Prediction of Glycerol-Effect on Antigen-Antibody Binding Affinity from Molecular Dynamics Simulations

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***Abstract*— Many biological and biotechnological processes are controlled by protein-protein interactions in solution. In order to understand, predict and optimize such processes, it is valuable to understand how additives such as salts, sugars, polyols and denaturants affect protein-protein interactions. Currently, no methodology to foretell the effect of additives on protein-protein interactions has been established and frequently and extensive empirical screening to identify additives beneficial to the protein process is resorted to.**

In this work, we developed a methodology enabling the prediction of the additive-effect on the protein reaction equilibrium. The only prerequisite is that the atomic structure of the protein reactants and products are known. The methodology is based on the thermodynamic model for preferential interactions and makes use of molecular dynamics simulations to gauge additive-protein interactions. In order to validate our methodology, the change in binding affinity of the antibody fragment Y32S Fv D1.3 for lysozyme in the presence of varying glycerol concentrations is being calculated and the results will be compared with experimental data from literature. Finally, our methodology will be used to predict the glycerol effect on the binding affinity of wild type Fv D1.3 and various mutants.

***Index Terms*— Binding affinity, glycerol, molecular dynamics, preferential interaction.**

I. Introduction

PROTEIN-protein interactions control a wide range of protein processes including *in vivo* protein complex formation, protein separation processes such as salting out and affinity chromatography, protein crystallization, and protein aggregation [1]. In these processes, protein-protein interactions typically occur in the presence of a fair amount of small molecules (> 100 mM) such as salts, sugars, polyols or denaturants, which have a similar magnitude of affinity for protein binding as water [2]. In the following, such weakly interacting small molecules are referred to as *additives* and their interactions with the protein as *additive-protein interactions*. For decades, it has been known that the additive type and concentration can affect protein-protein interactions, and the use of additives to manipulate protein processes is widespread [3-7]. Since the additive effect on protein-protein interactions is specific for each protein reaction [8, 9], a priori knowledge of the change in protein-protein interactions caused by an additive would be desirable. However, no methodology to predict the additive-effect on protein-protein interactions has been established, and consequently, extensive empirical searches for additives and their concentrations to understand or better the performance of a particular protein process are needed [10].

In this work a methodology is developed which is able to predict additive-effects on protein reactions. The type of the reaction is restricted to reversible reactions such as non-covalent protein association or folding, and the atomic structures for the protein reactants and products need to be known. The methodology is based on the thermodynamic model for preferential interaction of an additive with a protein. In this model, the preferential interaction coefficient is a measure for the change of the protein activity with respect to the additive activity under specific thermodynamic constraints [2]. For any protein reaction, the additive-effect on the reaction equilibrium is then a function of the difference of the preferential interaction coefficients of the protein reactants and the protein products [11]. The interpretation of preferential interactions has been revised many times [2, 12, 13], and in a number of recent publications the preferential interaction parameter has been derived by means of statistical thermodynamic functions representing the additive-protein interactions on a molecular level [14-23].

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Notwithstanding the theoretical advancements in the preferential interaction model, few attempts have been reported applying this model to understand the additive-effect on protein processes [24, 25]. This discrepancy can be attributed to the laboriousness to experimentally determine the preferential interaction coefficient [26, 27] together with the practical challenge to measure the preferential interaction coefficients for both the reactants and products of the protein reaction at the same additive concentration. As far as we know, the only successful attempt to circumvent this limitation was undertaken by Xie and Timasheff [24]. In this study, experimental determination of the preferential interaction with both the native and the denatured state was realized by varying a solution parameter which had no effect on preferential interaction. The difference in preferential interaction could quantitatively explain the effect of urea on the unfolding-equilibrium of RNAse A. The success of this attempt supports the assertion that additive-effects on protein reactions are the result of preferential additive-protein interactions. However, this experimental approach is demanding and restricted to this particular protein reaction. Baynes and Trout [28] demonstrated that the preferential interaction coefficient of an additive to a protein can be calculated from molecular dynamics simulations. In principle, their approach can be adopted for any protein for which the atomic structure is known.

Our methodology makes use of this approach to calculate the glycerol effect on the binding affinity of the monoclonal antibody fragment Y32S Fv D1.3 (D1.3) with hen egg-white lysozyme (HEL). The antigen-antibody complex HEL-D1.3 was chosen since high resolution crystal structures are available for both the monomers and the complex [29]. Glycerol was selected as weakly interacting additive as it is one of the most frequently used additives in various biotechnological and biochemical processes [30-35]. Glycerol is also known as an osmolyte preventing cells against osmotic stress [36], and its *in vivo* application has been reported [37, 38]. Moreover, the properties of glycerol in aqueous solutions [39-41], and its effect on the native protein state have been studied [42-44].

Based on a thermodynamic model for preferential interaction, an expression for the additive-effect on protein reactions is derived in Section II. This expression is then worked out in a format applicable to calculate the glycerol-effect on HEL-D1.3 binding affinity. Methods used for molecular dynamics simulations, the expression of D1.3 mutants, and measurement of the binding affinity of the HEL-D1.3-complex are discussed in Section III. In Section IV, results for the D1.3-HEL binding affinity under varying glycerol concentrations calculated by our methodology will be compared with experimental results from literature [9]. In addition, we will predict the glycerol effect on the binding affinity of various D1.3-mutants and compare the results with the experimentally measured results.

II. Preferential interaction model

A. Model derivation

In this section, different interpretations pertinent to preferential interactions of additives with a protein in dilute aqueous protein solutions are evaluated and eventually a model equation is presented which is of direct use in our methodology. Subscripts in the equations follow the Scatchard notation [45], where 1 stands for water, 2 for protein and 3 for additive.

Several decades ago, Wyman [11] presented an equation that linked the additive activity a_3 with the equilibrium constant of a protein reaction K_2:

$$\left(\frac{\delta \ln K_2}{\delta \ln a_3}\right)_{T,P,m_2} = \nu_3^P - \nu_3^R \qquad (1)$$

Where ν_3 is the number of additive molecules bound to the protein product (P) and reactant (R).

Whereas the Wyman linkage equation was originally derived for additives which tightly bind to specific locations on the protein surface, generalization followed soon for weakly binding additives by considering water molecules binding to the protein [12]:

$$\left(\frac{\delta \ln K_2}{\delta \ln a_3}\right)_{T,P,m_2} = (\nu_3^P - \nu_3^R) - \frac{m_3}{m_1}(\nu_1^P - \nu_1^R) \qquad (2)$$

Where ν_1 is the number of water molecules bound to the protein, and m_3 and m_1 are the molality of additive and water, respectively.

The term $\nu_3 - \frac{m_3}{m_1}\nu_1$ was interpreted as the preferential interaction coefficient Γ_{23} of the additive with respect to the protein: $\Gamma_{23} > 0$ implies $\frac{\nu_3}{\nu_1} > \frac{m_3}{m_1}$, indicating that the additive will be enriched near the protein surface with respect to the bulk solution. On the other hand $\Gamma_{23} < 0$ corresponds with additive depletion near the protein surface with respect to the bulk composition. For the derivation of (2), both additive and water were assumed to bind to the protein surface with a specific equilibrium constant [12], an assumption which was also made by various other authors [46-48]. However, the idea of specific binding sites on the protein surface might be misleading since additive and water molecules dynamically move with respect to the protein surface due to random diffusion, with minor perturbations caused by the weak interactions with the protein surface [28]. Since molecular dynamics simulations allow the sampling of relative molecular positions in a 3-component system consisting of

water, protein and additive, direct derivation of the macroscopic preferential interaction coefficient from molecular level insight is possible based on classical thermodynamics, combined with statistical thermodynamics.

A rigorous thermodynamic definition for the preferential interaction coefficient was introduced by Casassa and Eisenberg [49]:

$$(\Gamma^{m}_{23})_{T,P,\mu_3} \equiv \left(\frac{\delta m_3}{\delta m_2}\right)_{T,P,\mu_3} \tag{3}$$

This preferential interaction coefficient corresponds with the number of additive molecules δm_3 required to keep the chemical potential of the additive μ_3 constant upon addition of one protein molecule, and this under constant temperature T and pressure P.

Using the Euler reciprocity rule and the Maxwell relation, it is seen that the preferential interaction coefficient is a measure for the change of chemical potential μ_2 of the protein with respect to a change in chemical potential of the additive μ_3:

$$(\Gamma^{m}_{23})_{T,P,\mu_3} = -\left(\frac{\delta \mu_2}{\delta \mu_3}\right)_{T,P,m_2} \tag{4}$$

Combining (4) with a thermodynamic box for a protein reaction at two different additive activities [2], an equation similar to the Wyman linkage equation (2) is obtained:

$$\left(\frac{\delta \ln K_2}{\delta \ln a_3}\right)_{T,P,m_2\to 0} = \Delta_{P-R}\left(\Gamma^{m}_{23}\right)_{T,P,\mu_3} \tag{5}$$

Several recent publications have presented expressions of preferential interaction coefficients in 3-component systems based on statistical thermodynamics [14-23]. Although the expressions differ depending on the definition of the preferential interaction coefficient, they all make use of Kirkwood-Buff integrals [50]. KB-integrals are spatial integrals involving the pair correlation function:

$$G_{\alpha\beta} = \int (g_{\alpha\beta} - 1) dV \tag{6}$$

Where $G_{\alpha\beta}$ is called the KB-integral and $g_{\alpha\beta}$ is the pair correlation function of molecule β with respect to molecule α.

Smith [15] systematically derived different preferential interaction coefficients in function of KB-integrals in a straightforward way. Of direct interest for this work is the equation:

$$\left(\frac{\delta m_3}{\delta m_2}\right)_{T,P,\mu_3} = \frac{c_3}{c_1} + c_3(G_{23} - G_{12} + G_{11} - G_{13}) \tag{7}$$

With c_3 and c_1 the concentration of additive and water, respectively.

Combining (3) and (7) and taking the difference Δ_{P-R} between the products P and the reactants R of the protein reaction, one gets:

$$\Delta_{P-R}\left(\Gamma^{m}_{23}\right)_{T,P,m_2\to 0} = c_3 \Delta_{P-R}(G_{23} - G_{12}) \tag{8}$$

Equation (2), (6) and (8) can be combined into:

$$\left(\frac{\delta \ln K_2}{\delta \ln a_3}\right)_{T,P,m_2\to 0} = c_3 . \left[\int_P (g_{23} - g_{12}) dV - \int_R (g_{23} - g_{12}) dV\right] \tag{9}$$

Equation (9) shows that the additive effect on the protein reaction equilibrium constant can be calculated if the correlation functions of additive and water with respect to both protein reactants and protein products are known.

B. Application for Model System

Our methodology will be applied to calculate the glycerol-effect on the binding affinity K_2 of D1.3 with HEL. For this purpose, (9) is reformulated for this specific model system as follows.

With γ^{m}_{3} the molal activity coefficient for the additive, (9) is equivalent to:

$$\left(\frac{\partial \ln K_2}{\delta m_3}\right)_{T,P,m_2\to 0} = \frac{c_3\left[\int_P (g_{23} - g_{12}) dV - \int_R (g_{23} - g_{12}) dV\right] . \left(1 + \frac{\partial \ln \gamma^{m}_{3}}{\partial \ln m_3}\right)}{m_3} \tag{10}$$

Taking into account the activity data of aqueous glycerol solution [39], (10) can be simplified with an error of less than 1% up to a 10 molal glycerol into the following equation:

$$\left(\frac{\partial \ln K_2}{\delta m_3}\right)_{T,P,m_2\to 0} = \frac{c_3\left[\int_P (g_{23}-g_{12})dV - \int_R (g_{23}-g_{12})dV\right]}{m_3} \quad (11)$$

Furthermore, with HEL-D1.3-complex as the protein reaction product and HEL and D1.3 the reactants, we get:

$$\left(\frac{\partial \ln K_2}{\delta m_3}\right)_{T,P,m_2\to 0} = \frac{c_3}{m_3}.\left[\int_{HEL-D1.3} (g^S_{23}-g^S_{21})dV - \int_{HEL} (g^S_{23}-g^S_{21})dV - \int_{D1.3} (g^S_{23}-g^S_{21})dV\right] \quad (12)$$

As seen in Fig. 1, the assumption that $\left(\frac{\partial \ln K_2}{\delta m_3}\right)_{T,P,m_2\to 0}$ is constant up to 10 molal glycerol, is consistent with the experimental data for three different protein association reactions. Consequently, the association constant at any glycerol molality m_3 can be calculated as:

$$\ln\frac{K_2}{K_{2,m=0}} = \frac{c_3^S}{m_3^S}\left[\int_{HEL-D1.3} (g^S_{23}-g^S_{21})dV - \int_{HEL} (g^S_{23}-g^S_{21})dV - \int_{D1.3} (g^S_{23}-g^S_{21})dV\right].m_3 \quad (13)$$

With c_3^S, g^S_{23} and, g^S_{21} the additive concentration, the additive-protein correlation function and the water-protein correlation function, at a specific additive molality m_3^S.

III. Methods

A. Molecular Dynamics Simulations

Under progress

B. Expression of Fv D1.3 and Mutants in Yeast

Planned

C. Measurement of Binding Affinity

Planned

IV. Results

A. Validation of Methodology

B. Prediction of Binding Affinity for D1.3 Mutants

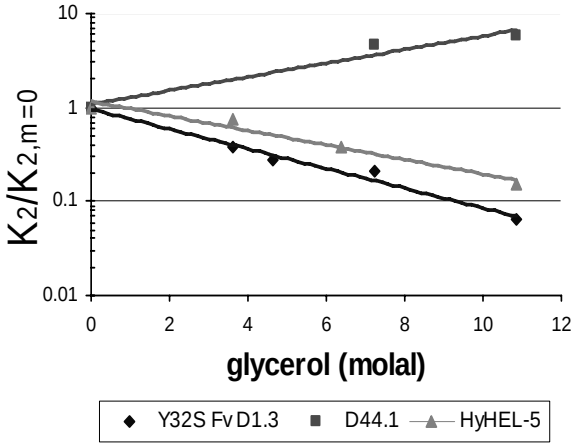

Fig. 1. The ratio $K_2/K_{2,m=0}$ in function of glycerol molality. K_2 is the binding affinity in glycerol-water solutions, and $K_{2,m=0}$ is the binding affinity in the absence of glycerol. The data for three different proteins binding to HEL are presented: Y32S Fv D1.3 [9], D44.1 [9], and HyHEL-5 [51].

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