

Preface

To act on a bad idea is better than not to act at all. Because the worth of the idea never becomes apparent until you do it. (Nick Cave, 20,000 Days on Earth)

The simulation of enzymatic processes is a well-established field within computational chemistry, as demonstrated by the 2013 Nobel Prize in Chemistry, awarded jointly to Martin Karplus, Michael Levitt and Arieh Warshel for the development of multiscale models for complex chemical systems. It is worth mentioning that this recognition by the Nobel Foundation of the capabilities of computational chemistry was preceded by previous awards to Walter Kohn and John Pople, who won the Nobel Prize in 1998 for their work on density functional theory and computational methods in quantum chemistry. The use of models and simulations to understand and predict chemical processes has been attracting increasing attention, especially in recent years due to the development of high-performance computers. Nowadays, computational chemistry has become an essential tool, complementary to other experimental techniques, to study enzyme reactivity due to their potential applications, in particular in complex systems where molecular details can be elusive to experimental approaches.

This book provides the basic knowledge for postgraduate students and researchers in chemistry, biochemistry and biophysics, who want a deeper understanding of how to get information on complex biological processes at the molecular level through computational simulations. The book is specifically centred on questions concerning the difficulties and recent advances in the computational modelling of enzyme reactivity. Enzymes are biological catalysts that speed up chemical reactions, making them compatible with life. Apart from this catalytic power, often these catalysts show important advantages with respect to non-natural catalysts such as their chemo-, regio- and stereoselectivity, or the ability to work under mild

conditions of temperature and pressure, which makes them the best environmentally-friendly catalysts to speed up the rate of chemical reactions. Nevertheless, although there have been numerous studies that have provided a solid understanding about some of the key factors of these biocatalysts, knowledge about the origin of enzymatic efficiency to catalyse chemical reactions is still not complete.

This book explores the theories, methodologies and applications in simulations of enzyme reactions divided in three sections: Theory, Methods and Applications. This division has been made from a pedagogical point of view although in some cases the three sections are unavoidably connected and some chapters cover aspects related to these three main sections. Our aim has been to gather together some of the most significant researchers in this field to each contribute a closely related topic. The book begins with an overview and perspective of the field written by the Nobel Laureate Arieh Warshel. Arieh Warshel and Ram Prasad Bora provide a perspective on modelling enzymatic reactions and discuss the main findings of simulations about the origin of the enormous catalytic power of enzymes. In doing so, the authors describe the development of the computational strategies for the study of chemical reactions in complex environments, the so-called hybrid quantum mechanics/molecular mechanics (QM/MM) methods where the system is divided into two subsystems described at different levels, as well as the need for careful sampling.

The first block of the book is called *Theory* and tries to provide the basic theoretical tools for the analysis of enzymatic reactions and the interpretation of experimental information for its use in the design of adequate computational simulations. This section devoted to theories begins, as it should, with an experimentalist approach to the problem of the rate of enzymatic processes. Richard L. Schowen briefly introduces or recalls the main ideas of enzyme kinetics and how computational simulations must be designed in connection to experimental approaches. Correct answers need the correct questions too. In the subsequent chapter James T. Hynes and coworkers present the basic principles of transition state theory. The assumptions of this theory are discussed, as are the refinements to account for possible failures, in particular for the study of enzymatic reactions involving the transfer of light particles (proton, hydride and hydrogen atom transfers), where a quantum description of their motion is required. The theory and its deviations are illustrated with applications to a number of enzyme-catalysed reactions. Electron transfer reactions are an important class of enzymatic reactions that are specifically analysed in a separate chapter written by Aurélien de la Lande, Fabien Cailliez and Dennis R. Salahub. Marcus-like approaches to the calculation of the reaction rate for electron transfer reactions in enzymes are presented and some applications are discussed. Apart from the rate constant itself, its variation with the mass of the atoms involved in the reaction, the kinetic isotope effect, provides valuable information where experiments and simulations meet. The next chapter, written by I. W. Williams and P. B. Wilson, analyses methods for

calculating kinetic isotope effects and their application to reactions catalysed by enzymes. The chapter emphasises one of the concepts that are omnipresent in the entire book – the importance of averaging in conformationally flexible systems, such as enzymes, in order to obtain magnitudes comparable to experimental determinations. The final chapter in this section, by J. Kästner and coworkers, is devoted to a key concept in the analysis of complex chemical processes: free energy. Free energy not only determines the spontaneity of a process, including obviously enzymatic reactions, but within transition state theory, the activation free energy is also related to the rate of the process. As mentioned above, averaging over important conformations of the system is fundamental to compute properties to be compared to experimental data and, in particular, to obtain free energies. Computational tools applied to reach this goal are reviewed in this chapter, establishing a natural link with the next section of the book.

The second block of the book is devoted to *Methods* or, in other words, to those computational strategies that can be used to calculate the magnitudes introduced in the first section, connecting experimental and theoretical approaches to enzymatic reactivity. Enzymes are proteins that may present different conformations during the catalytic cycle and the selection of the adequate one is a key step in the study of their reactivity. In their chapter, Pedro Sfriso and Modesto Orozco discuss simulations methods designed to study conformational transitions at a reasonable computational cost. The following three chapters of the book are devoted to different families of hybrid QM/MM methods. Keiji Morokuma and coworkers present the ONIOM (Our own N-layer Integrated molecular Orbital and molecular Mechanics) method and recent applications to enzymatic reactions. In the next chapter, Paolo Carloni, Ursula Rothlisberger and coworkers review the principles of QM/MM methods with particular emphasis to hybrid Car–Parrinello/molecular mechanics and continuum-based methodologies. Although all these methods consider a quantum treatment of some of the electrons of the system (while the rest are implicitly described), nuclear motion is generally assumed to be described accurately using classical mechanics. This approach breaks down when dealing with the transfer of light particles. To close this section of the book Dan T. Major and coworkers provide an overview of the different methods available for calculation of nuclear quantum effects in enzyme systems. Applications of these methods are discussed for the widely studied enzyme dihydrofolate reductase (DHFR), an enzymatic system that has attracted the attention of many experimental and theoretical researchers.

The last section of the book, centred on *Applications*, is closely related to those subjects presented in the two previous sections. In their contribution, Kara E. Ranaghan and Adrian J. Mulholland review different applications of QM/MM methods to the study of enzymatic reactivity, highlighting the role played by these methods in understanding fundamental issues related to enzymatic catalysis. The following chapter, by Joan Bertran and Antoni Oliva, is devoted to the study of reaction mechanisms catalysed by ribozymes,

biological catalysts that are not formed by a protein structure but by RNA. The large charge associated with these molecules and the presence of counterions introduces additional peculiarities that are analysed in this chapter. Damien Laage and coworkers focus in their contribution on an important aspect of enzymatic catalysis: the role of the solvent and the possibility of exploiting changes in its composition to acquire new catalytic functions in enzymes. The authors show that simulations do not support the simplistic and popular view of the solvent acting as a lubricant of protein motions. The next chapter, by M. Alfonso Prieto and Carme Rovira, is devoted to a special class of enzymes whose study requires specific methodological treatments: metalloenzymes, enzymes that catalyse chemical transformations making use of a metal centre present in the active site. The authors show how density functional theory-based simulations are able to unravel the molecular details in several relevant metalloenzymes. The final chapter in this section tries to complete the circle: if we have been able to understand and rationalise enzymatic reactivity using the tools provided by theoretical and computational chemistry, it might be possible to use all this knowledge and procedures to design new biological catalysts with new functions. In their chapter Agustí Lledós, Jean-Didier Maréchal and coworkers discuss this new and exciting research area at the frontier between organic and inorganic chemistry, protein engineering and structural biology that challenges molecular intuitions and where molecular modelling is a fundamental tool. The chapter analyses pros and cons of current strategies, reviewing the work carried out to date.

Advances in simulating enzyme reactivity are key not only to a better understanding of these complex biological machines but for their application in, for instance, the development of new drugs or new environmentally-friendly catalysts. In this regard, the combination of experimental techniques and computer simulations pave the way to a quicker and deeper understanding of their mode of action. The more we learn about the foundations of processes governing chemical processes in living organisms, including our bodies and cells, the better position we will be in to control them, with corresponding benefits in fields such as biomedicine, biotechnology, pharmacy, *etc.* Thus, one could say that the developments and advances in computer simulations are closely linked to the improvements in quality of life on our planet. We hope that this book will provide a reference for those researchers that want to dive into this amazing field.

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