An eBook on Thermodynamics

Chapter 1

Insights into Protein-Ligand Interactions: Thermodynamic Signatures

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Abstract

Non-covalent interactions play a central role in biochemical processes, mainly in the recognition of charged molecules or ligands to biological molecules such as proteins. This molecular recognition is driven by thermodynamics, through characterizing the binding energetics: the increment of binding free energy change (ΔG_b) and, therefore, equilibrium binding constant (K_b), binding enthalpy change (ΔH_b), binding entropy change (ΔS_b), and heat capacity change (ΔCp). To characterize a molecular interaction experimental techniques like fluorescence titration or calorimetry, are helpful. Experimental results can be correlated to a wide variety of computational methods in order to provide a qualitative or quantitative understanding of the driving forces responsible for the association process. The thermodynamical parameters have been used for the discovery of pharmaceuticals for various diseases as well as for the development of new biomaterials.

Keywords: Non-covalent interactions; molecular recognition; thermodynamical properties; experimental and computational methods.

Abbreviations: A: acceptor group; APBS: Adaptative Poisson-Boltzmaan Solver; ASA: solvent accessible surface area; D: donor group; $\Delta ASA_{non\text{-}pol}$: change in non-polar accessible surface area; ΔASA_{pol}: change in polar accessible surface area; ΔCp: heat capacity change; Δcp_{pol} and $\Delta cp_{non\text{-}pol}$: proportionality coefficients polar and non-polar for the heat capacity change, respectively; ΔG_b: binding free energy change; $\Delta G_{b,\ non\text{-}polar}$: non-polar energy; ΔG_{coul}: coulombic energy; ΔG_{sol}: solvation energy; ΔH_b: binding enthalpy change; Δh_{pol} and $\Delta h_{non\text{-}pol}$: proportionality coefficients polar and non-polar for the enthalpy change, respectively; ΔS_b: binding entropy change; ΔS_{conf}: conformational entropy change; $\Delta S_{or\text{-}t}$: is referred to the loss of translational and rotational degrees of freedom on the binding; Δs_{pol} and $\Delta s_{non\text{-}pol}$: proportionality coefficients polar and non-polar for the entropy change, respectively; ΔS_{sol}: solvation entropy change; DNA: Deoxyribonucleic acid;

γ: coefficient interfacial tension; IIcD: melatonin derivative; ITC: Isothermal Titration Calorimetry; K_b: equilibrium binding constant; K_d: dissociation constant; L-DOPA: L-3,4 dihydroxyphenylalanine; M: Molar; MTC: Multithermal Titration Calorimetry; NCl: Non-Covalent Interactions; QTAIM: Quantum Theory of Atoms in Molecules; R: gas constant; RNA: Ribonucleic acid; SBA15: Santa Barbara Amorphous; $Si0_2$: silica; T: temperature (in Kelvin); TIM: Triosephosphateisomerase from *Saccharomyces cerevisiae*; VMD: Visual Molecular Dynamics.

1. Introduction

Thermodynamics plays a very important role in biological reactions through energy transformations.Together with chemical kinetics, they conform a powerful body of knowledge around enzymes and their reactions. Through thermodynamics it has been possible to study molecular recognition in most biological processes such as: protein-ligand interactions [1], protein-protein interactions [2,3], protein-carbohydrate interactions [4,5], DNA-protein interactions[6], and protein-lipids interactions [7]; it has also been very important in the study of metal ions binding to ligands or proteins [8], and protein-nanomaterials interactions, and these fields have grown exponentially in recent years [9]. In the pharmaceutical development, thermodynamics has been applied to the design of drugs based on the knowledge of the biological target of interest. Recently, in the materials science, the thermodynamics of interactions between various nanomaterials and biomolecules is of utmost importance in the biomedical applications [10] as the tissue engineering [11].

The binding between a protein and a ligand involves non-covalent interactions such as: electrostatic interactions, hydrogen bonds, van der Waals interactions, hydrophobic interactions and π-cation interactions.

A brief description of these interactions is given below:

a) **Electrostatic interactions**. They can be classified into ion-ion, ion-dipole and dipole-dipole interactions. These interactions are adequately described by the Coulomb equation (based on attraction or repulsion between charges) [12]. Electrostatic interactions are very important in biological systems, for example, a negatively charged will interact with positively charged amino acids in the protein binding site, playing a very important role mainly in drug design. It is noteworthy that these interactions can be weakened when other ions are around, modifying the electric field. This has been observed experimentally, that is, in a cell containing a protein and a ligand, the electrostatic interactions are weakened in the binding by increasing the ionic strength in the medium[3].

b) **Hydrophobic effect or hydrophobic interactions.** These interactions refer to rejection of ligands or nonpolar molecules in aqueous solution. This effect is important in protein-ligand molecular recognition and can be divided into two energetic components: enthalpic hydrophobic and entropic hydrophobic effect. The interactions that are established with an enthalpic hydrophobic effect occur when a ligand releases water contained in a cavity, since this water does not interact strongly with the hydrophobic pocket of the protein. In this case,

there is also an entropic factor since the water that was previously ordered within the cavity becomes disordered in the surroundings (later in this chapter we will discuss in more detail on the enthalpic and entropic contributions in the association) [13,14].

c) **Hydrogen bonds.** These interactions are the result of an attraction between a bound proton to a donor group (**D**) and an acceptor group (**A**, Lewis base), *i.e.* **D-H···A**. Besides, they play an important role in the stability, binding, catalysis and conformational changes in a protein [15,16].

d) **van der Waals interactions.** These dispersion forces are typically of short range, and contribute to the interactions of proteins with other ligands (nanotubes, graphene) or with surfaces in water. Their strength increases as the distance decreases and they are effective up to the nanometer scale [17].

e) **Cation-π interactions.** These non-covalent interactions are established between aromatic rings (π electron-rich) and a cation. The contribution of cation-π interactions are of outmost importance in the secondary structure of proteins and in the binding of ligands to diverse proteins. In proteins, cation-π interactions can be established between the aromatic amino acids (tryptophan, tyrosine and phenylalanine), as the π component, and the positively charged amino acids (lysine, arginine) as the cation [18]. Another example is the molecular recognition of arginine or lysine, as the cation, and the ligands or molecules that contain aromatic rings in their chemical structure like dopamine, L-DOPA, or melatonin derivatives as the π component.

These interactions are responsible for the stability when a complex is formed (Figure 1).

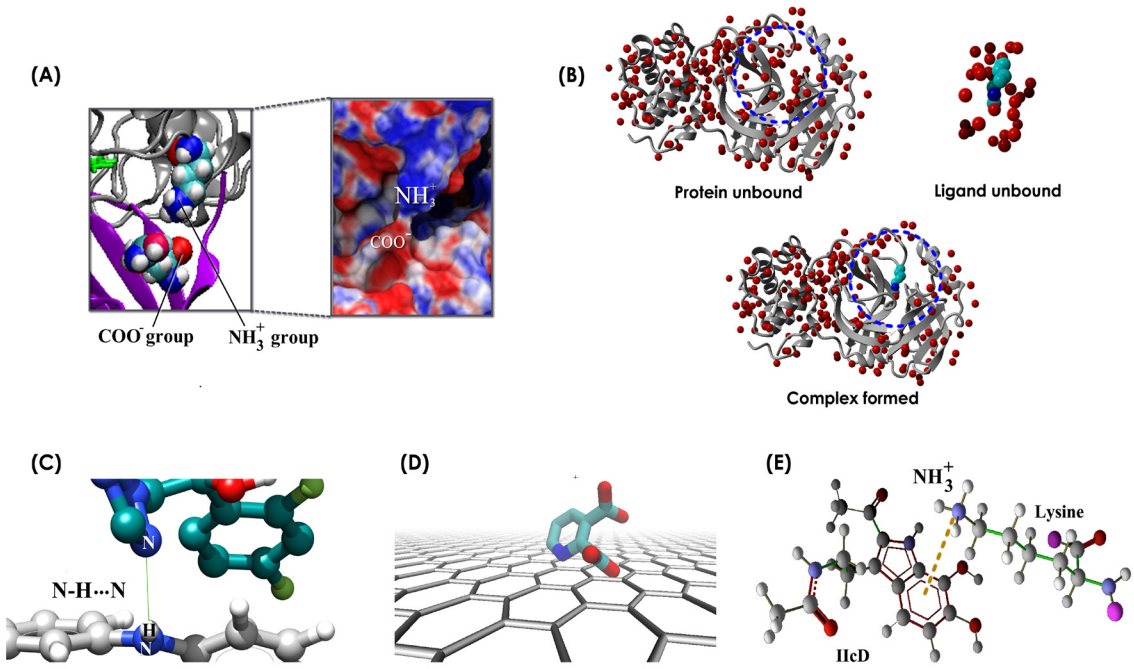

Figure 1: Non-covalent interactions in biological systems. (A) Electrostatic interactions (between charged groups); the electrostatic surface potential is shown in the right panel, where the negative charges are represented in red color and positive charges in blue color. (B) Hydrophobic interactions, these structures do not relate very favorably with water; the water molecules, represented as red spheres, are released in the binding site of a protein by hydrophobic groups of the ligand. (C) Hydrogen bond interactions: attractive interaction between a Hydrogen atom (white color) and an electronegative, Nitrogen atom (blue color). (D) van der Waals interactions are weaker than any other type of chemical bond (sheets of graphene or carbon nanotubes with a drug molecule). (F) Cation-π interactions are established between aromatic rings (π electron-rich) as a melatonin derivative (IIcD) [19] and a positively charged amino acid (lysine) that would act as cation.

2. Thermodynamic Signatures

The binding is a thermodynamic driven process, which is mainly influenced by non-covalent interactions, as well as desolvation, residual mobility and dynamics [20]. The thermodynamic signatures are derived from chemical equilibrium of a reaction, where the rate of the forward reaction (r_1) and rate of the reverse reaction (r_2) are equal:

$$aA + bB \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} cC + dD$$

Forward reaction : $r_1 = k_1[A]^a[B]^b$ (1)

Reverse reaction : $r_2 = k_{-1}[C]^c[D]^d$ (2)

At the equilibrium state, equations (1) and (2) are equal ($r_1 = r_2$)

$$k_1[A]^a[B]^b = k_{-1}[C]^c[D]^d \quad (3)$$

$$\frac{k_1}{k_{-1}} = \frac{[C]^c[D]^d}{[A]^a[B]^b} \quad (4)$$

The equilibrium constant K_{eq} is expressed as:

$$K_{eq} = \frac{[C]^c[D]^d}{[A]^a[B]^b} \quad (5)$$

This equation is fundamental in the study of molecular recognition such as the receptor-ligand interaction.

2.1 Binding constant/ Binding free energy

The binding affinity or binding constant (K_b) presents information about the strength of interaction between a protein, antibody, carbohydrate, DNA, RNA or nanomaterial with ligands (drugs, analogues of substrates, small molecules) [21]. K_b reflects the effects of non-covalent interactions (mentioned above) between protein-ligand (**Figure 2**), and is a particular case of the equilibrium constant (K_{eq}) eq (5).

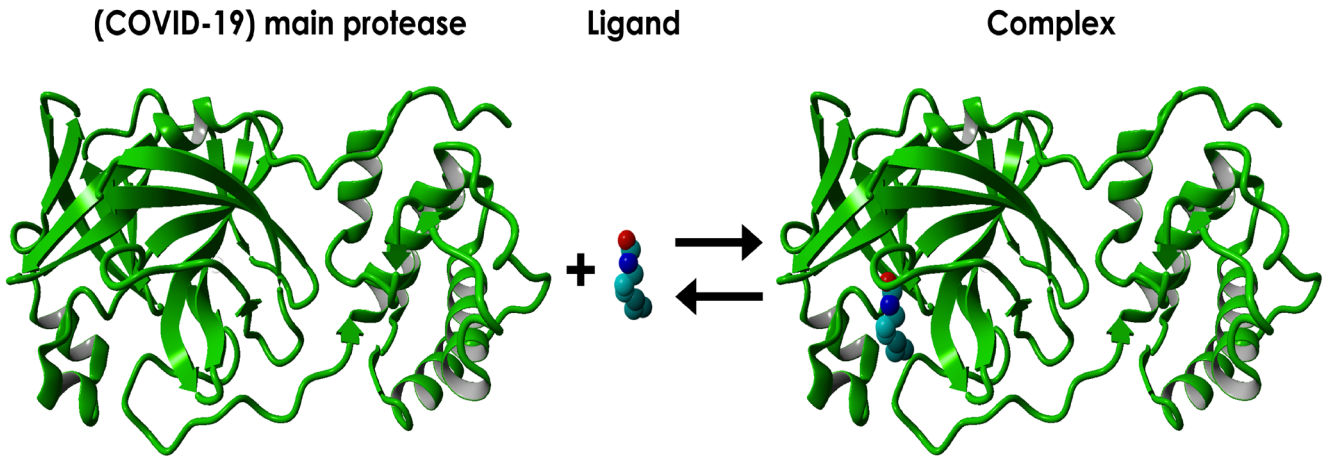

Figure 2: Proteinase-ligand interaction. The crystal structure of COVID-19 main protease in complex with carmofur (ID 7BUY) [22].

According to the **Figure 2**, the equilibrium nature of the protein and ligand is expressed in equation (6)

$$K_b = \frac{[Complex]}{[Protease][Ligand]} \quad (6)$$

The determination of K_b is the basis for the design of drugs. A clear example is the situation that we are currently experiencing, a pandemic, and that several research groups are working urgently to develop new drugs against coronavirus SARS-CoV-2 and thermodynamics plays a very important role in these efforts.

Additionally, the equilibrium (6) can be expressed by the dissociation constant (K_d), which is the inverse of K_b :

$$K_d = \frac{1}{K_b} \quad (7)$$

Fortunately, K_b has been classified as: low ($K_b<10^4$ M^{-1}), moderate ($10^4<K_b<10^8 M^{-1}$) and high ($K_b>10^8$ M^{-1}), through protocols determined by Vazquez-Campoy et al., (2004)[23].

By determining K_b, the Gibbs free energy of binding (ΔG_b) is obtained through the known expression:

$$\Delta G_b = -RT\ln K_b \quad (8)$$

in which R is the gas constant, and T is temperature (in Kelvin).

Furthermore, ΔG_b is related to two important thermodynamic quantities: the binding enthalpy change (ΔH_b) and binding entropy change (ΔS_b):

$$\Delta G_b = \Delta H_b - T\Delta S_b \quad (9)$$

A negative ΔG_b indicates the spontaneity of the binding process. Besides, these thermodynamic parameters are a fundamental key to optimizing the development of drugs, biomaterials, etc.

2.2 Binding enthalpy

The binding enthalpy change (ΔH_b) is given by the interactions that are responsible for the formation of the protein-ligand complex and also for the dehydration of the contact surfaces. Non-covalent interactions van der Waals forces, hydrogen bonds, and electrostatic interactions, are favorable in the binding interface [21,24] causing an exothermic reaction, $\Delta H_b<0$. Conversely, the dehydration of the contact surfaces is an endothermic reaction with $\Delta H_b>0$.

2.3 Binding entropy

The entropic contribution is associated to three important contributions, as expressed in the equation (10):

$$\Delta S_b = \Delta S_{sol} + \Delta S_{conf} + \Delta S_{or-t} \quad (10)$$

where the entropy change (ΔS_b) is related to the solvation entropy (polar and non-polar part, ΔS_{sol}) due to the burial of water-accessible surface area on binding, which is the most important component. The polar part of the solvation entropy consists of the displacement of the water molecules from the binding pocket when the ligand binds to protein. The conformational entropy change, ΔS_{conf} is grounded in the freezing of interfacial side chains of the protein on the binding with the ligand. The ΔS_{or-t} term is referred to the loss of translational and rotational degrees of freedom on the binding. The decrease in degrees of freedom in ΔS_{or-t} and freezing rotatable bonds in ΔS_{conf} cause an unfavorable, and negative entropy in these processes [21,24,

25].

2.4 Enthalpy/entropy compensation.

The binding protein-ligand causes a phenomenon of compensation between enthalpy and entropy. An entropy gain is accompanied by a positive enthalpy change (endothermic) due to the requirement of energy for the disruption of non-covalent interactions in the binding. In the same way, a negative enthalpy change involves a negative entropy change (unfavorable) due to the loss of degrees of freedom that are established between the interacting molecules [21,24].

3. Experimental trend of thermodynamic parameters with computational models

Thermodynamic signatures contribute with powerful insights into protein-ligand interactions. Experimental techniques exist to acquire these thermodynamic properties. For instance, through fluorescence titration, we can determine the K_d, and according to the equations (7) and (8) it allows us calculate $K_b$and ΔG_b. It is noteworthy that through the variation of K_b with the temperature, ΔH_b and ΔS_b can be determined, and these parameters are calculated through the van't Hoff equation [26].

$$\ln K_b = -\frac{\Delta H}{RT} + \frac{\Delta S}{R} \qquad (11)$$

where K_b is the binding constant of complex at absolute temperature (T) and R is the gas constant. The values of $-\Delta H/R$ and $\Delta S/R$ are determined through the slope and intercept, respectively, of the ln K_b vs 1/T plot.

The integrated van't Hoff equation includes an important parameter which is the change in heat capacity (ΔCp) [27]. The description of this parameter is very extensive and will not be covered in this chapter. In our research group, we have determined the thermodynamic parameters, including the ΔCp, within a study of protein-ligand interactions; recently Serratos et al., (2011) [3] and Serratos et al., (2018) [28] reported important characteristics of ΔCp in the binding.

Fluorescence titration is known to be a sensitive technique for the acquisition of thermodynamic parameters. The advantage of this technique is that it requires less than 1.0 mg of protein to carry out an experiment. Another powerful technique is Isothermal Titration Calorimetry (ITC) since it allows the determination of several parameters in a single experiment: $K_b/\Delta G_b$, ΔH_b, ΔS_b and stoichiometry *n*. Multithermal Titration Calorimetry (MTC) is an extension of ITC; is a novel technique that permits to obtain, in a single experiment, not only the parameters already mentioned but also ΔCp [29]. We have recently applied this technique in the study of binding energetics of Triosephosphate isomerase (TIM) from *Saccharomyces cerevisiae* with two of its inhibitors with variations in ionic strength and

osmolality concentrations of the medium [3,28].

Once the experimentally thermodynamic properties are obtained for a given system the signs (>0, <0 or ~0) are an indication of what type of non-covalent interactions participate in the association process. Ross and Subramanian [30] carried out an exhaustive analysis of how no-covalent interactions contribute to the association process.

Also, there are several studies that have addressed the problem of relating the binding thermodynamic parameters experimentally obtained with those obtained by computational methods. To carry out the computational calculations, you must have files that contain the crystallographic coordinates of the molecules involved in the formation of the complex and the complex itself, or files obtained by other techniques that have a .pdb extension.

To estimate the structural ΔH, ΔS and the ΔCp some models are based on empirical parameterizations that correlate the magnitude of the solvent accessible surface area (ASA) that is hidden at the interface of the complex: $\Delta ASA = ASA_{complex} - ASA_{protein} - ASA_{ligand}$. The parameterizations are divided into two contributions (polar and non-polar). The corresponding areas are denoted as ΔASA_{pol} and $\Delta ASA_{non\text{-}pol}$ respectively. Proportionality coefficients such as Δh_{pol}, $\Delta h_{non\text{-}pol}$ for ΔH and Δs_{pol}, $\Delta s_{non\text{-}pol}$ for ΔS have been obtained through the analysis of protein-ligands complexes [31], protein-protein complexes and protein unfolding [32] and protein-carbohydrate complexes [33]. These coefficients of Δcp_{pol}, $\Delta cp_{non\text{-}pol}$ for ΔC_p have been obtained by Murphy and Freire (1992) [34], Spolar and Record Jr (1994) [35], Makhatadze and Privalov (1995) [36], and Myers et al., (1995) [37].

$$\Delta H = \Delta h_{pol} * \Delta ASA_{pol} + \Delta h_{non-pol} * \Delta ASA_{non-pol} \quad (12)$$

$$\Delta Cp = \Delta cp_{pol} * \Delta ASA_{pol} + \Delta cp_{non-pol} * \Delta ASA_{non-pol} \quad (13)$$

According to the eq (10), and as mentioned above, the solvation entropy is based on a polar and non-polar part multiplied by ASA:

$$\Delta S = \Delta s_{pol} * \Delta ASA_{pol} + \Delta s_{non-pol} * \Delta ASA_{non-pol} + \Delta S_{conf} + \Delta S_{or-t} \quad (14)$$

The entropic term is difficult determine in silico because it is computationally expensive. However, Singh and Warshel [25] provided the first microscopic estimate of the magnitude of all the contributions to binding entropy based on solving very complex thermodynamic cycles for each component.

Fogolari et al., (2018) [38] calculated the free energy, enthalpy and entropy from implicit solvent end-point simulations making the estimation of the binding energy more feasible. In recent years, implicit solvent models have shown to be accurate in treating protein-ligand thermodynamics. Unfortunately some contributions (including entropic contributions and

water molecules in the binding) to the calculation of binding energy are omitted, causing a disparity with the experimental ΔG_b. However, in some systems there is an agreement between the experimental and the computational results, since ΔG_b can be evaluated directly, without enthalpic and entropic contributions. This also depends on the study system or sometimes an experimental and computational correlation is established, since ΔG_b is a term that has a greater contribution in the protein-ligand interaction [39].

In our research group, we estimate the binding energies with the Adaptive Poisson-Boltzmaan Solver (APBS) program, which has an implicit solvent model. According to Baker et al., (2001) [40] the binding free energy ΔG_b is a function of the solvation energy ΔG_{sol} and of the coulombic energy ΔG_{coul}, as the electrostatic component. This relationship is given by eq (15):

$$\Delta G_{b,elec} = \Delta G_{sol} + \Delta G_{coul} \qquad (15)$$

Wagoner and Baker [41] also implemented the calculation of the non-polar solvation free energy. We evaluate the non-polar energy $\Delta G_{non\text{-}polar}$ through the energy released by hiding the interface area to the solvent when the complex is formed through the following expression:

$$\Delta G_{b,non-polar} = \gamma(ASA_{complex} - ASA_{protein} - ASA_{ligand}) \qquad (16)$$

In eq (16) the ΔASA is multiplied by coefficient γ, which is an interfacial tension of 5 cal•mol^{-1} Å^{-2} [42, 43]. The calculations of ASA for each species are done with the Visual Molecular Dynamics (VMD) program [44], implying a probe radius of 1.4 Å[1]. Finally, the binding energy, ΔG_b is obtained with equation (17):

$$\Delta G_b = \Delta G_{sol} + \Delta G_{coul} + \Delta G_{non-polar} \qquad (17)$$

Of course, there are many methods for tracking down thermodynamic parameters either experimentally or computationally. In our research group, we have mainly used the methodology described by Baker et al., (2001) for ΔG_b, through the APBS [40] and VMD programs [44] (as mentioned above). We have carried out docking assays or clustering analysis by molecular dynamics to determine the ΔG_b in several systems, and the results have been correlated with the ΔG obtained experimentally by fluorescence titration or ITC . In other systems, the calculated ΔG_b complements other types of experimental studies in order to explain molecular recognition. **Table 1** shows some works by our group.

[1]The probe radius represents a solvent molecule (such as water) in order to calculate the solvent accessible surface area on the protein surface, a value of 1.4 Å is usually assigned to describing hydration effects [45,46].

Table 1: Studies carried out on the protein-ligand interaction applied to different study areas.

Type of interaction	System under study
Protein-inhibitors	Triosephosphate Isomerase with two phosphorylated inhibitors [3].
Protein-dyes	Tau with anionic and cationic dyes [47].
Granular activated carbon-antibiotics	Graphene layers of granular activated carbon with two antibiotics [48].
Organic polymer matrix-antifungal	Polyaniline with fluconazol [49].
Mesoporous material-antifungal	SBA15 with fluconazol. It is noteworthy that SBA15 is a $Si0_2$ matrix; fortunately force fields for Si and metals have been implemented in some programs. In this work, we added interaction studies with QTAIM and NCl analysis, which are correlated with those obtained by APBS and VMD [50].
Protein-protein	Cathepsins and their prosegments [51]
Protein-organic polymer	Integrins with pyrrole structure [52].

4. Conclusion

To conclude, the study of protein-ligand interactions provides data that shed light on the function of a target (proteins). The knowledge of the thermodynamic parameters that govern such process is extremely useful since with the information obtained from the characterization of the recognition site it is possible, on the one hand, to establish the relationships between structure and function and, on the other hand, to develop strategies to interfere with or to facilitate the binding of the protein with the ligand. This is the foundation of the stages involved in the design of drugs, in which diverse areas of knowledge converge; and as we mentioned in this chapter, these procedures are currently being used in the development of biomaterials too. However, much work remains to be done experimentally as well as computationally in order to refine and provide a better explanation to molecular recognition through thermodynamic parameters.

5. References

1. Peck EM and Smith BD. Chapter 1: Applications of Synthetic Receptors for Biomolecules, in Synthetic Receptors for Biomolecules: Design Principles and Applications. From Book Series: Monographs in Supramolecular Chemistry. 2015; pp. 1-38. ISBN: 978-1-78262-206-2.

2. Paketurytė V, Zubrienė A, Ladbury JE, Matulis D. Intrinsic Thermodynamics of Protein-Ligand Binding by Isothermal Titration Calorimetry as Aid to Drug Design. Methods Mol Biol. 2019; 1964:61-74.

3.Serratos IN, Pérez-Hernández G, Garza-Ramos G, Hernández-Arana A, González-Mondragón E, Zubillaga RA. Binding thermodynamics of phosphorylated inhibitors to triosephosphate isomerase and the contribution of electrostatic interactions. J Mol Biol. 2011; 405:158-172.

4. García-Hernández E, Zubillaga RA, Chavelas-Adame EA, Vázquez-Contreras E, Rojo-Domínguez A, Costas M. Structural energetics of protein carbohydrate interactions: Insights derived from the study of lysozyme binding to its natural saccharide inhibitors. Protein Sci. 2003; 12:135-142.

5. Reynolds M, Marradi M, Imberty A, Penadés S, Pérez S. Multivalent gold glycoclusters: high affinity molecular

recognition by bacterial lectin PA-IL. Chemistry. 2012; 18: 4264-4273.

6. Lee S, Lee SH, Heo H, Oh EH, Shin JH, Kim HS, Jung JH, Choi SY, Choi KD, Lee H, Lee C, Choi JH. Impaired DNA-binding affinity of novel PAX6 mutations. Sci Rep. 2020; 10:3062.

7. Swamy MJ, Sankhala RS, Singh BP. Thermodynamic Analysis of Protein-Lipid Interactions by Isothermal Titration Calorimetry. Methods Mol Biol. 2019; 2003:71-89.

8. Quinn CF, Carpenter MC, Croteau ML, Wilcox DE. Isothermal Titration Calorimetry Measurements of Metal Ions Binding to Proteins. Methods Enzymol. 2016; 567:3-21.

9. Yang LY, Hua SY, Zhou ZQ, Wang GC, Jiang FL, Liu Y. Characterization of fullerenol-protein interactions and an extended investigation on cytotoxicity. Colloids Surf B Biointerfaces. 2017; 157:261-267.

10. Huang R, Lau BLT. Biomolecule nanoparticle interactions: Elucidation of the thermodynamics by isothermal titration calorimetry. Biochim Biophys Acta. 2016; 1860:945-956.

11. Kabiri M and Unsworth LD. Application of isothermal titration calorimetry for characterizing thermodynamic parameters of biomolecular interactions: peptide self-assembly and protein adsorption case studies. Biomacromolecules. 2014; 15:3463-3473.

12. Dill KA and Bromberg S. Molecular Driving Forces: Statistical Thermodynamics in Chemistry and Biology. Garland Science Publishing, New York 2003.

13. Berg L. Exploring non-covalent interactions between drug-like molecules and the protein acetylcholinesterase. Doctoral Thesis, 2017.

14. Southall NT. Dill KA, Haymet ADJ. A View of the Hydrophobic Effect. J Phys Chem. 2002; 106: 521-533.

15. Jeffery GA. An Introduction to Hydrogen Bonding, Oxford University Press, Oxford, UK, 1997.

16. Hobza P and Havlas Z. Blue-Shifting Hydrogen Bonds. Chem Rev. 2000; 100: 4253-4264.

17. Roth CM, Neal BL and Lenhoff AM. Van der Waals Interactions Involving Proteins. Biophys J. 1996; 70: 977-987.

18. Dougherty DA. Cation-pi interactions in chemistry and biology: a new view of benzene, Phe, Tyr, and Trp. Science. 1996; 271:163-168.

19. Galano A. Computational-aided design of melatonin analogues with outstanding multifunctional antioxidant capacity. RSC Adv. 2016; 6: 22951-22963

20. Klebe G. Applying thermodynamic profiling in lead finding and optimization. Nat Rev Drug Discov. 2015; 14: 95–110.

21. Du X, Li Y, Xia YL, Ai SM, Liang J, Sang P, Ji XL, Liu SQ. Insights into Protein-Ligand Interactions: Mechanisms, Models, and Methods. Int J Mol Sci. 2016;17: 1-34.

22. Zhao Y, Zhang B, Jin Z, Liu X, Yang H, Rao Z. COVID-19 virus main protease complex with carmofur. To be published.

23. Velázquez-Campoy A, Ohtaka H, Nezami A, Muzammil S, Freire E. Isothermal Titration Calorimetry. Curr Protoc Cell Biol. Editorial board, Juan S. Bonifacino ... [et al.], 2004; Chapter 17, Unit 17.8.

24. Perozzo R, Folkers G, Scapozza L. Thermodynamics of Protein–Ligand Interactions: History, Presence, and Future Aspects. J Recept Sig Transd. 2004; 24: 1-52.

25. Singh Nand Warshel A. A comprehensive examination of the contributions to the binding entropy of protein-ligand complexes. Proteins. 2010; 78:1724-1735.

26. Sharma G and First EA. Thermodynamic Analysis Reveals a Temperature-dependent Change in the Catalytic Mechanism of Bacillus stearothermophilus Tyrosyl-tRNA Synthetase. J Biol Chem. 2009; 284: 4179–4190.

27. Liu Y and Sturtevant JM. Significant discrepancies between van't Hoff and calorimetric enthalpies. II. Protein Sci. 1995; 4: 2559–2561.

28.Serratos IN, Millán-Pacheco C, Garza-Ramos G, Pérez-Hernández G, Zubillaga RA. Exploring interfacial water trapping in protein-ligand complexes with multithermal titration calorimetry. Biochim Biophys Acta Proteins Proteom. 2018; 1866:488-495.

29. Chavelas EA, Zubillaga RA, Pulido NO, García-Hernández E.Multithermal titration calorimetry: a rapid method to determine binding heat capacities. Biophys Chem. 2006; 120:10-14.

30. Ross PD and Subramanian S. Thermodynamics of protein association reactions: forces contributing to stability. Biochemistry, 1981, 20: 3096-3102.

31. Luque I and Freire E. Structural parameterization of the binding enthalpy of small ligands. Proteins: Struct Funct Genet (2002) 49, 181–190.

32. Luque I and Freire, E. Structure-based prediction of binding affinities and molecular design of peptide ligands. Methods Enzymol. 1998; 295:100–127.

33. García-Hernández E and Hernández-Arana A. Structural bases of lectin-carbohydrate affinities: Comparison with protein-folding energetics. Protein Sci 1999; 8:1075–1086.

34. Murphy KP and Freire E. Thermodynamics of structural stabilityandcooperative folding behavior in proteins. Adv. Protein Chem. 1992; 43:313–361.

35. Spolar RS and Record JMT. Coupling of local folding to site-specific binding of proteins to DNA. Science. 1994; 263:777–784.

36. Makhatadze GI and Privalov PL. Energetics of protein structure. Adv Protein Chem. 1995; 47:307–425.

37. Myers JK, Pace CN and Scholtz JM. Denaturant m values and heatcapacity changes: Relation to changes in accessible surface areas of proteinunfolding. Protein Sci. 1995; 4:2138–2148.

38. Fogolari F, Corazza A, Esposito G. Free Energy, Enthalpy and Entropy from Implicit Solvent End-Point Simulations. Front Mol Biosci. 2018; 5:11.

39. Chodera JD and Mobley DL. Entropy-enthalpy compensation: role and ramifications in biomolecular ligand recognition and design. Annu Rev Biophys 2013;42: 121-142.

40. Baker NA, Sept D, Joseph S, Holst MJ, McCammon JA. Electrostatics of nanosystems: application to microtubules and the ribosome. Proc Natl Acad Sci USA. 2001; 98:10037-10041.

41. Wagoner JA and Baker NA. Assessing implicit models for nonpolar mean solvation forces: The importance of dispersion and volume terms Proc Natl Acad Sci U S A. 2006; 103: 8331–8336.

42. Sitkoff, D, Sharp KA, Honig B. Accurate Calculation of Hydration Free Energies Using Macroscopic Solvent Models. J Phys Chem. 1994;98: 1978-1988.

43. Levy RM, Zhang LY, Gallicchio E, Felts AK. On the nonpolar hydration free energy of proteins: surface area and continuum solvent models for the solute-solvent interaction energy. J Am Chem Soc. 2003; 125:9523-9530.

44. Humphrey W, Dalke A, Schulten K. VMD: visual molecular dynamics. J Mol Graph. 1996; 14:33-38

45. Lee B, Richards FM. The interpretation of protein structures: estimation of static accessibility. J Mol Biol. 1971; 55: 379–400.

46. Durham E, Dorr B, Woetzel N, Staritzbichler R, Meiler J. Solvent Accessible Surface Area Approximations for Rapid and Accurate Protein Structure Prediction. J Mol Model. 2009; 15:1093-1108.

47. Lira-De León, KI, García-Gutiérrez P, Serratos IN, Palomera-Cárdenas M, Figueroa-Corona MP, Campos-Peña V, Meraz-Ríos MA. Molecular mechanism of tau aggregation induced by anionic and cationic dyes. J Alzheimers Dis. 2013;35: 319–334.

48. Moral-Rodríguez AI, Leyva-Ramos R, Ocampo-Pérez R, Mendoza-Barron J, Serratos-Alvarez IN, Salazar-Rabago JJ. Removal of ronidazole and sulfamethoxazole from water solutions by adsorption on granular activated carbon: equilibrium and intra particle diffusion mechanisms. Adsorption. 2016; 22, 89–103.

49. Bustos-Terrones V, Serratos IN, Castañeda-Villa N, Vicente EscobarJO, Romero Romo MA, Córdoba G, Uruchurtu Chavarín J, Menchaca Campos C, Esparza SchulzJM, Domínguez OrtizA. Functionalized coatings based on organic polymer matrix against the process of corrosion of mild steel in neutral medium. Progress in Organic Coatings. 2018; 119:221–229.

50 Bustos-Terrones V, Serratos IN, Vargas R, Landeros-Rivera BC, Bustos-Terrones YA, Soto Estrada AM, Vicente Escobar JO, Romero Romo MA, Uruchurtu J, Menchaca C, Esparza Schulz JM, Domínguez A. SBA15–Fluconazole as a Protective Approach Against Mild Steel Corrosion: Synthesis, Characterization, and Computational Studies ChemistryOpen. 2018; 7: 984-994.

51. Martínez-Hernández JC, Serratos IN, Millán-Pacheco C, Rojo-Domínguez A, Padilla-Zúñiga J. What comparisons of natural and chimeric contacts reveal about inhibition of human cathepsins K, L and S by their prosegments. J Mex Chem. Soc. 2019; 63: 13-24.

52. Serratos, I.N., Olayo, R., Millán-Pacheco, C. Morales-Corona J, Vicente-Escobar JO, Soto-Estrada AM, Córdoba-Herrera JG, Uribe O, Gómez-Quintero T, Arroyo-Ornelas MA, Godínez-Fernández R. Modeling integrin and plasma-polymerized pyrrole interactions: chemical diversity relevance for cell regeneration. Sci Rep. 2019; 9: 7009.