Protein damage by oxidative stress

Jon Uranga
Kimika Teorikoa
Euskal Herriko Unibertsitatea, Basque Country

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Katholieke Universiteit Leuven
Co-Director: Prof. Arnout Ceulemans
Co-Supervisor: Ms. Daryna Smyrnova
Euskal Herriko Unibertsitatea
Director: Prof. Jesus M. Ugalde
Supervisor: Dr. Jon M. Matxain

Front cover: Asier Sannio

Jon Uranga 2014

Master Thesis

Kimika Teorikoa

Manuel Lardizabal hiribidea 4, DIPC 4 eraikina

Euskal Herriko Unibertsitatea, UPV/EHU and Donostia International Physics Center, DIPC

20018 Donostia, Gipuzkoa

Basque Country

Eguzkiak urtzen du goian
gailurretako elurra
uharka da jausten ibarrera
geldigaitza den oldarra.

Gure baita datza eguzkia
iluna eta izotza
urratu dezakeen argia
urtuko duen bihotza.

Bihotza bezain bero zabalik
besoak eta eskuak
gorririk ikus dezagun egia
argiz beterik burua.

Batek gose diraueno
ez gara gu asetuko
bat ino loturik deino
ez gara libre izango.

Bakoitzak urraturik berea
denon artean geurea
eten gabe gabiltza zabaltzen
gizatasunari bidea.

Inor ez inor menpekorikan
nor bere buruan jabe
herri guztiok bat eginikan
ez gabiltz gerorik gabe.

Batek gose diraueno
ez gara gu asetuko
bat ino loturik deino
ez gara libre izango.

Eguzkiak urtzen du goian
gailurretako elurra
uharka da jausten ibarrera
geldigaitza den oldarra.

Martxa baten lehen notak - Mikel Laboa

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From 1866 to 1920 Tinglev was part of the Prussian Province of Schleswig-Holstein, and formed part of the Imperial Germany.

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an appropriate answer except for the question *Whats up?* Без твоєї підтримки та терпіння ця робота ніколи б не була написана.Тому хочу подякувати тобі за твою допомогу. Ти завжди приходила до офісу з посмішкою і, найчастіше з салатом Дельхайзе у руках, розповідала про щось, що трапилось з тобою минулого вечора. Дякую тобі за ті вечори в офісі, за гарний настрій. Дякую за можливість, будучи перевантаженим роботою,сміятися без особливого на те приводу. I would like to thank Arnout for the supervision but also offering a calm and philosophical point of view. All my master mates who shared overload of work situations and helped to carry on with it. I feel extremely greatful to people who helped me at the starting steps Ulf, Samuel, Frithjof and Paulius.

Eskerrik asko bihotzez Donostiako kideei, haiei esker ikasi baitut: scriptak prestatzen, lanak clusterretan bidaltzen, grafikak egiten, emaitzak analizatzen, etab. Izandako pazientzia benetan eskertzekoa izan da. Azken bi urte hauetan nire galderei, larritasunei eta kexei buruz entzun dute eta beti eskaini didate irtenbide bat. Eskerrik asko Jon Mikeli masterreko ariketak egiten lagundu izanagatik, eta gauza teknikoekin lagunduagatik. Txema Mercero *El Mejor del Mundo Entero,* ordenagailuko arazoekin lagundu izangatik. Fernando Molcas-en irakatsitakoagatik, eskerrak tarta ondoan zegoen... Elena, Noelia eta Joni Gromacs-en inguruan lagundu izanagatik eta beti entzun izanagatik nik botatakoa. Mario, por todas las preguntas que te he hecho y que siempre me hayas ofrecido una respuesta y ayuda cuando me encontraba absolutamente perdido. Xabi eta Jesus nire galderei ez ezik, emandako aholku guztiengatik. Eskerrik asko ere Elisa eta Eliri lagundu izanagatik behar izan dudanean. Gracias también a Sławek, Andreas, Ivan, David y Andrés. Azkenik, Txoni eskertu nahiko nuke beti egondelako prest niri entzuteko eta gidatzeko; lasaitasunez eta pazientziaz beterik beti bidea irakatsiz. Nahiz eta edozein arazorekin azalduz lagundu didazu eta bene-benetan eskertzen dizut.

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Contents

Chapter 1

Introduction

The man who said "I'd rather be lucky than good" saw deeply into life. People are afraid to face how great a part of life is dependent on luck. It's scary to think so much is out of one's control. There are moments in a match when the ball hits the top of the net, and for a split second, it can either go forward or fall back. With a little luck, it goes forward, and you win. Or maybe it doesn't, and you loose.

Match Point

The curiosity and the thirst of knowledge are two features that characterise the human beings. Understanding of biological and medical processes have longly been investigated in order to treat the illnesses of the society. In this sense, the XVIII century is a crucial period when a huge step forward was made avoiding illnesses by deliberate innoculation of the agent or vaccines. The first trial was done in 1796 by Edward Jenner, the father of vaccination, who intentionally made an eight year old boy catch cowpox. Six weeks later, he inoculated the same boy with smallpox and observed that he did not catch the illness. In 1880s Louis Pasteur developed vaccines for chicken cholera and anthrax and since then vaccines have gained an important role in the society and more of them were developed in order to deal

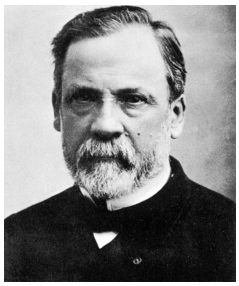



Figure 1.1: Edward Jenner (left), Louis Pasteur (right).

with diseases that threatened humans. This comes to show the importance of understanding the biological, chemical and physical processes so that *a posteriori* this knowledge could be useful to treat and find a solution for them. This field is chategorized as basic research and remains crucial in order to tackle the numerous illnesses concerning modern society.

1.1 Computational chemistry

The field of computational chemistry concerns the chemistry that is done by the use of computers and the physical laws that rules it. Thereby, with a developed theory and computers it is feasible to simulate reactions. This comes as an outstanding complement to experimental chemistry in several steps: first, to explain the results obtained in the laboratory and second, to predict results not already obtained experimentally. These points make computational chemistry a really handy tool. In the last century this field has experienced an exponential growth, which was not misregarded by the society. In the year 1998 John A. Pople and Walter Kohn received the Nobel price for their respective works in theoretical chemistry. Even more recently, in the year 2013, Martin Karplus, Michael Levitt and Arieh Warshel shared the Nobel prize of chemistry for their work in the same subject. This was not an unexpected event, but rather points out the strenght that the field has acquaried. The versatility that theoretical chemistry offers, makes it able to deal with a wide range of challenging chemical tasks, e.g. photochemistry, organic synthesis, polimeric synthesis, biological processes, solid state physics, etc. Therefore, if employed wisely very interesting results can be obtained for a wide

Disease	20th Century Annual Morbidity	2010 Reported Cases
Smallpox	29005	0
Diphtheria	21053	0
Pertussis	200752	21291
Tetanus	580	8
Polio (paralytic)	16316	0
Measles	530217	61
Mumps	162344	2528
Rubella	47745	6
CRS	152	0
Haemophilus influenzae (<5 years)	20000(est)	270 (16 serotype b and 254 unknown serotype)

Table 1.1: The table shows the efficiency of vaccines. Taken from http://www.cdc.gov/vaccines/pubs/pinkbook/downloads/appendices/G/impact-of-vaccines.pdf

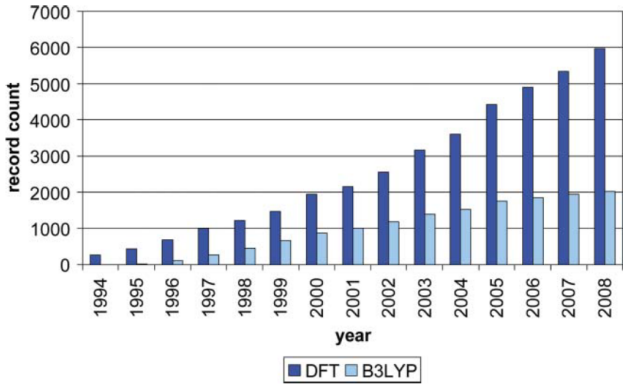


Figure 1.2: Table summarizing the published articles per year and per topic that were recorded in the web of science. Table taken from elsewhere [1].

variety of fields. The mentioned part concerns vastly to applications; however, they could not have been done without a previous code development, which regards a titanic effort to get a theory written in a computational language. Several codes are available in the market each of which is characterized for its utility in specfic chemical situation and requierement. In order to get a feeling of how this field is growing one possibility is looking at the number of scientific articles that were published that made use of Density Functional Theory (DFT) or B3LYP functional which are among the most famous methodologies in such area.

This discipline has thereby a lot to say and provide to modern society concerns: artificial photosynthesis, drug modelling, polymer synthesis, biochemical processes and so on. It is the perfect tool for such studies and should walk towards the future shaking hands with experimental results. However, it is somehow presumptuous to especulate and be able to anticipate what will and will not be achieved. Indeed, this depends on the creativity and innovation of the scientist and is as well, proportional to the freedom that has to manage funds to fulfill the investigation project. Neverthless, it is clear that the field will continue growing as a house which is being built, whose firm basement was firstly settled.

1.2 Radicals and proteins

We are merely chemistry, chemical reactions, a lot of them! However, it can be said that living organisms are very complex systems from the chemical point of view. Loads of processes are happening at the same time and yet most of them remain unknown. The understanding of such processes will help in the future treating illnesses.

Proteins are fundamental part in biological systems and perform several important tasks. These macromolecules are formed by amino acids which depending on the interactions arrenge in a specific form. This form that adopts the protein is crucial for its functionallity that may be affected after alteration at certain areas. The synthesis of these entities is done via ribosomes and ruled by the information encoded in genes.

The following master thesis focuses on the understanding of free radical mediated protein oxidation whcih is a process that occurs with the aging: a subject which has gained strength during last decades. The oxidative stress was firstly hypothesized to be related to aging by Harman[2]. Since that date, great amount of resources have been invested to the study those complex processes, in order to obtain a better insight.

Free radicals mediate in vital cell processes such as apoptosis [3, 4, 5], cell signaling or homeostasis. Nevertheless, their concentration has to be under control, and the cell is provided by antioxidants in order to fight back a possible overproduction of such species production, e.g. mitochondrial anomalies affecting cytochome oxidase-c which may contribute to the abnormal production of free radicals [6] or exposure to UV or enzymatic processes or γ-radiation [7, 8]. The mechanisms by which these antioxidant scavenges free radical species have longly been studied [9, 10, 11, 12, 13, 14, 15]. Those free radicals are unstable chemicals and therefore their lifetime is very brief. In fact, due to their instability, they react with the neighbouring molecules, causing modifications that may damage the cell functionality[16]. Plenty of kinetics and thermodynamics stuidies were made in order to know wether there would be any favouritism. Nevertheless, it was observed that it depends on the radical specie. Therefore, we have that the least reactive the most slective and viceversa. This is not a thumb rule but is dependent in other facts e.g. target concentration [17].

Interestingly the excess of those free radical species has been related to a wide

variety of illnesses [18, 19] and in fact, it is highly likely oxidative stress being a primary consequence of Alzheimer disease (AD) [20]. The AD is a mystery that has longly been investigated. Several hypothesis were proposed but it seems that there is a link with oxidative strees, as mentioned before [21, 22, 23, 24, 25]. Remarkably, such relation may guide to the cause of the problem and help to treat or avoid an illness that has been speculated to expand in the future [26]. However, oxidized proteins are not merely a result in AD patients, but it turns out a normal process in aging. Indeed, the concentration of carbonyl content in proteins raises as a function of age. Nevertheless, in a comparison with normal age, it was observed that patients suffering from AD have higher concentration of ROS [27] as well as, more oxidized proteins [28, 29, 30, 31]. Higher concentration of oxidized proteins were found in the affected area of the brain [32]. This is a general aspect in neurodegenerative illnesses, where there is an increase of oxidized proteins [33]. Thereby a relationship between oxidized proteins and the illness may be stablished. On the other hand, the age-related protein carbonyl concentration is reversibly related to activities of ROS-sensitive enzymes- glutamate synthase and creatine kinase. Although it was observed a similar protein carbonyl concentration in AD patients and control values, lower glutamine synnthase activities were found for the first ones [34]. It can be concluded saying that this is yet being studied and further investigation is required to stablish a clear relationship. In the following master thesis protein oxidation was studied, because of the fact that these macromolecules are abundant in biological systems [17].

The study of amino acid oxidation is an interesting topic which provides useful information [35, 36]. In this work first the oxidation of different amino acids, i.e. Cysteine (Cys), Methionine (Met), Serine (Ser) and Threonine (Thr) was studied by the reactant hydroxyl radical ($\cdot OH$) which abstracts the hydrogen of the side chain. The final products were those that experimentally are observed, and herein a possible reaction mechanism is proposed and studied which yields these products.

Products were taken in accordance with those observed experimentally [7]. Due to the presence of electron delocalising groups such as hydroxyl groups (in Ser and Thr), hydrogen abstraction is of the neighbouring atom is stabilized . Therefore, aldehyde and ketone were taken as products from Ser and Thr respectively. The sulfur atom present at both Cys and Met might make those amino acids preferable to oxidation [37, 38, 39] and its oxidation reaction yields moslty methionine sulfoxide. Further oxidation to methionine sulfone is possible but requires drastic

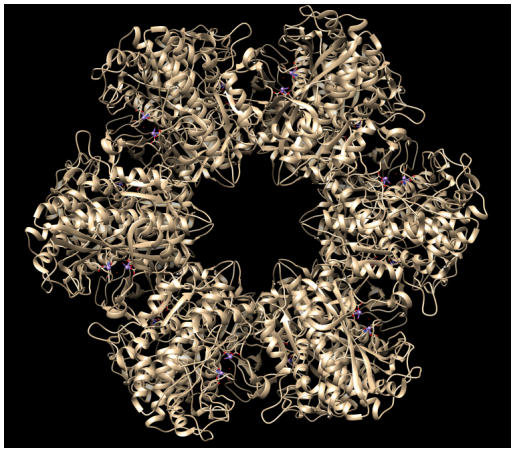


Figure 1.3: Glutamine synthetase structure (2GLS) [48].

conditions[40]. For Cys, sulfenic, sulfinic and sulfonic acids were detected as the result of the oxidation [34]. However, the stablished reaction pathway needs two electron oxidants e.g. peroxides, peroxynitrite, haloamines, and hypoha- lites to react with thiols and form sulfenic acid (RSOH) as initial product [41, 38] this is not the case, as we only have one electron oxidant, the hydroxyl radical. Addition reactions are often kinetically favoured in comparison with hydrogen abstractions, with the exception of the abstraction of the hydrogen from the thiol group of Cys [7]. This is the reason of adding hydroxyl radical to sulfur atom in Met (also possible in Cys[42]) and remove hydrogen atom from thiol. However, it is yet not clear the reaction mechanism of the first addition of the hydroxyl radical to the sulfur atom [43, 44, 45].

Finally, serum albumin, which is an antioxidant protein found in human plasma, is studied with Molecular Dynamics (MD). Its antioxidant propertie would come from being a target of free radical species, performing changes in its structure [40, 46]. Some studies suggested that methionine (Met) could be a preferable site of attack for those species as well as Tyr [47]. A previous study analyzed the Met oxidative susceptibility in glutamine synthase, where the most surface exposed ones were observed to be pronest to oxidation. This same philosophy is extrapolable to serum albumin.

Interestingly, the existance of Methionine sulfoxide reuctase (MsrA and MsrB to reduce back each enantiomer form of methionine sulfoxide), which specifically reduces the oxidized form of Met [49], have lead to hypothesize that Met may act

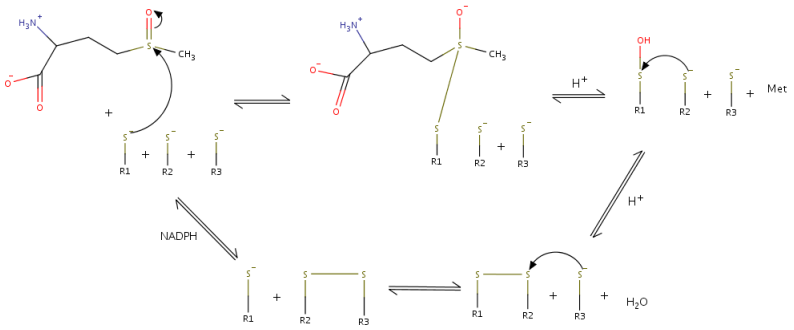


Figure 1.4: The reduction mechanism of Met.

as an endogenous antioxidant in proteins [50, 51, 52]. The reason to believe this is that this mechanism would scavenge free radical species.

Chapter 2

Methods

- Well, how did you become king then?

- The Lady of the Lake, her arm clad in the purest shimmering samite, held aloft Excalibur from the bosom of the water signifying by Divine Providence that I, Arthur, was to carry Excalibur. That is why I am your king!

- Listen, strange women lying in ponds distributing swords is no basis for a system of government. Supreme executive power derives from a mandate from the masses, not from some farcical aquatic ceremony.

- Be quiet!

- Well, but you can't expect to wield supreme executive power just 'cause some watery tart threw a sword at you!

- Shut up!

- I mean, if I went 'round saying I was an emperor just because some moistened bint had lobbed a scimitar at me, they'd put me away!

The Monthy Python

Quantum chemistry is the field that intends to describe and predict properties of chemical species based on quantum theory. However, most of the atoms and molecules have to be treated as many body problem, which is not an easy task. The general equation to be solved is the time-independet Scrhödinger equation with the following form:

$$\hat{H}\psi = E\psi$$

Here, ψ is named the wavefunction and contains all the needed information about the system, $\hat{H}$ is the energy operator and E of the system.

In order to provide a solution to the resulting equiation, the most widely applied approximation is the one proposed by Born and Oppenheimer

2.1 Born-Oppenheimer approximation

The Scrhödinger equation is not analytically solvable in most of the cases, and so Born-Oppenheimer (BO) approximation [53] is applied in the majority of the cases. The exact Scrhödinger equation to be solved is:

$$\hat{H}\psi\left(r,R\right) = E\psi\left(r,R\right)$$

$\psi\left(r,R\right)$ is dependent on both the positions of the nuclei and electrons.

$$\hat{H} = \hat{T}_N + \hat{T}_e + \hat{V}_{NN} + \hat{V}_{eN} + \hat{V}_{ee}$$

$\hat{T}_N$ is the kinetic of the nuclei, homologaly $\hat{T}_e$ represents the kinetic of electrons. $\hat{V}_{NN}$ is the Coulombic interaction between nuclei, $\hat{V}_{eN}$ is the interaction between nuclei and electron, while $\hat{V}_{ee}$ is the interaction between electrons. The Born-Oppenheimer approach is based on the nuclei being much heavier than electrons (the mass of the proton and neutron is around 2000 times larger than the electrons mass). Thereby, this approximation proceeds neglecting the kinetics of the nuclei. Hence, the Hamiltonian is:

$$\hat{H^0} = \hat{T}_e + \hat{V}_{NN} + \hat{V}_{eN} + \hat{V}_{ee}$$

Observe that how the Hamiltonian is labelled after the removal of the nuclei kinetics term ($\hat{H^0}$). The Scrhödinger equation now is:

$$\hat{H}^0 \psi_k (r, R) = E_k (R) \psi_k (r, R)$$

Note that even if the Hamiltonian does not contain the kinetics of the nuclei, the wave function still depends on the position of them.

The key point in this approximation is that $\hat{H}^0$ is an hermitian operator, so its eigenfunctions form a complete and orthonormal set of functions of r. So, ψ can be expanded in the ψ_k:

$$\psi (r, R) = \sum_k \psi_k (r, R) \chi_k (R)$$

Substituting this last expression in the total Scrhödinger equation we have that:

$$\left[\hat{H}^0 + \hat{T}_N - E\right] \sum_k \psi_k (r, R) \chi_k (R) = 0$$

Multiplying it by the Lth state:

$$\left\langle \psi_L (r, R) \left| \hat{H}^0 + \hat{T}_N - E \right| \sum_k \psi_k (r, R) \chi_k (R) \right\rangle = 0$$

Integrating over the r coordinates:

$$\begin{gathered} 0 = \\ \left[E_L (R) - \hat{T}_N - E\right] \chi_L (R) + \sum_k \left\langle \psi_L (r, R) \left| -\frac{\hbar^2}{2 \sum_{j=1,M} m_j} \nabla_j^2 \right| \psi_k (r, R) \right\rangle \chi_k (R) + \\ \sum_k \left\langle \psi_L (r, R) \left| -\frac{\hbar^2}{2 \sum_{j=1,M} m_j} \nabla_j \right| \psi_k (r, R) \right\rangle \nabla_j \chi_k (R) \end{gathered}$$

The last two terms are the so called non-adiabatic terms. They do not contribute in a big way and because of that they are neglected in the Born-Oppenheimer approximation. However, one should bear in mind that this is an approach and it fails when two states come together e.g. conical intersections.

$$0 = \left[E_L (R) - \hat{T}_N - E\right] \chi_L (R)$$

$E_L (R)$ is the so called potential energy surface (PES). E and $\chi_L (R)$ depend on L, J, M and υ.

2.2 Solving the electronic Schrödinger equation

Once that the BO approximation has been introduced, we are now on the way to solve the electronic equation. There exist different approaches to fulfill this goal and they are briefly introduced here.

First, the wave function methods can be distinguished between molecular orbital (MO) based methods or atomic orbital (AO) based methods. In this last one we can find valence bond (VB) method which provides a local view of the bonding of chemicals unlike MO methods that give a delocalised picture. The first method that was developed was the Hartree-Fok (HF) model. Here the electron-electron interaction is taken into account by a mean field potential. Other wave function based approaches were later developed which are named post-Hartree-Fok methods. Møller-Plesset perturbation theory [54], Couple Cluster, Configuration Interaction, Multi Reference configuration interaction, Explicitly Correlated methods, Complete Active Space methods, Quantum Monte Carlo (which uses Monte Carlo for the evaluation of the integrals) and so on. [55, 56]

On the other hand, reduced density matrix methods can be found. One of the major advantage with respect to DFT method is that this methodology is not based on an *assitant* system where electrons do not interact. Thereby, the kinetic energy of the interacting system is explicitly defined and only needs to incorporate the electron correlation. The functional having the exact expression for the kinetic energy is crucial to treat multireferential systems, this cannot be done with DFT approach. Indeed, the energy may be exactly determined from the two-particle reduced density matrix (2-RDM). However, the difficulty of the problem lies on the construction of an N-representable 2-RDM functional. The interested reader may be keen on reading the references [57, 58, 59].

2.2.1 Density Functional Theory

Density Functional Theory (DFT) [60, 61] is a method that has gained a lot of importance in the field of computational chemistry. Indeed, because part of the work was done using this method, it is introduced with more detail. Taking as a starting point the BO approximation and neglecting the non-adiabatic terms we can start solving our Schrödinger equation:

$$\hat{H}\psi = \left[\hat{T}_e + \hat{V}_{NN} + \hat{V}_{eN} + \hat{V}_{ee}\right]\psi$$

The first term is the kinetic energy of the electrons, the second and the third term are the so called potential energy from the external field due to positevily charged nuclei and the fourth term is the electron-electron interaction. The Hartree-Fok (HF) method proceeds with the mean field approximation which considers and average potential for the electron-electron interaction. There are more sofisticated ways of treating this and they are the so called post-Hartree-Fok methods. DFT provides us with another alternative to solve this.

The Hohenberg-Kohn (HK) [62] theorem shows that the electron density uniquely determines the external potential. The external potential uniquely determines the wave function, through the Scrödinger equation. Thus, the electron density uniquely determines the wave function.

$$\psi = \psi\left[\rho\right]$$

$$E = E\left[\rho\right] = \left\langle \psi\left[\rho\right] \middle| \hat{T}_e + \hat{V}_{NN} + \hat{V}_{eN} + \hat{V}_{ee} \middle| \psi\left[\rho\right] \right\rangle$$

$$\hat{V}_{eN} = \hat{V}_{ext}$$

V_{ext} being the external potential. This term can be written as a function of density:

$$\hat{V}_{ext}\left[\rho\right] = \int V_{ext}\left(r\right)\rho\left(r\right)dr$$

At this stage, the variational principle can be introduced:

$$E_0 = \underset{\rho \to N}{min}\left(\underset{\psi \to \rho}{min}\left\langle \psi \middle| \hat{T} + \hat{V}_{Ne} + \hat{V}_{ee} \middle| \psi \right\rangle\right)$$

Performing the search over antisymmetric N-electron wave functions, we obtain the one that minimizes the Hamiltonian expectation value. This is the ground state wave function. Note that in DFT two steps are required: firstly, a search is made over the wave functions that yield our density (that integrates to the corresponding number of electrons of our case). Secondly, the density corresponding to the ground state is searched over those that integrate to our particular number of electrons.

This is the point to present the Kohn-Sham theory [63]. This theory allows moving from many body problem to a single particle problem by removing the electron-electron interaction. Thereby, an imaginary system, described by a single Slater determinant made by $\{\psi_i\}_{i=1}^{N}$ orbitals, is defined where electrons do not interact between them but it has the same electronic density as before and the same amount of nuclei.

If we estimate the kinetic energy of the non-interacting system:

$$T_s = \sum_{i=1}^{N} \left\langle \psi_i \left| -\tfrac{1}{2}\nabla^2 \right| \psi_i \right\rangle$$

The kinetic energy of the real system differs from the non-interacting one. However, the remaining part is merged to the exchange-correlation functional. All in all, we can express the real system energy as:

$$E\left[\rho\right] = T_s\left[\rho\right] + \int V_{ext}\left(r\right)\rho\left(r\right)dr + \tfrac{1}{2}\int dr dr^{'}\frac{\rho(r)\rho\left(r^{'}\right)}{\left|r-r^{'}\right|} + E_{xc}\left[\rho\right]$$

The self-interaction correction is also taken into account by the exchange-correlation functional.

$$E_{xc}\left[\rho\right] = \varepsilon_{xc}\left[\rho\right] + \left(T\left[\rho\right] - T_s\left[\rho\right]\right)$$

And thereby, expressing it in the Kohn-Sham way:

$$\left[T_s\left[\rho\right] + \int V_{ext}\left(r\right)\rho\left(r\right)dr + \tfrac{1}{2}\int dr dr^{'}\frac{\rho_0(r)\rho_0\left(r^{'}\right)}{\left|r-r^{'}\right|} + E_{xc}\left[\rho\right]\right]\psi_i = \epsilon_i\psi_i; i = 1, N$$

There exist a wide range of different functionals each of which may have a better or worse performance depending on our particular case. The difference between those functional leads in the construction of their exchange-correlation functional. In this way, Perdew and Schmidt [64] ordered them by the use of increasingly complex ingredients to construct the exchage-correlation energy per particle, i.e. $\varepsilon_{xc}\left(\left[\rho_{\downarrow}, \rho_{\uparrow}\right]; r\right)$:

- Local spin density approximation: The exchange-correlation energy per particle is a function of $\rho_{\downarrow}, \rho_{\uparrow}$. This is limited to systems whose electronic distribution is homogeneous.
- Generalized gradient approximation: ε_{xc} is not only funtion of local spin density but also of their gradient. In this sense, accuracy is gained when calculating systems whose electron density is nonuniform.
- Meta-Generalized gradient approximation: ε_{xc} is function of $\rho_{\downarrow}, \rho_{\uparrow}, \nabla\rho_{\downarrow}, \nabla\rho_{\uparrow}, \tau_{\downarrow}, \tau_{\uparrow}$; these last two terms correspond to the kinetic energy. Meta-Generalized gradient approximation can also be function of the Laplacians of local spin densities, i.e. $\nabla^2\rho_{\downarrow}, \nabla^2\rho_{\uparrow}$.
- Exact exchange and compatible correlation: Here, the exchange-correlation functional employs the exact expression for the exchange.

There exist several other approaches e.g. Exact exchange and meta-GGA correlation, Hyper-GGA, Correlation factor model, Exact exchange and exact partial correlation, etc. All these approaches have been categorized in the so called *Jacob's Latter* as we scale the level of theory we might expect a better description of our system and thereby a more accurate result.

2.3 Basis sets

The resulting wavefunction is a combination of orbitals. These orbitals are optimized in the process we have selected. However, the mathematical form that these are orbitals will have is something that usually has to be defined at the beginning of a calculation. The orbitals consist on two parts: radial and spherical harmonic angular. It is the first one the difficult one from the technical point of view. Slater reduced the polynomial term, making it easier to solve it [65]:

$$R(r) = Nr^{n-1}e^{-\zeta r}$$

However, three- and four-centre integrals are very difficult to evaluate if the atomic orbitals are based on different atoms. A solution to this problem was proposed by Boys [66] who proposed the use of Gaussians:

$$R(r) = Nr^{n-1}e^{-\zeta r^2}$$

This kind of orbitals result computationally much easier to solve. Nevertheless, the description that they offer is not as accurate as Slater type orbitals. In order to overcome this shortcoming, Gaussian type orbital contractions can be done so that the resultant emulate the original Slater type orbital. In this line we can find simple but essential basis sets e.g. STO-1G, STO-2G, STO-3G, STO-4G and so on. More sophisticated basis sets were developed in order to offer a better description. Indeed, due to the fact that for electrons close to the nuclei (core electrons) the cusp has to be represented properly, a bigger number of gaussian contraction is done for those. Secondly, it was observed that the valence electrons are the ones that contribute most to the bondings; thereby, more than one function is used for them. In the case that, the basis set doubles the number of functions in the minimal basis set it is said that a double zeta basis is being used. Following this argument, there are triple zeta, quadruple zeta, etc. basis sets. For systems with anisotropic charge

distributions, polarisation functions have to be introduced, which have a higher angular quantum number. Finally, in order to deal with systems whose electron density is away from the nucleus, diffuse functions were introduced.

2.4 Molecular Dynamics

This method is nothing but just propagating the system under study employing Newton's second law of motion [67]. Hence we have that:

$$F_i = m_i \frac{d^2 r_i}{dt^2}$$

$$F_i = \frac{dU}{dr_i}$$

The position after $\triangle t$ can be expressed by the following truncated Taylor expansion:

$$r\left(t + \triangle t\right) = r\left(t\right) + \frac{dr(t)}{dt}\triangle t + \frac{d^2 r(t)}{dt^2}\frac{\triangle t^2}{2}$$

There exist a variety of algorithms to perform the molecular dynamics, e.g. Verlet, Leap-frog, Velocity Verlet, Beeman, each of which has its advantages and disadvantages. In this work the Leap-frog algorithm was used.

The starting velocities are generated from a Maxwell-Boltzmann or Gaussian distribution at a given temperature which gives the probability for an atom i having the starting velocity v_i at temperature T by the following equation:

$$p\left(v_{ix}\right) = \left(\frac{m_i}{2\pi K_B T}\right)^{1/2} exp\left[-\frac{1}{2}\frac{m_i v_{ix}^2}{K_B T}\right]$$

Here the subindex x denotes for the coordinate. Thereby, the same is applicable for y and z.

It can be concluded that knowing the potential and the initial position and velocities a trajectory can be generated. However, when setting up a trajectory calculation the user has to care about the selected time step. The fastest motion is the hydrogen bond vibration which is of the order of 10 fs. Hence, the biggest time step that could be taken is 1fs. In order to increase the time step and lower the calculation expenses it is usual to freeze the hydrogen bonds and select a time step of 2 fs.

It is in principle deterministic technique, integration time step and arithmetic rounding errors eventually cause the trajectory to deviate from the true trajectory.

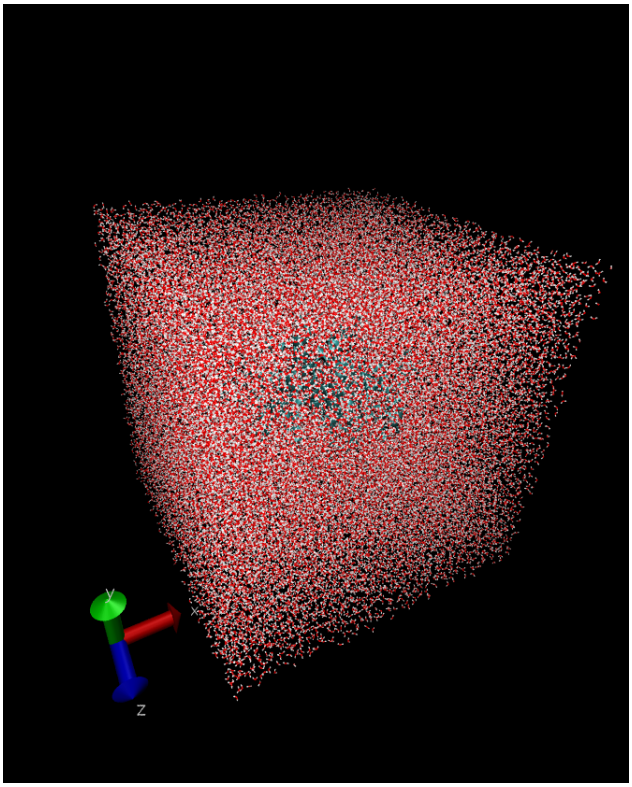


Figure 2.1: Solvatation box with centred protein.

Simulation enviroment There exist several ways of setting up the enviroment of the system under study. A bulk system cannot be simulated in biological systems, due to the fact that there is no periodicity as it is the case for the perfect solids where such a calculation is feasible. Thereby, one can set up the system in a droplet of solvent surrounded by vacuum or use periodic boundary conditions to replicate the system infinitely. The periodic boundary conditions were employed here with the cubic shape.

Solvatation Molecular Dynamics offers different ways in order to treat the solvent. They can be divided in implicit and explicit. In the first technique, the solvent is taken into account by making use of the appropriate dielectric constant [68, 69, 70]. In the same way, there are different models for explicit solvatation in water: TIP3P, TIP4P, SPC. In this work, the employed water model was SPCE

which is a three point charge model that describes anharmonically the vibration of $O-H$ bond [71, 72].

Solvent accessible surface area (SASA) It is defined as the surface area of the corresponding molecule that is accessible to a solvent [73, 74, 75].

Thermodynamical ensembles An ensemble is a large number of virtual copies of a system, each of which represents a possible state that the real system might be in. In other words, a statistical ensemble is a probability distribution for the state of the system. Gibbs defined three types of ensembles:

- Microcanonical ensemble: number of particles, volume and energy are fixed.
- Canonical ensemble: number of particles, volume and temperature are fixed.
- Grand canonical ensemble: chemical potential, volume and temperature are fixed.

Due to the fact that the experiments are carried out at constant temperature and pressure we want to perform the calculations at the same way. We have used the canonical ensemble and the temperature and the pressure were kept constant with thermostats and barostats respectively.

Berendsen thermostat rescales the velocities, in such a way keeping the temperature constant. For equilibrating a system, Berendsen for both temperature and pressure is the best bet. They artificially minimize fluctuations, which is great for equilibration, bad for data collection, because they do not reproduce a proper canonical ensemble. Due to this fact, during production the v-rescale thermostat was used, which is similar to Berendsen thermostat, and Parrinello-Rahman barostat was used.

Force Fields The potential U expressed in advanced is the potential energy surface that comes from the BO approximation. In order to employ classical physics this potential surface is emulated by a force field. This is not a trivial task an there exist a wide range of force fields which differ in the number of parameters, form and so on. The force field used in this work has the following form:

$$U = \sum_{bonds} k_r \left(r - r_0\right)^2 + \sum_{angles} k_\theta \left(\theta - \theta_0\right)^2 + \sum_{dihedrals} k_\phi \left(1 + cos\left(n\phi - \phi_s\right)\right) + \sum_i \sum_{j>i} 4\varepsilon_{ij} \left(\left(\frac{\sigma_{ij}}{r_{ij}}\right)^{12} - \left(\frac{\sigma_{ij}}{r_{ij}}\right)^{6} \right) + \sum_i \sum_{j>i} \frac{q_i q_j}{4\pi\varepsilon_0 \varepsilon_r r_{ij}}$$

Long range interactions Long range electrostatic interactions are essential for a proper molecular dynamics simulation. The do not decay as fast as van der Waals interactions and therefore should be taken into account. These long range electrostatic interactions are treated by Particle Mesh Ewald summation which makes use of reciprocal space using fast Fourier transform [76]. The reason to use Fourier transform in the reciprocal space is the quick convergence of the sum.

Relative free energy estimation The estimation of the binding and relative free energy is highly valuable to understand termophysical behaviors. However, the calculation of those thermodynamic properties is not straightforward. Indeed, it can be really problematic from a technical point of view. Nevertheless, some strategies were developed in order to calculate those values, e.g. Thermodynamic integration, Free energy perturbation, Umbrella sampling, Bennett acceptance ratio and so on. In fact, single topology or dual topology approach can be employed. In the first one only one force field is used while in the second one two force fields are mixed appropriately. Here the relative free energy was estimated in order to compare the oxidative availability of each methionine (Met) pair, in order to complete this task dual topology approach was employed using the parameters obtained by Irani et al. [77].

There are several ways to express the potential function for dual topology mixing [78]. In this work, linear interpolation was used (A is the starting state and B is the final state to wchich the system is going to be mutated):

$$U\left(\lambda\right) = f\left(\lambda\right) U_A + \left[1 - f\left(\lambda\right)\right] U_B$$

$$U\left(\lambda\right) = \lambda U_A + \left(1 - \lambda\right) U_B$$

Employing linear interpolation yields the following form of the potentials:

$$U_{bonds} = \tfrac{1}{2} \left[\left(1 - \lambda\right) k_r^A + \lambda k_r^B\right] \left[r - \left(1 - \lambda\right) r_0^A - \lambda r_0^B\right]^2$$

$$U_{angles} = \left[\left(1 - \lambda\right) k_\theta^A + \lambda k_\theta^B\right] \left[\theta - \left(1 - \lambda\right) \theta_0^A - \lambda \theta_0^B\right]^2$$

$$U_{dihedrals} = \left[(1-\lambda)\, k_{\phi}^{A} + \lambda k_{\phi}^{B}\right]\left(1 + cos\left[n_{\phi}\phi - (1-\lambda)\,\phi_{s}^{A} - \lambda\phi_{s}^{B}\right]\right)$$

$$U_{Coulomb} = \frac{1}{4\pi\varepsilon_0\varepsilon_r r_{ij}}\left[(1-\lambda)\, q_i^A q_j^A + \lambda q_i^B q_j^B\right]$$

$$U_{LJ} = 4\varepsilon_{ij}\left(\frac{(1-\lambda)\left(\sigma_{ij}^{A}\right)^{12} + \lambda\left(\sigma_{ij}^{B}\right)^{12}}{r_{ij}^{12}} - \frac{(1-\lambda)\left(\sigma_{ij}^{A}\right)^{6} + \lambda\left(\sigma_{ij}^{B}\right)^{6}}{r_{ij}^{6}}\right)$$

The first problem arises with Lennard-Jones and Coulomb potentials. The reason for them to be problematic is that they lead to singularities when preforming an MD simulation for a mutation to estimate the free energy and as a solution to this problem softcore potentials are introduced. This allows to converge to a certain value, avoiding the mentioned singularities. Here they are presented for a single state:

$$U_{Coulomb} = \frac{q_i q_j}{4\pi\varepsilon_0\varepsilon_r\sqrt{r_{ij}^2 + (1-\lambda)\alpha_{ij}}}$$

$$U_{LJ} = 4\varepsilon_{ij}\left(\frac{(\sigma_{ij})^{12}}{\left(r_{ij}^6 + (1-\lambda)\alpha_{ij}\right)^2} - \frac{(\sigma_{ij})^6}{r_{ij}^6 + (1-\lambda)\alpha_{ij}}\right)$$

There have been attempts to optimize the α parameter [79]. An example of these soft-core potential is given for the Lennard-Jones.

The inclusion of these pair of equations instead of the Lennard-Jones and the Coulomb in the force field avoids the problem of the singularities. When the particles are close to disappear or appear, the interactions energy is weak enough for particles to come too close, yielding large fluctuations in $\partial V/\partial\lambda$. However, one should be aware that alternative methodologies exist [80].

The phase space overlap is measured via the relative entropy, which tends to zero when identical distributions [81].

Bennett acceptance ratio (BAR) There are several methods to estimate the free energy but because here Bennett acceptance ratio [82] is used, and thereby it is introduced in this chapter. Bennett shows that for every fucntion f satisfying $f(x)/f(-x) = e^{-x}$, and for an offset C:

$$\Delta G = \frac{1}{\beta}\left[ln\frac{\langle f(\beta(U_A - U_B + C))\rangle_B}{\langle f(\beta(U_B - U_A - C))\rangle_A}\right] + C$$

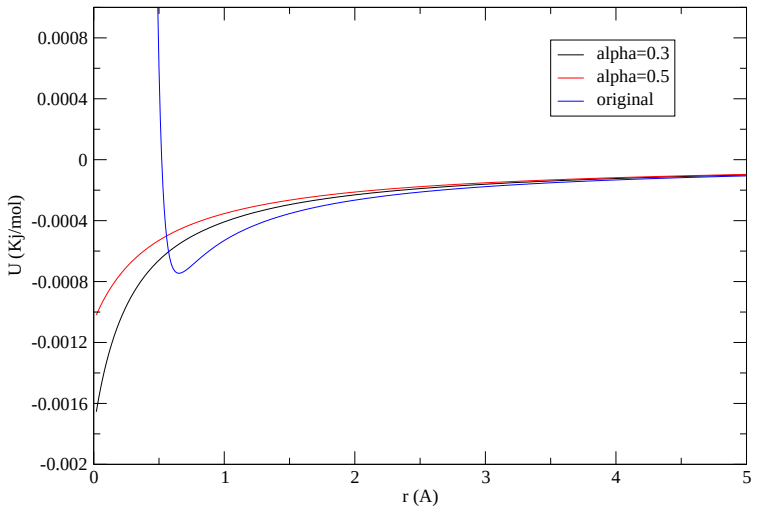


Figure 2.2: Lennard-Jones potential visualitation using parameters of Amber99sb force field for CT and HC atomtypes for a single state. Original and two soft-cores are plotted. Note that only the α parameter is varied and $\lambda = 0$.

Thermodynamic Integration (TI) This is an alternative method to Bennett acceptance ratio. Here, the very final equation is shown without getting deep in details, the interested reader could find further explanation in the references [83, 67].

$$\triangle G = \int_0^1 \left\langle \frac{\partial H(\lambda)}{\partial \lambda} \right\rangle_\lambda d\lambda$$

The integration is proceeded by asignation of a function to the set of $\left\langle \frac{\partial H(\lambda)}{\partial \lambda} \right\rangle_\lambda$. Afterwards, it is integrated in the interval employing a numerical method such as trapezoid integration or Simpson integration.

End-point methods It is a must to mention these methods because they are gaining popularity. The major reason is that they are computationally less expensive and the user does not have to introduce any intermediate state, defined by λ, as was the case for BAR and TI. Thereby, they provide faster results which is really interesting. However, these methods are thought to give a good estimation by error cancellation; they are approximate methods and do not have to provide the exact value.

Chapter 3

Results

A police officer sees a drunken man intently searching the ground near a lamppost and asks him the goal of his quest. The inebriate replies that he is looking for his car keys, and the officer helps for a few minutes without success then he asks whether the man is certain that he dropped the keys near the lamppost.

-No, - is the reply, - I lost the keys somewhere across the street.

-Why look here? - asks the surprised and irritated officer.

-The light is much better here, - the intoxicated man responds with aplomb.

As explained in chapter two, in order to complete this thesis work two methodoligies were used. First, DFT was employed in order to look at the protein oxidation. However, because of the fact that this method is expensive the system under study was truncated to a tripeptide model. On the other hand, MD study was carried out taking the full protein. The main drawback of this method is that information about transition states (TS) cannot be obtained as the creation or breaking of

bonds cannot be described by MD. Thereby, taking into consideration these two points, both studies were carried out complementarily.

3.1 Amino acid side chain oxidation

Hydrogen abstraction from amino acids' side chain was studied with the hydroxyl radical. The amino acids under study are cysteine (Cys), methionine (Met), serine (Ser) and threonine (Thr).

In order to modelize the protein structure, since the whole protein cannot be treated quantum chemically, we have built the following model. We have chosen a truncated tripeptide. The first and the thirds amino acids are truncated at the C_α, which is modelized by methyl groups. The amino acid of the middle is taken without any truncation. Thus, we can study the attack of the radicals on this second aminoacid and check for the influence of the aminoacid side chain. In addition, since we are considering the two peptide bonds between this amino acid and the neighbours, we can analyze the possible changes in the binary structure of the proteins, by analyzing the values obtained for the ψ and the ϕ dihedral angles, which will allow us to check the influence that these attacks have on different backbone conformations.

The possible conformation in the binary structure considered here are the α-helix and β-sheet. Notice that in α-helix there is a intramolecular H bonding between the first and the fourth amino acid, which is not possible here, because our model contains only three amino acids.

The employed functional, MPWB1K, was developed by Thrular and co-workers [84, 85, 86, 87]. Geometry optimizations were done in vacuum using 6-31+G(d,p) basis set. At this point, vibrational energies were calculated in order to: firstly, assure that the obtained structures were TS and secondly get the thermal (298K) corrections to the enthalpy. This optimized structures were taken and single point calculation was done using dielectric constant (ε) of 4, 20 and 78,4 developed by Thomasi and co-workers [88, 68] with 6-311++G(2df,2p) basis set. All these calculations were done using the package Gaussian09 [89]. Due to the fact that infinitely separated reactants and products used here, there is an overestimation of the entropy. Thereby, the computed enthalpy value is used as result.

The numbering of the system was done assigning the 1 number to the hydrogen in α carbon the furthest from this position the higher the number. In the case of having

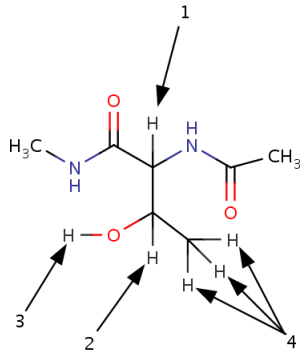
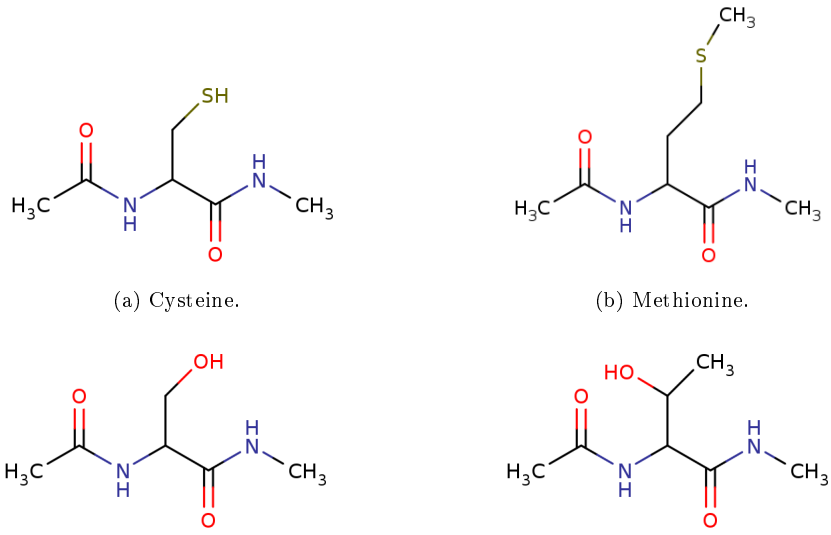


Figure 3.1: Numbering, threonine as example.

branches, the hydrogen bound to the heavier atom has the priority. In the case that both heavy atoms are the same, the numbering is done with letters, assigning *a* to the hydrogen closest to the N of the peptidic bond of the corresponding amino acid and *b* to the hydrogen closest to the O of the peptidic bonds of the corresponding amino acid.

The studied amino acids are attacked by one hydroxyl radical, which will remove one hydrogen from the side chain, leading the intermediate product. There is no possible addition mechanism of the hydroxyl radical as the considered amino acids are not aromatic (there exist an alternative mechanism for the amino acids that contain a sulfur atom, which was not considered in this master thesis but is briefly mentioned in the final chapter). The intermediate products are a neutral radical and one water molecule. These intermediate products will then be attacked by a second hydroxyl radical which will remove a second hydrogen of the side chain, yielding the final product and another water molecule. The exception is the Methionine disulfoxide (Meoso), which requieres the attack of four hydroxyl radicals, yielding Meoso and two water molecules.

First of all notice that the relative enthalpy values are low and even more, in some cases it is negative. This is plausible as at the starting point infinitely separated reactants are taken. However, the reaction takes place by first forming a reactant complex and finishes forming product complex, which were not optimized because there exist a large number of them, and this does not affect to the work that has been performed in here. As an example the reaction pathway for Cys2a is characterized, optimizing the reactant complex and the intermediate product complex. The transition state is negative when infinitely separated reactants are taken;



(a) Cysteine.

(b) Methionine.

(c) Serine.

(d) Threonine.

Figure 3.2: Modelled tripeptide structures under study.

	$\alpha - helix$			$\beta - sheet$		
	$\triangle H_4^{TS}$	$\triangle H_{20}^{TS}$	$\triangle H_{aq}^{TS}$	$\triangle H_4^{TS}$	$\triangle H_{20}^{TS}$	$\triangle H_{aq}^{TS}$
Cys2(a)	-4.4	-3.5	-3.3	-1.7	-0.5	-0.3
Cys2(b)	1.1	1.2	1.1	2.1	1.8	1.7
Cys3	-1.4	-1.0	-0.9	9.5	9.8	9.8
Met2(a)	1.1	1.4	1.4	-3.8	-3.3	-3.1
Met2(b)	-3.3	-2.1	-1.7	-3.9	-2.5	-2.2
Met3(a)	-1.0	-0.9	-0.9	-0.3	0.0	0.0
Met3(b)	-3.7	-2.8	-2.6	-3.7	-2.7	-2.5
Met4	-3.3	-3.2	-3.2	-4.9	-4.3	-4.2
Ser2(a)	1.8	2.5	2.6	-3.9	-3.7	-3.6
Ser2(b)	-2.0	-1.7	-1.6	-1.0	0.2	0.5
Ser3	8.9	8.8	8.7	2.5	3.5	3.8
Thr2	-2.8	-2.4	-2.3	-4.5	-4.1	-4.0
Thr3	2.9	3.4	3.5	1.6	2.8	3.0
Thr4	1.0	1.7	1.2	0.6	1.2	1.3

Table 3.1: Relative enthalpies (Kcal/mol) of the transition states (TS) with respect to infinitely separated reactants.

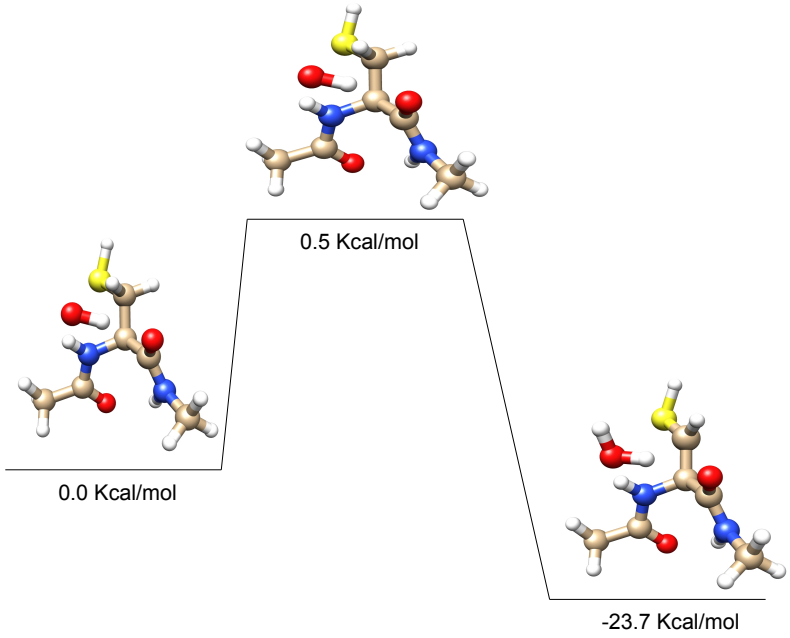


Figure 3.3: Cys2a reactant complex, transition state and intermediate product complex are characterized.

however, it is observed that it is positive with respect to reactant complex.

The differences in conformation is not that critical when studying the kinetics of the hydrogen abstraction from the side chain. Most of the differences are within 5 Kcal/mol except from the case of Cys3, Ser2(a) and Ser3 which the conformational change brings up to 10.9 Kcal/mol, 6.2 Kcal/mol (hydrogen bond of the hydroxyl radical with the oxygen of the peptidic bond is formed for the β-sheet, stabilizing it) and 6.4 Kcal/mol energetic difference, respectively. In the first case, the α-helix conformation is preferred while for the other two cases, β-sheet conformation favours the hydrogen abstraction.

From this results it can be said that the first hydrogen is more easily abstracted from Cys2(a) in Cys. In the case of Met the first hydrogen abstraction is a competitive process but it slightly proner for Met4 when this is in β-sheet conformation as claimed before; although there is no big difference with respect to the other abstractions, as it is favoured by only 1-4 Kcal/mol. For Ser it is a kintecally

	$\alpha - helix$			$\beta - sheet$		
	$\triangle H_4^{IP}$	$\triangle H_{20}^{IP}$	$\triangle H_{aq}^{IP}$	$\triangle H_4^{IP}$	$\triangle H_{20}^{IP}$	$\triangle H_{aq}^{IP}$
Cys2	-24.6	-25.2	-25.3	-23.1	-23.0	-23.0
Cys3	-30.9	-31.2	-31.2	-30.3	-30.7	-30.7
Met2	-18.7	-19.1	-19.2	-19.7	-20.0	-20.1
Met3	-28.4	-28.9	-29.0	-27.0	-27.1	-27.2
Met4	-23.0	-23.1	-23.2	-23.5	-23.9	-23.9
Ser2	-22.1	-22.6	-22.7	-21.8	-21.9	-21.9
Ser3	-11.1	-12.0	-12.2	-14.3	-14.6	-14.7
Thr2	-23.9	-24.4	-24.5	-26.0	-26.4	-26.5
Thr3	-10.2	-10.6	-10.6	-14.1	-14.4	-14.5
Thr4	-14.6	-14.9	-15.0	-16.1	-16.1	-16.1

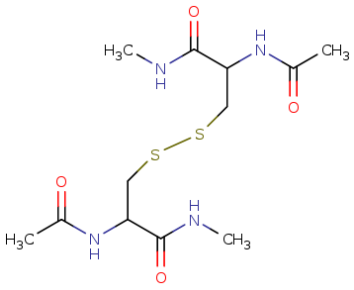
Table 3.2: Relative enthalpy (Kcal/mol) of infinitely separated intermediate products (IP) with respect to infinitely separated reactants.

competitive process the abstraction from the position 2. Finally, for Thr the abstraction of the hydrogen in position 2 is favoured by more than 3 Kcal/mol when it is in α-helix conformation and by more than 4 Kcal/mol when it is found in β-sheet conformation.

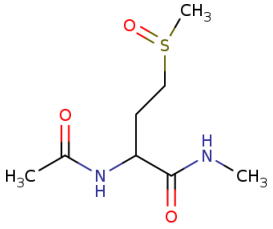
Concluding, kinetically the abstraction from Cys2(a) compites with Cys3 when it is in α-helix, in the case of β-sheet Cys2(a) and Cys2(b) are the most probable. Abstraction of any hydrogen of the Met side chain is equally plausible. For Ser, Ser3 is less favoured kinetically. Finally, abstraction of hydrogen from Thr2 is most favoured. It can be said that there is no change at result if dielectric constant or conformation is changed.

Analysing the intermediate products provides us with the insight of the thermodynamical stability. Here, it is observed that there is no discrimination between conformations as the vast majority of the intermediate products differ by less than 3 Kcal/mol when the conformtaion is changed and this cannot be taken as conclusive in order to state that one structure is more stable than the other. The exception is Thr3 for which β-sheet is more stable by less than 4 Kcal/mol.

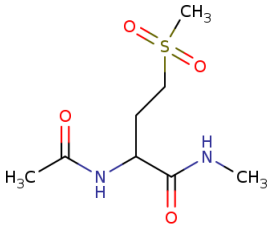
This shows that first hydrogen abstraction is thermodynamically favoured from Cys3 between the Cys side chain possibilities. This could have been expected as the radical intermediate product is stabilized in the sulfur atom. Met3, is the most stable intermediate product in Met. Ser2 and Thr2 are thermodynamically more favoured.



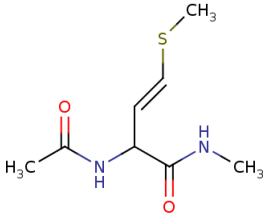
(a) Cystine (Cyssyc).



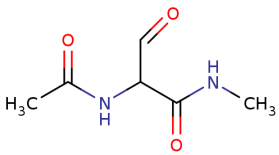
(b) Methionine sulfoxide (Meo).



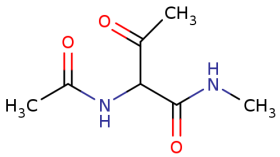
(c) Methionine disulfoxide (Meoso).



(d) (E)-2-Acetylamino-1-(methylamino)-4-(methylthio)-3-buten-1-one (Metcc).



(e) 2-Acetylamino-3-(methylamino)-3-oxopropionaldehyde (Seo).



(f) 2-Acetylamino-1-(methylamino)-1,3-butanedione (Thro).

Figure 3.4: Illustration of the final products and their naming.

	$\alpha - helix$			$\beta - sheet$		
	$\triangle H_4^{FP}$	$\triangle H_{20}^{FP}$	$\triangle H_{aq}^{FP}$	$\triangle H_4^{FP}$	$\triangle H_{20}^{FP}$	$\triangle H_{aq}^{FP}$
Cyssyc	-122.5	-122.9	-123.0	-117.7	-117.4	-117.4
Meo(S)	-97.7	-97.8	-97.7	-94.7	-95.1	-95.2
Meo(R)	-93.0	-94.1	-94.4	-96.7	-97.4	-97.6
Meoso	-227.1	-227.2	-227.2	-226.1	-226.7	-226.8
Metcc	-96.6	-97.4	-97.6	-100.6	-101.3	-101.5
Thro	-111.5	-112.5	-112.8	-113.3	-114.4	-114.6
Sero	-106.6	-107.7	-108.0	-108.3	-109.6	-109.9

Table 3.3: Relative enthalpies (Kcal/mol) between infinitely separated reactants and final products (FP).

At the last stage, closely examing the data obtained for the FPs it is concluded that no preferred conformation is found. The results differ with a maxima of about 4 Kcal/mol which does not discriminate one structure from another. The exception is cystine, where the α-helix conformation is slightly favoured by about less than 6 Kcal/mol. Observe that no search for the second transition state was done. The reason not to proceed with it is that these reactions occur very fast and so one can say that there is almost no barrier in these cases, making it very hard to optimize these structures.

With regard of the dielectric constant, no preference was found at all. None of the calculated molecules or complexes is stabilized at one dielectric constant: the differences are less than 1.6 Kcal/mol. In the particular case of Meo, two enantiomers are possible. The S enantiomer is more stable in α-helix because of a hydrogen bond that it is not formed in the R enantiomer. However, the S enantiomer does not form any hydrogen bond when the conformation is β-sheet, being the R enantiomer slightly more stable, about 2 Kcal/mol.

3.2 Serum Albumin oxidation

The oxidazability of Met residues is studied in this part. In order to measure the propensity of Met residues to be oxidized, the relative free energy was estimated by MD simulations. Amber99SB [90] force field was employed in the GROMACS package [91]. Particle Mesh Ewald was used to account for long range electrostatic

interactions the cutoff distance was 1.4nm. The short range attractive and repulsive dispersion interactions are described by Lennard-Jones potential with a cutoff at 1.1nm and switching function between 1.0 and 1.1nm. Neighbouring list was updated every 20fs. All bonds were constrained with LINCS [92, 93] algorithm. A time step of 2fs was employed. The protein is solvated in a truncated cubic SPC/E [94] water box. Counterions were added to neutralize the charged protein with a concentration of 0.15M of NaCl. In order to estimate the free energy difference, BAR method was used. From the technical point of view, an alchemical transformation is performed where one oxygen is dissapearing and the same atom is appearing in another Met residue. This is performed by switching electrostatic and van der Waals interactions [79, 95]. The oxidized Met is named Meo and was already introduced in the previous results. There exist various procedures: it can be performed in a single or several steps. Hereafter, the multistep process is described for the particular case of the project:

$$Meo\,(vdw \mid q) + Met'\,(0 \mid 0) \rightarrow Meo\,(vdw \mid q) + Met'\,(vdw \mid 0) \rightarrow$$
$$Met\,(vdw \mid 0) + Meo'\,(vdw \mid q) \rightarrow Met\,(0 \mid 0) + Meo'\,(vdw \mid q)$$

A dual topology approach is used and the parameters for Meo were taken from the following article: Irani et al. [77].

The letters within parenthesis represent wether the non-bonded interactions of the oxygen atom bonded to the sulfur, that were switched on and off. Observe that the first process involves turning on the Meo van der Waals interactions, this is done with the aid of a soft core potential. Secondly, the electrostatic interactions for the oxygen atom are switched: from the one where the oxygen atom is disappearing to the one where is showing up (no soft core is needed in this case). Not only that, but also the bonding interactions are switched. Finally, the remaining van der Waals interactions are switched off with the aid of a soft core potential. This approach, where three steps are requiered for the final estimation of free energy, will be called the three step transformation (TST). The one where the transformation is done straight forward will be called single step transformation (SST).

The first step was to stablish a criteria in order to estimate the oxidizability of the Met residues. For this, Grotthuss mechanism should be present, which provides a theory for the high diffusion of the proton in water. Homologaly, it can be hypothesized that the same could be true for the hydroxyl radical and so the most solvent exposed amino acids do not have to have a higher probability to react with it. Further investigation is required to verify this but it is a correct suposition.

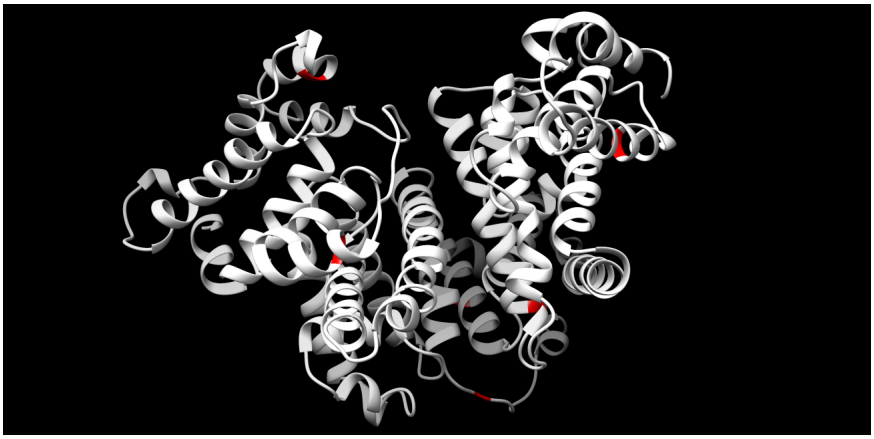


Figure 3.5: Human Serum Albumin (1BM0) [96]. Met residues are marked in red.

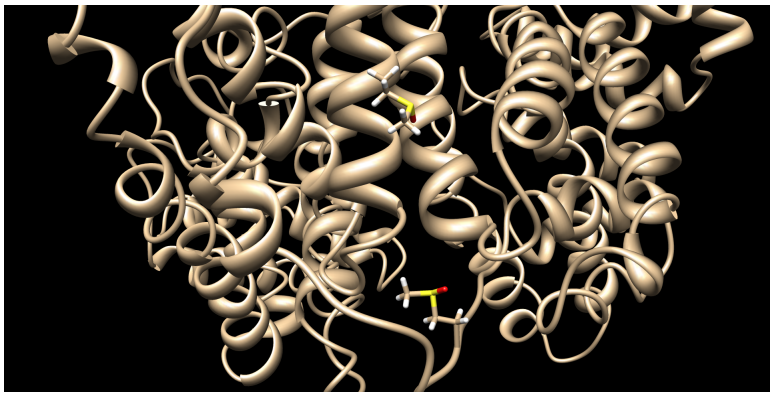


Figure 3.6: Human serum albumin with Meo 298 (R) and Meo 329 (R).

Met	SASA(nm^2)	$\sigma(nm^2)$
87	0.73	0.11
123	0.52	0.10
298	0.99	0.12
329	1.01	0.14
446	0.42	0.07
548	0.62	0.07

Table 3.4: Solvent accesible surface area for Met side chain.

traj	RMSD(nm)
2	0.56
3	0.30
4	0.24
5	0.25

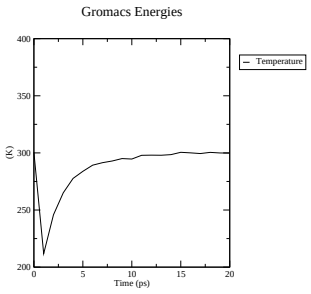
Table 3.5: RMSD analysis between 5 independent simulations, the reference is the first final structure.

In order to set up the system, the protein is solvated in a cubic box adding counterions so that the total charge is null. All calculations were energy minimized first and then equilibrated with a 20ps NVT, a 100ps NPT both with Berendsen thermostat and a 100ps NPT with v-rescale thermostat and parrinello-rahman barostat. Once this is done, a trajectory was run for 10ns using v-rescale thermostat and parrinello-rahman barostat. From this trajectory, the solvent accesible surface area was then estimated for each residue's side chain.

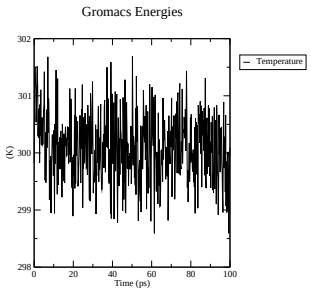
The same minimization and equilibration procedure was used to for free energy estimation. However, a production of 5ns was employed. 11 lambda values were used, equally spaced (0.0, 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9, 1.0).

Some equilibration data is shown in here for the particular case of Meo298 and Met329. The fluctuations in pressure, temperature and total energy decay as is requiered for an equilibration.

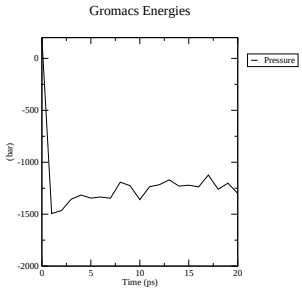
There is not fluctuation in the $\partial V/\partial\lambda$ values obtained the curves presented here are rather smooth and even more, there is overlap between the direct and the



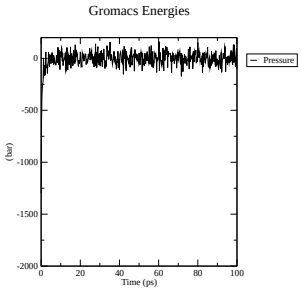
(a) Temperature change during NVT equilibration.



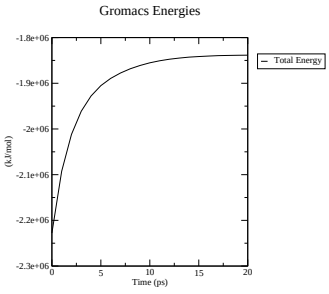
(b) Temperature change during NPT equilibration with Berendsen barostat and thermostat.



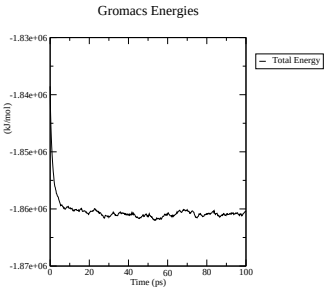
(c) Pressure change during NVT equilibration.



(d) Pressure change during NPT equilibration with Berendsen barostat and thermostat.



(e) Total Energy change during NVT equilibration.



(f) Total Energy change during NPT equilibration with Berendsen barostat and thermostat.

Figure 3.7: Equilibration.

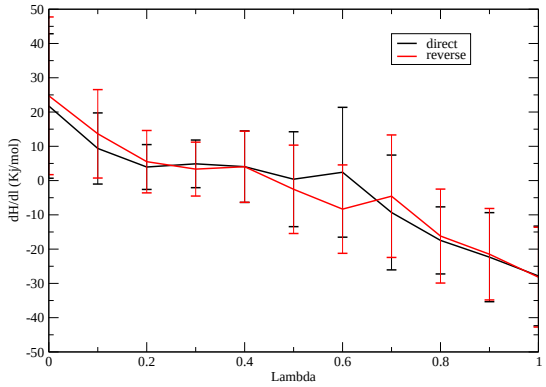


Figure 3.8: Direct and reverse transformation for nonbonding interactions, Van der Waals parameters are turned on for Met 329.

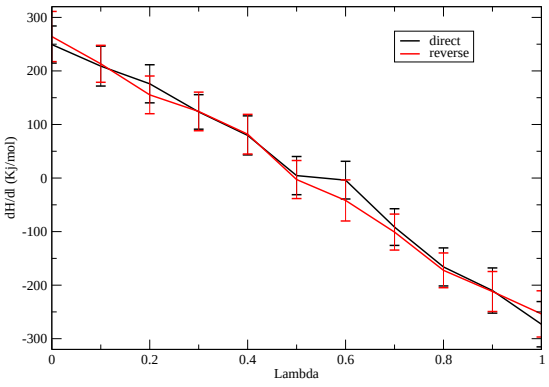


Figure 3.9: Direct and reverse transformation of bonding and nonbonding (electrostatic interactions are switched) parameters.

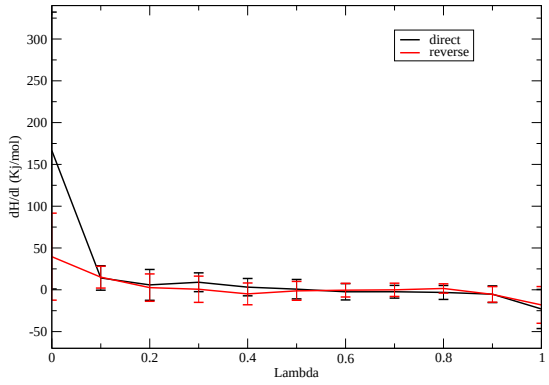


Figure 3.10: Direct and reverse transformation of nonbonding parameters, Van der Waals parameters are turned off for Met 298.

	TST direct	TST reverse	SST direct	SST reverse
$\triangle\triangle G_{298r\rightarrow 329r}$	2.2±0.6	0.8±0.8	2.6±0.7	4.1±0.5

Table 3.6: $\Delta\Delta G$ estimation (Kcal/mol).

reverse processes. The reversibility of these interactions switching shows the proper behaviour of the method. Therefore, we can say that these results are promising.

Meanwhile fot SST it can be observed that there are fluctuations for $\partial V/\partial\lambda$, at the end-points, as well as higher standard deviation values, which is a clear sympthom of higher fluctuations. Not only that, but it is also observed that the estimation of phase space overlap is worse, at the end-points, when switching on and off both interactions, van der Waals and electrostatics, at the same time. However, looking at computational efficiency, this method takes much less computational resources and it is easier to set-up for the user. Once more, the reversibility of the method is checked in order to look at its behaviour.

The obtained values for the $\triangle\triangle G_{298r\rightarrow 329r}$ were very small and it can be said that remain inside the methods' error. However, it is interesting to note that for the particular case, the same conclusion can be extracted from both methods: Met 298 and Met 329 are equally probable to be oxidized. The performace of both methods, SST and TST, is pretty similar and thereby such conclusion is consistent.

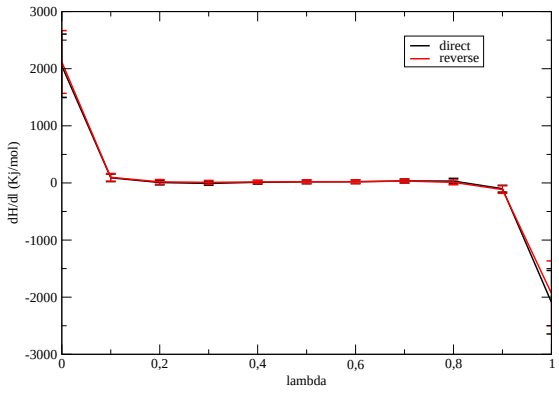


Figure 3.11: Direct and reverse SST for Met residues 298 and 329.

A possible explanation to this phenomena could be that Met is a hydrophobic amino acid and when it gets oxidized it is more hydrophilic. Thereby, one might expect that Meo is more stable in the surface whereas Met is more stable inside the protein where the dielectric constant is lower.

The obtained results do not show any preference for one enantiomer form. This means that no critical interaction is broken or created when switching from (R) to (S) and viceversa.

	SST
$\triangle\triangle G_{298s\rightarrow 329r}$	4.9±0.8
$\triangle\triangle G_{298r\rightarrow 329s}$	4.3±0.9
$\triangle\triangle G_{298s\rightarrow 329s}$	1.2±0.4
$\triangle\triangle G_{298r\rightarrow 446r}$	12.9±0.6
$\triangle\triangle G_{298r\rightarrow 446s}$	7.4±0.3
$\triangle\triangle G_{298s\rightarrow 446r}$	8.8±0.7
$\triangle\triangle G_{298s\rightarrow 446s}$	6.1±0.3
$\triangle\triangle G_{298r\rightarrow 87r}$	4.0±0.9
$\triangle\triangle G_{298r\rightarrow 87s}$	12.0±1.4
$\triangle\triangle G_{298s\rightarrow 87r}$	6.1±1.1
$\triangle\triangle G_{298s\rightarrow 87s}$	7.6±1.2

Table 3.7: $\Delta\Delta G$ estimation (Kcal/mol).

Chapter 4

Conclusion

Fortunately, the world has not been designed with a view to such instincts that only good-natured herd animals could find their narrow happiness in it: to demand that all should become "good human beings," herd animals, blue-eyed, benevolent, "beautiful souls" -or as Mr. Herbert Spencer would have it, altruistic- would deprive existence of its great character and would castrate men and reduce them to the level of desiccated Chinese stagnation. -And this has been attempted!- Precisely this has been called morality.

Friedrich Nietzsche

4.1 Concluding remarks

The work done here provides a better insight about protein oxidation a subject that has gained interest in the last years. As mentioned in the first chapter those oxidated proteins are related to neurodegenerative illnesses and therefore their study is crucial for the development of pharmaceuticals to fight back. In this

sense, it has been shown that computational chemistry has much to aport. In this section the obtained results are summarized and a final conclusion is extracted.

Amino acid side chain oxidation The performed calculations pointed out that a change in the dielectric enviroment does not affect the results. A note has to be done, because implicit solvatation was used and in the case of explicit solvatation some change may be expected as a result of direct interactions. In the same line, hardly no difference was observed for the conformational change in the system. Taking all this into account it can be said that the attack of $\cdot OH$ is equally plausible in low and high dielectric and in most of conformations. For the analysed amino acids, the kinetic barriers were very low, this may be a result of the reactivity of the $\cdot OH$. The thermodynamical study for IP and FP provide us with the most favoured reaction pathway and the most favoured final products.

Serum albumin oxidation First of all, the TST and SST approaches were compared. The obtained results show that for the particular case they perform equally and thereby the extracted conclusion is the same in both cases. At the same time, the MD calculations were conclusive pointing out that Met residue 298 and 329, whcih are the most solvent exposed from all Met residues, are equally probable to be oxidized. The solvent exposure was analyzed by the estimation of the free energy between different pairs of Met and it was noted that the oxidation of the solvent exposed Met is less favoured. A possible answear to this might be provided by the higher hidrophilicity that is acquired when Met is oxidized. Therefore, Met is more stabilized in lower dielectric and Meo is more stable when it is at higher dielectric so that when it is more solvent exposed. This is remarked to be a possible explanation, because the enviroment of each Met residue is crucial when performing MD studies. The reason is that the stabilization of each residue also depends on the interactions that may be created or broken with the neighbouring residues. In the same way no preference was observed, for the studied cases, for one enantiomer form.

4.2 Future Work

It was brief but intense. This master thesis presents some preliminary results for a work that needs to be extended. However, it is rather difficult to anticipate what

exactly is going to be done as it is common to encounter with problems while working on a project. The intention in the near future is first to complete the quantum mechanics study with the remaining amino acids in order to observe wether there exist agreement with what was observed experimentally. Secondly, proposing a reaction mechanism for the oxidation of the Met residue, which is already a subject of deep interest [45, 44, 43], yielding the final product that was introduced here, Meo. It is known that hydroxyl radical can be added to the sulfur atom of the Met residue forming hydroxysulfuranyl radicals. However, not very deeply was studied and a severe reflexetion about this issue is needed. This might be the first step of a future reaction mechanism. There exist several strategies to proceed with such study. Firstly, the reaction mechanism can be depicted with a QM/MM method for the Human Serum Albumin as a complementary project to the one expossed here. On the other hand, if it is case that multireferential methodology has to be used to obtain an insight of the reaction, the initial plan consists on considering a radius from the Met of the Human Serum Albumin and freeze the atoms in the edge of it. In that way, a multireferential calculation would be plausible. Indeed, an energetic study could be perdormed for oxidized Met residues, in order to confirm which one is going to be the most prone to react, confirming the results obtained with free energy estimation. In this work, Bennett acceptance ratio method was used to compute the free energy. Therefore, a comparison with other methodologies such as Thermodynamic Integration, Free Energy Perturbation or end point methods e.g. MMPBSA, MMGBSA would provide with interesting data about their performance. Another interesting topic could be performing a study on a protein behaviour whose amino acids have been oxidized. Lastly, it has been considered to perform further oxidation studies for Tyr, Cys and Trp which have been mentioned here as other very favorable targets for free radicals. Thereby, a comparison would be really interesting between Met, Tyr, Trp and Cys. There are ideas, but a deeper bibliographic search is needed before giving a step forward. They will have to be discussed and pinpoint the possible technical or chemical inconvenients. Then, before proceeding with the calculations, a scrupulous methodology and plan should be stablished.

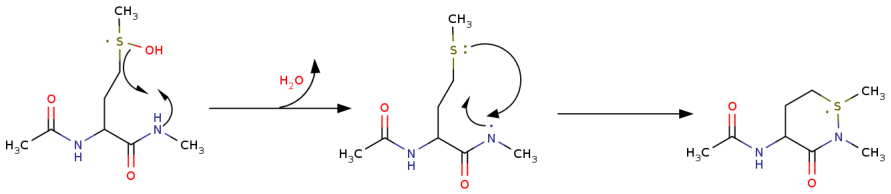


Figure 4.1: Tripeptide model of the hydroxysulfuranyl (first image). A possible rearrangement is shown proposed by Schöneich et al. and the final product is stabilized by radical delocalization between sulfur and nitrogen atoms.

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