predictions of the present theory is in a very good agreement with corresponding computer simulation and appears to be much more accurate than that of the conventional TPT1 approach. An important advantage of the developed theory is its simplicity and ability to provide a completely analytical description for the general case of any number of the functional groups at polymer beads and enzymes molecules as well as for different size ratios of them.

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Fig. 4 Number of bonds per number of enzyme patches $X_e$ (left panel) and per total number of polymer functional groups $X_p$ (right panel) depending on the number density of enzyme molecules $\rho_e$ for Model C2.

Fig. 5 Same as in Fig. 4 but for Model C3.

Fig. 6 Same as in Fig. 4 but for Model C4.
Fig. 7 Same as in Fig. but for Model C5.

Fig. 8 Same as in Fig. but for Model C7.