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DISSERTATION

COMPUTER SIMULATIONS OF POLYMER-ENZYMES COMPLEXES AND CHELATE STRUCTURES OF HEAVY METAL IONS

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The dissertation contains the results of my own research.

The use of other authors' ideas, results and texts have references to the relevant
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АНОТАЦІЯ

Бутович Г.А. Комп'ютерне моделювання полімерно-ферментних комплексів та хелатних структур іонів важких металів.— Кваліфікаційна наукова праця на правах рукопису.

Дисертація на здобуття ступеня доктора філософії за спеціальністю 104 — Фізика та астрономія. — Інститут фізики конденсованих систем НАН України, Львів, 2025.

Дисертація присвячена вивченню утворення комплексів в полімерно-ферментних сумішах та хелатних структур іонів важких металів. Зв'язування молекул ферментів із полімерними ланцюгами-каркасами, яке відіграє важливу роль у біокаталітичних процесах при виробництві біопалива, було вивчене в рамках крупнозернистої моделі за допомогою комп'ютерного моделювання методом ланжевенової динаміки. Формування хелатних комплексів іонів важких металів у воді, що становлять інтерес з точки зору очищення стічних вод, досліджувалося на атомістичному рівні деталізації з використанням методів класичної молекулярної динаміки та квантово-хімічного моделювання, застосовуючи теорію функціоналу густини.

Робота складається з чотирьох розділів та організована таким чином: огляд літератури, дослідження утворення полімерно-ферментних комплексів, вивчення хелатування іонів ртуті лінійним поліетиленіміном у водному розчині та аналіз хелатування іонів деяких важких металів етилендіамінтетраоцтовою кислотою.

Перший розділ оглядає літературу, в якій розкриваються проблеми, що знаходяться у фокусі представленої роботи, розповідає, якими методами вони вирішуються і які є досягнення в цих напрямках.

У другому розділі запропоновано крупнозернисту модель та теоретичні основи для опису комплексоутворення у двокомпонентній системі полімеру та ферменту як систему плямистих ланцюгів та плямистих мономерів, яка успішно описується за допомогою модифікованої версії термодинамічної теорії збурень Вертгайма першого роду. Розраховані ступені зв'язаності між молекулами

ферментів та полімерів в залежності від концентрації ферментів у системі при двох значеннях температури дозволили встановити, що запропонована модифікація теорії суттєво покращує результат порівняно із немодифікованою версією. Комп'ютерне моделювання, виконане методом ланжевенової динаміки при різних умовах, показує добру точність теорії, проте виявляє також і недоліки її у випадку щільно функціоналізованих ланцюгів. Особливістю проведеного дослідження процесу зв'язування ферментів з полімерами є аналіз впливу розташування функціональних груп посередині ланцюга і на його краях. Показано, що врахування цього фактору є ключовим для коректного опису комплексоутворення в системі в рамках запропонованого теоретичного підходу. Оскільки запропонована модель підходить для опису формування полімерно-ферментних комплексів, її можна використовувати для комп'ютерного моделювання в галузі виробництва біопалива, наприклад для кращої оптимізації параметрів промислових процесів. Крім того, цю модель можна використовувати для моделювання і інших макромолекулярних гібридів, підібравши відповідні параметри міжчастинкової взаємодії. Теоретичний підхід, застосований в даній роботі, дає можливість робити аналогічні, водночас швидкі, передбачення із досить доброю точністю.

У третьому розділі вивчається хелатування іонів ртуті (Hg^{2+}) молекулою поліетиленіміну (PEI) у водному середовищі. Для випадку лінійного електронейтрального полімеру PEI різної довжини (від 4, 5 та 10 азотовмісних ланок) проаналізовано структуру утворених комплексів PEI-Hg та їхню стійкість. Для цього проводилось комп'ютерне моделювання методом молекулярної динаміки (МД) при постійній температурі та тиску (нормальні умови), а розрахунки теорії функціоналу густини (ТФГ) використовувались для уточнення структури хелатів та валідації результатів МД. Таким чином, отримано детальні атомарні структури комплексів, на основі чого розраховано характерні відстані між іонами ртуті та атомами азоту в PEI, між іонами ртуті то молекулами води в першій координаційні оболонці, а також зроблено оцінку конформаційних характеристик самого полімеру на основі відстаней між атомами азоту. Проаналізовано вплив довжини ланцюгів PEI на хелатування іонів ртуті і насичення кількома катіонами одного

полімерного ланцюга. Стійкість досліджуваних комплексів визначався значеннями енергії адсорбції, отриманими як з МД, так і з ТФГ. Показано, що лінійний РЕІ здатний утворювати стійкі комплекси з іонами ртуті, і найбільш стійким виявився комплекс із РЕІ довжиною у п'ять азотовмісних ланок. Отримані результати розширюють розуміння хелатуючих властивостей РЕІ і можуть бути використані у конструюванні функціональних наноматеріалів на основі молекул РЕІ для ефективного вилучення іонів ртуті з водного середовища.

Четвертий розділ зосереджується на особливостях механізмів хелатування важких металів етилендіамінтетраоцтовою кислотою (EDTA) у воді за нормальних умов. Для цього спочатку проводиться дослідження структури та стабільності хелатних комплексів іонів ртуті ($\text{EDTA} + \text{Hg}^{2+}$) залежно від рівня протонації EDTA. Потім виконується порівняння властивостей структури та її стабільності для іонів двовалентних металів Hg^{2+} , Cd^{2+} та Pb^{2+} , які є найбільш токсичними елементами та надзвичайно шкідливими навіть у найменших дозах. Показано, що EDTA найбільш ефективно зв'язується з іонами, коли є у повністю депротонованому стані, що спостерігається в лужному середовищі. З ростом кислотності до карбоксильних груп EDTA приєднуються атоми водню, послаблюючи при цьому хелатаційну здатність цього ліганду. Як і у випадку РЕІ, дослідження проводилися за допомогою комп'ютерного моделювання методом МД та квантово-хімічних розрахунків ТФГ. МД дозволило отримати хелатні комплекси, оцінити їх структуру та обчислити енергію адсорбції. Конфігурації досліджуваних комплексів потім були уточнені шляхом оптимізації, виконаної ТФГ у різних наближеннях, на основі чого була також розрахована вільна енергія Гіббса комплексоутворення. Результати порівнювалися з експериментальними даними доступними в літературі, та аналізувалися в контексті розрахунків, проведених іншими дослідниками. Суттєвий вплив рівня протонації на хелатуючу здатність EDTA дозволяє контролювати його комплексоутворення з іонами за допомогою рН. Цей підхід може знайти застосування у різних технологічних процесах при очищенні стічних вод, в хімічній промисловості та медицині.

У підсумку, це дослідження покращує розуміння механізмів, які супрово-

джують явища хелатування іонів важких металів у водних середовищах та дозволяє передбачати ступені зв'язаності в полімерно-ферментних системах. Результати цієї дисертації дають цінну інформацію для наукових досліджень та практичних застосувань націлених, для прикладу – на очистку води від токсичних елементів та покращення ефективності біокаталізу при виробництві біопалива з целюлозної сировини. За результатами виконаної роботи опубліковано дві статті, препринт і зроблено три доповіді на конференціях.

Ключові слова: іони важких металів, хелатування, полімери, комплексоутворення, поліетиленімін, етилендіамінтетраоцтова кислота, ферменти, молекулярна динаміка, ланжевенівська динаміка, теорія функціоналу густини, термодинамічна теорія збурень Вертгайма.

ABSTRACT

Butovych H. Computer Simulations of Polymer-Enzyme Complexes and Chelate Structures of Heavy Metal Ions. — Qualifying scientific work on the rights of the manuscript.

Thesis for the Degree of Doctor of Philosophy on the speciality 104 — Physics and Astronomy. — Institute for Condensed Matter Physics of the National Academy of Sciences of Ukraine, Lviv, 2025.

This thesis is devoted to the study of complex formation in polymer-enzyme mixtures and the chelate structures of heavy metal ions. The binding of enzyme molecules to polymer chain scaffolds, which plays an important role in biocatalytic processes during biofuel production, was investigated using a coarse-grained model and computer simulations based on Langevin dynamics. The formation of heavy metal chelation complexes in an aqueous environment, relevant to wastewater treatment, was studied at the atomistic level using classical molecular dynamics (MD) and by quantum-chemical modelling using the density functional theory (DFT).

The thesis consists of four chapters and is organised as follows: a literature review, a study of polymer-enzyme complex formation, an investigation of mercury ion chelation by linear polyethyleneimine (PEI) in an aqueous solution, and an analysis of the chelation of heavy metal ions by ethylenediaminetetraacetic acid (EDTA).

Chapter 1 reviews the literature addressing the problems that are central to this thesis, the methods used to tackle them, and the progress achieved in the respective research areas.

Chapter 2 introduces a coarse-grained model and the theoretical approach to describing complexation in a polymer-enzyme system, represented as patchy chains and patchy monomers. This system is effectively described using a modified version of Wertheim's first-order thermodynamic perturbation theory. The calculated degrees of binding between polymer and enzyme molecules as a function of the enzyme concentration at two temperatures show that the proposed theoretical mod-

ification significantly improves the predictions compared to the unmodified version. Computer simulations performed using Langevin dynamics at different parameters confirm an accuracy of the theory, while also highlighting its limitations in the case of closely functionalised chains. A particular feature of this study is the analysis of how the arrangement of functional groups, either at the intermediate or at the terminal monomers of the polymer chain, affect enzyme binding. It is shown that accounting for this factor is essential for the accurate description of complex formation within the proposed theoretical approach. Since the model is suitable for describing polymer-enzyme conjugates, it may be applied to computer simulations in the field of biofuel production, for example, to optimise industrial process parameters. Moreover, the model is adaptable to other types of macromolecular hybrids, provided the interaction parameters are appropriately chosen. The theoretical approach used in this study allows for similarly accurate yet computationally inexpensive predictions.

Chapter 3 investigates the chelation of mercury ions (Hg^{2+}) by polyethyleneimine (PEI) in an aqueous solution. For linear, electroneutral PEI chains of varying lengths (4, 5, and 10 nitrogen-containing units), the structures and stability of the formed PEI-Hg complexes were analysed. To this end, MD simulations were performed under ambient conditions, and DFT calculations were used to refine the chelate structures and validate the MD results. This approach yielded detailed atomic structures of the complexes, allowing the calculation of characteristic distances between Hg ions and nitrogen atoms in PEI, as well as between Hg ions and water molecules in the first coordination shell. Conformational features of the polymer were also evaluated based on nitrogen-nitrogen distances. The effect of the PEI chain length on mercury chelation and multi-ion binding to a single PEI chain were investigated. Complex stability was evaluated based on adsorption energy values obtained from both MD and DFT calculations. It was shown that linear PEI forms stable complexes with mercury ions, and the PEI chain with five nitrogen-containing units demonstrated the highest stability. These findings enhance our understanding of the chelating properties of PEI and may be used in designing functional nanomaterials based on PEI for efficient mercury ion removal

from aqueous environments.

Chapter 4 focuses on the mechanisms of heavy metal chelation by ethylenediaminetetraacetic acid (EDTA) in water under ambient conditions. It begins with an investigation of the structure and stability of mercury-EDTA complexes depending on the protonation state of the ligand. This is followed by a comparison of the structural features and stability of EDTA complexes with the divalent heavy metal ions Hg^{2+} , Cd^{2+} , and Pb^{2+} , which are extremely toxic even in very low doses. It is shown that EDTA binds most effectively to metal ions when it is fully deprotonated, which occurs in alkaline environments. As acidity increases, protons bind to EDTA's carboxyl groups, weakening its chelating ability. Similarly to the PEI case, the study involved MD simulations and quantum-chemical DFT calculations. MD simulations enabled the formation and analysis of chelate complexes and the estimation of their adsorption energy. The configurations of these complexes were then refined using DFT optimisations based on several approximations, enabling also the calculation of the Gibbs free energy of complexation. The results were compared with experimental data available in the literature and analysed in the context of other theoretical studies. The strong dependence of EDTA's chelating ability on its protonation state enables the pH control of complex formations with metal ions. This approach has potential applications in various technological processes, including wastewater treatment, the chemical industry, and medicine.

To summarise, this study improves the understanding of the mechanisms underlying heavy metal ion chelation in aqueous environments and enables the prediction of binding degrees in polymer-enzyme systems. The findings provide valuable insights for both fundamental studies and practical applications, for example, in the removal of toxic elements from water and in enhancing the efficiency of biocatalysis during biofuel production from cellulosic biomass. Based on this work, two research articles, a preprint, and three conference presentations have been produced.

Keywords: heavy metal ions, chelation, complex formation, polymers, polyethylenimine, ethylenediaminetetraacetic acid, enzymes, molecular dynamics, Langevin dynamics, density functional theory, Wertheim's thermodynamic perturbation theory.

PhD publications

1. *H. Butovych, Y.V. Kalyuzhnyi, T. Patsahan, J. Ilnytskyi* Modeling of polymer-enzyme conjugates formation: Thermodynamic perturbation theory and computer simulations. // *J. Mol. Liq.* — 2023. — Vol. 385. — P. 122321. Scopus (Q1), DOI: 10.1016/j.molliq.2023.122321
2. *H. Butovych, F. Keshavarz, B. Barbiellini, E. Lähderanta, J. Ilnytskyi, T. Patsahan* Role of EDTA protonation in chelation-based removal of mercury ions from water. // *Phys. Chem. Chem. Phys.* — 2024. — Vol. 26. — P. 25402-25411. Scopus (Q2), DOI: 10.1039/D4CP02980A
3. *H. Butovych, J. Ilnytskyi, E. Lähderanta, T. Patsahan*, Chelation of the mercury ions by polyethyleneimine: Atomistic molecular dynamics study // arXiv preprint arXiv:2506.18835. 2025.
4. *H. Butovych, T. Patsahan, J. Ilnytskyi, E. Lähderanta, B. Barbiellini, F. Keshavarz*, “Chelation of heavy metals by ethylenediaminetetraacetic acid in aqueous solution”, EMLG-JMLG Annual Meeting 2024, Structure and Dynamics of Hydrogen-Bonded Systems, 8-13 September, 2024, Trieste, Italy, Book of Abstracts, p. 96. <https://indico.elettra.eu/event/39/>
5. *H. Butovych, F. Keshavarz, J. Ilnytskyi, B. Barbiellini, E. Lähderanta, T. Patsahan*, “Computer simulation of complexes formed by heavy metal ions and EDTA molecules in aqueous solution”, 1st annual workshop on the use of computer simulations in nanophysics “Nanophysics meets computer simulations”, November 8, 2024, Lviv, Ukraine, Book of Abstracts, p. 9. <https://icmp.lviv.ua/nanosim>
6. *Г. Бутович, Ф. Кешаварз, Б. Барб’єлліні, Е. Ляхдеранта, Я. Ільницький, Т. Пацаган*, “ЕДТА при різних рівнях протонації для хелатування іонів ртуті у водному розчині”, 24-та Всеукраїнська школа-семінар молодих вчених зі статистичної фізики та теорії конденсованої речовини, 24-25 жовтня 2024 р., Львів, Збірка тез, с.32. <https://icmp.lviv.ua/konkurs/2024/index.html>

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List of Abbreviations

APT	—	atomic polar tensor,
CG	—	coarse-grained,
CN	—	running coordination number,
DFT	—	density functional theory,
EDTA	—	ethylenediaminetetraacetic acid,
FF	—	force field,
LD	—	Langevin dynamics,
LJ	—	Lennard-Jones,
PEI	—	polyethyleneimine,
RDF	—	radial distribution function,
TPT1	—	first-order multi-density thermodynamic perturbation theory.

INTRODUCTION

Justification of the choice of research topic. This work consists of modelling polymer-enzyme and heavy metal-chelator complexes. Enzymes are known for their biocatalytic properties and are used in the processing of cellulose and biowaste to produce biofuel, an eco-friendly alternative to traditional fuels. However, the current cost of biofuel is not yet competitive with conventional fuel alternatives. The chemical industry requires the optimisation of bioethanol production to reduce both the processing time of biowaste and the cost of materials. One approach to address this challenge is the use of cellulosome-polymer-enzyme hybrids formed as polymer scaffolds grafted with various types of enzymes. The self-assembly of enzyme molecules and bottlebrush polymers, as well as the formation of such conjugates, is investigated in this study. Another issue explored in this study concerns the removal of heavy metal ions from aqueous environments. Pollution of soil and aquatic ecosystems with heavy metals such as Pb, Hg, and Cd is extremely hazardous to human and animal health due to their high toxicity even at low concentrations. Bioaccumulation further complicates the problem by leading to long-lasting health effects. Chelation provides an effective strategy for heavy metal removal used to tackle this problem at different levels: from eliminating the pollution at its sources to remediating already contaminated environments and treating the consequent illnesses. Chelating agents are applied in the areas of wastewater cleaning and chelation therapy as is or as building blocks of various nanomaterials. One of the chelating agents considered in this study is ethylenediaminetetraacetic acid (EDTA), a strong and broadly utilized ligand known for its high efficiency in binding metal ions. Another example is polyethyleneimine (PEI), which is less commonly used and has been comparatively less studied as a chelating agent. A deeper understanding of the chelation

mechanism and its associated properties is required to enhance industrial processes and mitigate the side effects arising from the application of chelating agents. This thesis will contribute to environmental sustainability in the fields of heavy metal decontamination and clean energy, and possibly also to medical and pharmaceutical research.

Relationship with academic programmes, plans, themes. The thesis research was conducted at the Institute for Condensed Matter Physics of the National Academy of Sciences of Ukraine and Lappeenranta-Lahti University of Technology, Lappeenranta, Finland, under the cotutelle agreement between the two institutions. It has been supported by the Institute for Condensed Matter Physics of the National Academy of Sciences of Ukraine (research theme funded by the NAS of Ukraine: “Statistical theory of complex liquids: Influence of spatial restriction and anisotropy, mesoscopic ordering and anomalous effects”, 2021-2024, 0120U100202), the National Research Foundation of Ukraine (project “Patchy colloids in porous media: theory and computer simulations”, 2021-2023, No. 2020.02/0317), Finnish National Agency for Education (EDUFI Fellowship for doctoral students from Ukraine, ref. no. OPH-4602-2022; the Team Finland Knowledge programme, ref. no. 12/221/2023), Lappeenranta-Lahti University of Technology, Scholarship of the President of Ukraine for young scientists (2024-2025, Ukraine). CSC (Finnish IT Center for Science) is acknowledged for computing resources and the “EMLG-JMLG Annual Meeting 2024” Organizing Committee at Elettra Sincrotrone Trieste for the support in the conference participation.

The purpose and objectives of the study.

Object of study. Complexes formed in polymer-enzyme mixtures and chelation structures of heavy metal ions in aqueous environments.

Subject of study. The binding degree due to complexation in polymer-enzyme systems, as well as the stability of structures formed due to the chelation of heavy metal ions with polyethyleneimine (PEI) and ethylenediaminetetraacetic acid

(EDTA) ligands, investigated using computer simulations.

Purpose of study To analyse the complexation behaviour and binding degree in polymer–enzyme systems, as well as the chelation of heavy metal ions with polyethyleneimine (PEI) and ethylenediaminetetraacetic acid (EDTA), using computer simulations to gain insight into their binding efficiency and structural stability.

Research methods. Computer simulations using molecular dynamics in the NPT ensemble, Langevin dynamics in the NVT ensemble, and quantum-chemical calculations based on density functional theory (DFT).

Scientific novelty of the obtained results. A theoretical and computational framework was developed to describe complex formation in polymer–enzyme systems using a coarse-grained patchy particle model combined with a modified version of Wertheim’s thermodynamic perturbation theory. The influence of functional group distribution along the polymer chain on the binding degree was systematically studied for the first time using Langevin dynamics simulations.

At the atomistic level, a detailed structural and energetic characterisation of chelate complexes formed between heavy metal ions and polyethyleneimine (PEI) or ethylenediaminetetraacetic acid (EDTA) in aqueous environments was obtained through a combination of molecular dynamics and quantum-chemical (DFT) calculations. The effect of polymer chain length and ion loading on complex stability was quantitatively evaluated. Furthermore, the role of EDTA protonation state in determining chelation efficiency was elucidated, highlighting the importance of pH in controlling complex formation.

The practical significance of the results. The results of this study contribute to the optimisation of polymer-enzyme systems for use in biocatalytic processes, such as the enzymatic conversion of cellulosic biomass into biofuels. The proposed coarse-grained modelling framework enables the prediction and tuning of polymer-enzyme binding efficiency, which can support the rational design of enzyme immobilisation

strategies in industrial applications.

In the context of environmental remediation, the atomistic modelling of heavy metal chelation by PEI and EDTA provides molecular-level insight into the structure and stability of the resulting complexes in aqueous media. These findings can be applied to the development of more efficient materials and protocols for the removal of toxic metal ions (e.g., Hg^{2+} , Cd^{2+} , Pb^{2+}) from contaminated water. The demonstrated influence of the polymer structure and ligand protonation on chelation efficiency offers valuable guidance for the design of next-generation chelating agents and nanomaterials in environmental and medical technologies.

Personal contribution of the PhD. The PhD researcher personally:

- performed molecular dynamics simulations of chelate complexes in water using the NPT ensemble [1, 2];
- conducted quantum-chemical calculations of chelates using density functional theory (DFT) [1, 2];
- carried out coarse-grained Langevin dynamics simulations of polymer–enzyme complexes in the NVT ensemble [3];
- analysed and visualised the simulation data [1–3].

The objectives, methods, models and parameters for them were chosen and results were discussed and interpreted together with the co-authors of the published works.

Approbation of obtained results Key results of this research were presented at the conferences: EMLG-JMLG 2024 annual meeting 2024: Structure and dynamics of hydrogen bonded systems, Trieste, Italy (8-13 September, 2024), 1st annual workshop on the use of computer simulations in nanophysics “Nanophysics meets computer simulations”, Lviv (November 8, 2024), 24-та Всеукраїнська школа-семінар молодих вчених. зі статистичної фізики та теорії конденсованої речовини, Lviv (24-25 October, 2024), as well as the ICMP seminars.

Publications. According to the results of the research, 2 scientific articles were published [1, 3] in international journals indexed in the Scopus and WoS databases, belonging to the Q1 and Q2 quartiles; 1 preprint [2]; and 3 conference abstracts [4–6].

Structure and scope of the thesis. The thesis consists of an Introduction, a literature review chapter, and three chapters presenting the main research results. It also includes the final conclusions, a bibliography, 4 appendices, 40 figures, and 9 tables. It is 97 pages long (total length: 130 pages). The bibliography contains 166 scientific references.

The Introduction outlines the motivation for the study, the purpose and objectives of the research, the scientific novelty, and the practical significance of the obtained results. A brief overview of the thesis content is also provided.

Chapter 1 presents a literature review of the research topic. It covers the current state of the art in the field of polymer-enzyme complexes and their applications, as well as approaches to the removal of heavy metal ions from wastewater using chelating agents, particularly ethylenediaminetetraacetic acid (EDTA) and polyethyleneimine (PEI). The computational and theoretical methods applied in the study, along with their alternatives, are discussed in detail.

Chapter 2 describes the coarse-grained model developed for polymer-enzyme complexes, the theoretical approach used to characterise binding in such systems, and Langevin dynamics simulations performed under various conditions. The chapter presents the results of both the theoretical predictions and simulations, along with a comparison and analysis of their consistency.

Chapter 3 is devoted to the study of mercury ion chelation by linear polyethyleneimine in an aqueous solution. It includes the atomistic model of the PEI chain, details of the molecular dynamics simulation protocol, the Lennard-Jones parameters used, and the results of simulations and density functional theory (DFT) calculations. The structural and energetic characteristics of the resulting complexes are analysed in detail.

Chapter 4 addresses the chelation of heavy metal cations, Hg^{2+} , Cd^{2+} , and

Pb^{2+} , by EDTA in an aqueous solution. The chapter discusses the protonation states of EDTA, the corresponding Lennard-Jones parameters used in the simulations, and the rationale for selecting specific DFT levels of theory. Molecular dynamics simulation results are presented for mercury-EDTA complexes at various protonation levels, along with the corresponding DFT results. Similar analyses are provided for complexes of Hg^{2+} , Cd^{2+} , and Pb^{2+} with fully deprotonated EDTA. A comparison with available experimental data and a discussion of the results are included.

The thesis concludes with a summary of the main findings in the **Conclusions**, followed by the **Bibliography** and supporting material in the **Appendices**.

CHAPTER 1

LITERATURE REVIEW

1.1. Introduction

This chapter presents a literature review on molecular complexation in the context of the research topics addressed in this thesis. The focus is on two key issues: the formation of polymer-enzyme hybrids and the chelation of heavy metal ions by molecules such as polyethyleneimine and ethylenediaminetetraacetic acid. Both topics are not only important but also highly relevant, as evidenced by the current scientific literature reviewed in this section. In particular, the complexation study of polymer-enzyme hybrids or conjugates is of interest due to its implications for predicting the conditions required for enzyme immobilisation, especially for cellulase-type enzymes on polymeric scaffolds. This contributes to improving the efficiency of cellulose hydrolysis in biofuel production from waste biomass. Computational modelling and appropriate theoretical approaches can offer valuable tools for predicting the physicochemical properties of such systems. The contamination of wastewater with heavy metal ions necessitates the development of effective purification technologies. Adsorption of these ions and their subsequent removal from aqueous environments using chelator-based composite materials is a promising strategy for addressing this issue. Designing such advanced materials with tunable adsorption properties requires a deep understanding of the complex interaction mechanisms between metal ions and chelating agents, which can be achieved through both classical computer simulations and quantum chemical calculations. Despite the importance of the problems addressed in this work, they remain insufficiently studied in the literature. In particular, although some experimental studies are available, there

remains a lack of data necessary for the validation of theoretical models. A number of relevant literature sources will also be discussed in the course of this chapter.

1.2. Polymer-enzymes complexes

Various enzymes are used in the field of waste recycling. For example, biowaste can be utilised to produce ethanol. The biowaste processing and biofuel production industries are developing and have great potential in both Ukraine [7] and Finland [8]. One of the most promising and environmentally friendly approaches in biofuel production is the enzymatic depolymerisation of cellulose into glucose. This method is based on the use of cellulase enzymes. The full process cycle includes preliminary raw material preparation, enzymatic hydrolysis, fermentation, distillation, and dehydration. The feedstock used for the production of cellulosic ethanol is ligno-cellulose, a material that is difficult to decompose due to the crystalline structure of cellulose, the presence of lignin polyphenols, and its water insolubility [9, 10]. Ligno-cellulose processing is an attractive method due to the low cost and wide availability of the raw material. However, this type of fuel production remains still less economically feasible compared to conventional fossil fuel extraction. Within this process, more than a half of the total cost is spent on hydrolysis in the case of biomass and 20% in the case of cellulose input material. That is why extensive research is currently being conducted to make enzymatic recycling more efficient. This includes recycling of the enzymes themselves for repeated use, improvement of their biocatalytic properties, and optimisation of the balance of different types of cellulases. The most common composition of cellulase includes endoglucanases (EG), which break the glycosidic bonds within cellulose, CBH1 and CBH2 cellobiohydrolases, and β -glucosidases, which catalyse the hydrolysis of cellobiose into glucose.

One way to improve the process is seen in some microorganisms which produce cellulosomes, an arrangement of various enzymes on a protein scaffold [11]. The reasons for the high efficiency of cellulosomes are not fully understood due to the large variety of enzymes and their structures. The enzymes of a cellulosome typ-

ically constitute a combination of active components for cellulose depolymerisation (such as endo- and exoglucanases, as well as oxidative enzymes), cellobiohydrolases, and enzymes responsible for the degradation of polysaccharides other than cellulose [12]. Another commonly mentioned feature of cellulosomes is their structural flexibility and their ability to bind to the surface of crystalline cellulose through a carbohydrate-binding module (CBM) protein acting as an anchor [12]. Because naturally obtained cellulosomes are insufficient for industrial use, research is being conducted on synthetically immobilised enzymes on a scaffold, such as polymer-enzyme conjugates. Synthetic cellulosomes can be produced either through biological synthesis, based on recombinant protein expression mechanisms, or through chemical synthesis using biomimetic technologies to attach enzymes to chemically prepared scaffolds. The promising advantages of the biomimetic method include lower cost at scale and the ability to design the structure of the conjugates according to specific requirements, such as improved thermal and chemical stability, as well as enhanced ease of extraction and reusability. For instance, Yadavalli et al. [13] demonstrated that molecular brushes (bottlebrush polymers) can significantly enhance the thermal stability of enzymes without compromising their catalytic activity. By grafting polymers onto enzyme surfaces, they created a protective environment that preserved the protein structure at elevated temperatures. The study showed that the architecture and composition of the polymer brushes play a key role in maintaining enzyme functionality under thermal stress, offering a promising strategy for designing robust biocatalysts for industrial applications. Zholobko et al. [14] investigated the construction of biomimetic cellulosomes using molecular brush polymers as synthetic scaffolds for enzyme immobilisation. The study compared randomly assembled enzyme-polymer complexes with simple enzyme mixtures, demonstrating that the molecular brush-based scaffolds enhanced the enzyme proximity and stability. This resulted in improved efficiency of cellulose hydrolysis. The work confirms the potential of bottlebrush polymer architectures for designing advanced multienzyme systems that mimic natural cellulosomes in industrial biocatalysis.

Alongside experimental studies, polymer-enzyme complexes are also investi-

gated using computer simulation techniques. However, the multi-step nature of cellulose hydrolysis, its extended timescale, and the size of the involved molecules make its computational modelling particularly challenging. Even in the case of free cellobiohydrolase enzymes, the hydrolysis process involves several steps: attachment of the enzymes to the cellulose surface; diffusion along the surface until they encounter the ends of the cellulose chains; translocation of the chain into the active site of the enzyme; formation of the enzyme-substrate complex; the hydrolysis reaction itself, producing cellobiose; and finally, release of the product. Related atomistic simulations would require enormous computational resources and considerable time. In addition, the modelling of cellulosomes would require complex and precise parameter fine-tuning. Therefore, it is reasonable to consider a coarse-grained (CG) modelling approach in this case. However, to refine the coarse-grained (CG) model, certain aspects of the problem may be modelled in greater detail, such as the atomistic simulation of the crystalline cellulose structure [15], cellulose-protein interactions during the straightening of the cellulose chain [16], interactions between CBH-1 and cellodextrins within cellulose microfibrils [17], and the investigation of the CBM-cellulose interaction mechanism [18].

To reduce the use of computing resources in protein simulations, the solvent is usually not modelled explicitly, and the molecules are represented in a coarse-grained manner. For instance, in the study by Bomble et al. [19], a coarse-grained modelling approach was developed to investigate the self-assembly of cellulosome-multienzyme complexes responsible for cellulose hydrolysis. The authors constructed a specialised model in which individual domains of enzymes and a scaffold are treated as rigid bodies interacting via affinity-based potentials that reflect experimentally determined cohesin-dockerin specificity. The simulations allowed assessing how multivalency, linker flexibility, and steric effects influenced the formation of stable cellulosome architectures. In another study, Poma et al. [20] developed a coarse-grained (CG) modelling approach to describe protein-polysaccharide complexes in a unified way and investigated their behaviour with reduced computational costs, while preserving essential structural and interaction features. In their model, each glucose unit was

represented by a single bead, and proteins were modelled using a $C\alpha$ -based representation. The CG interaction parameters in [20] were derived using the Boltzmann inversion for bonded terms and an energy-based approach for tuning non-bonded interactions from short atomistic molecular dynamics simulations. The study demonstrated that this approach can effectively capture the binding behaviour and stability of protein-cellulose complexes, and showed that the length of the polysaccharide chain significantly affects complex formation. Despite the coarse-graining in such models, they are still quite difficult to generalise to large systems involving many molecules. Nevertheless, these models effectively reproduce the behaviour of individual molecular complexes.

For significantly large systems, further simplification is required. This enables not only large-scale simulations but also the application of theoretical approaches. Models similar to the one proposed here have earlier been used to describe aggregation and association effects in systems such as surfactants, alkanols, organic acids, amino acids, and others (see [21–25] and references therein). In these studies, system particles were modelled as fully flexible associative chains composed of spherical monomers interacting via Lennard-Jones (LJ) potentials. Similarly to our model, association occurred through interaction sites (associative centres) located on specific monomers, modelled by a square-well potential. For the theoretical description, the authors employed TPT1 with a reference system represented by LJ monomers. This theory provides reasonably accurate predictions for polymer models in which binding sites are located at the chain ends. However, when the binding sites are located in the middle of the chain, such theoretical predictions become unreliable [26, 27]. This drawback of the theory is related to the so-called “single-bond approximation” used in TPT1, wherein the binding ability of a given interaction site is assumed to be independent of the bonding state of other sites on the same monomer [28–30].

1.2.1. Patchy particles model

For the description of the abovementioned polymer-enzyme complexes the so called patchy particles approach was utilized. The patchy particles model is a conceptual colloidal model that describes heterogeneous particles with specific surface regions exhibiting properties distinct from the rest of the surface [31, 32]. Such regions are referred to as patches. A patchy particle is commonly represented as a spherical core bearing interacting sites on its surface that correspond to functional interactions (see Fig. 1.1). This model is widely adopted for describing molecules with specific interaction sites that allow binding only between certain chemical groups, for example, in modelling DNA, gels, crystals, and water molecules. The application of this model was motivated by recent advances in nanomaterial design and the study of the spatial organisation of biomolecules [31]. It has been applied to describe self-assembly processes, the formation of clusters which lead to non-trivial phase behaviours in systems and their unique mechanical, optical or electric properties [33–35]. The interactions between such particles are anisotropic and in the behaviour may be orientation dependent, so the resulting system properties are of great interest.



Figure 1.1. A model of a patchy particle (left) and a graphical description of an established bond between 2 patchy particles. The patches are shown in red and yellow.

Patchy particle models, combined with computer simulations, have become a powerful approach for investigating the self-assembly of biomolecules such as proteins, their phase behaviour and interaction specificity. Simulations incorporating directional and limited-valence interactions reproduce key features of protein solutions, such as the broad vapor-liquid coexistence observed experimentally [36]. By

tuning the number, orientation, and strength of the patches, models can capture how asymmetric interaction patterns affect crystallisation kinetics and polymorphism, as demonstrated for globular proteins [37]. Atomistic and coarse-grained simulations have also been used to probe how engineered mutations or chemical modifications modulate the effective patchiness of real proteins, leading to predictable shifts in their phase diagrams [38, 39].

Theoretical and simulation studies illustrate the general applicability of patchy models to describe protein self-assembly across a range of systems, from simple monomers to multicomponent condensates [40, 41]. Experimental SAXS data from protein systems provides validation for patch-based models by showing that partially unfolded proteins form network structures consistent with limited-valence interactions [42]. Additionally, simulations of patchy protein-like particles interacting with polymers or nucleic acids have shed light on clustering and compaction mechanisms relevant to cellular organization [43]. In multicomponent mixtures, patchy particle simulations reveal how network connectivity governs the composition and stability of protein-rich condensates [44]. Together, these computational studies demonstrate that patchy particle models, grounded in physical interaction anisotropy, provide an essential toolkit for simulating and predicting protein behaviour in complex environments.

A notable study that applies the patchy particle model to enzymes is the article by Audus et al. [45]. This research explores how patchy particle models can effectively describe the phase separation and self-assembly behaviours of globular proteins, which include many enzymes. In their work, the authors investigate systems of patchy particles with varying valences and interaction strengths to understand the universal behaviours governing self-assembly processes. They find that the probability of a patch being bound, the valence (number of patches), and the particle density are key parameters that determine the mass distribution and shape of self-assembled clusters. This approach provides insights into how enzymes, modelled as particles with specific interaction sites, can form complex structures through self-assembly mechanisms.

Another important study is published by Kalyuzhnyi with collaborators [46], where a comprehensive investigation is carried out of aggregation, liquid-liquid phase separation, and percolation behaviour in a model system of monoclonal antibodies (mAbs), using the patchy particle model. Antibodies are represented as Y-shaped molecules composed of seven tangent hard spheres, with directional interaction sites (patches) placed on the Fab and Fc domains to describe specific domain-domain binding. This design captures the essential valence-limited and anisotropic interactions characteristic of real mAbs. Theoretical predictions were obtained using Wertheim’s thermodynamic perturbation theory (TPT1), allowing the authors to compute phase behaviour and cluster size distributions. The results of the theory were validated through Monte Carlo simulations. This work effectively demonstrates how patchy particle models, when combined with statistical mechanics and simulations, can provide mechanistic insight into the phase behaviour and aggregation in the system of complex, anisotropic biomolecules.

1.3. Chelation of heavy metal ions

The accumulation of heavy metal ions in the human body poses a serious threat to the functioning of the liver, kidneys, brain, heart and lungs [47–49]. In addition, it causes harm to animal health and affects plant growth and development [49]. Therefore, the removal of these toxic contaminants from the environment is a highly relevant task at the intersection of physics, chemistry, and medicine. Heavy metal pollution happens as a result of industrial activities, mining, wars [50] and naturally. The techniques of heavy metal removal are chelation, adsorption, flotation, ion exchange, precipitation, coagulation, flocculation, phytoremediation, membrane and electrochemical methods [51]. Among these methods of purification, adsorption techniques, particularly those based on the chelation of metal ions by magnetic particles functionalised with polymer chelators [52], have proven to be the most effective. A substantial body of experimental data has been accumulated for such systems, which now require further development aimed at improving their

efficiency and cost-effectiveness. This calls for the establishment of the physico-chemical mechanisms underlying chelation and their dependence on the molecular architecture of the chelating agents.

Chelation is the process where two or more donor atoms of a ligand form coordinate bonds with a metal ion, resulting in the formation of a cyclic complex (Fig. 1.2). Chelating ligands are characterised by their denticity, which refers to the number of coordinate bonds a ligand can form with a metal ion [53]. Multidentate ligands (with a denticity > 1) typically form more stable chelate complexes than monodentate ones [54]. Certain atoms within a ligand, such as nitrogen and oxygen, possess lone pairs of electrons that can be donated to a metal centre when they are unprotonated. The mechanism of chelation is based on the acceptance of electron pairs by metal ions from electron-donating ligands, with the formation of covalent bonds as a result [53, 55]. The strength of chelation of a metal ion M by a ligand L is measured by an (absolute) formation/stability constant, defined as $K = \frac{[ML]}{[M][L]}$, where $[ML]$, $[M]$ and $[L]$ denote the concentrations of the ML complex, the nonbound metal ion M and the free ligand L in a solution, respectively [54].

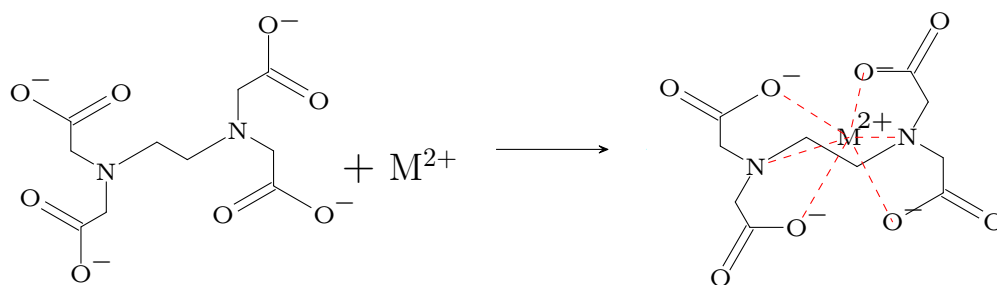


Figure 1.2. Formation of an EDTA-metal complex

Chelation is applied in various industries, including analytical chemistry, wastewater treatment, cleaning, pharmaceuticals, medicine, and agriculture. Examples of chelation applications include preventing metal ions from participating in undesirable reactions, enhancing the bioaccessibility of metal ions, dissolving precipitates, removing rust by chelating iron cations, and performing titrations to determine metal ion concentrations [53]. Well-known examples of chelating agents include ethylenediaminetetraacetic acid (EDTA), hydroxyethylethylenediaminetri-

acetic acid (HEDTA), nitrilotriacetic acid (NTA), diethylenetriaminepentaacetic acid (DTPA), hydroxyethyliminodiacetic acid (HEIDA), and glutamic acid diacetic acid (GLDA) [56]. These compounds are capable of forming stable complexes with a wide range of metal ions through multiple donor atoms, typically nitrogen and oxygen. EDTA is one of the most widely used chelating agents, applied in industries ranging from analytical chemistry and medicine to water treatment and agriculture. DTPA, a structurally extended analogue of EDTA, is particularly effective in chelating trivalent and tetravalent metal ions. NTA and HEDTA are used in similar applications but exhibit different stability and biodegradability profiles. More recently, biodegradable alternatives such as GLDA and HEIDA have gained attention due to increasing environmental concerns. These newer agents are designed to maintain high chelating efficiency while offering enhanced biodegradability, making them suitable for sustainable industrial and environmental applications.

1.3.1. Polyethylenimine

Another example of a chelating agent is polyethyleneimine (PEI) – an efficient ligand capable of binding metal ions through its numerous amine groups. It is used in the medical and pharmaceutical fields for gene delivery and antimicrobial wound treatment, as well as in the environmental sector [57]. It is a water-soluble polymer, although its solubility depends on the composition of PEI, as well as on the pH and temperature. PEI is constructed from repeating elementary units of ethyleneimine, including $\text{CH}_2\text{CH}_2\text{NH}-$ (containing a secondary amine group), $-\text{CH}_2\text{CH}_2\text{NH}_2$ (a terminal unit with a primary amine group), and $-\text{CH}_2\text{CH}_2\text{N} <$ (a branching unit with a tertiary amine group). During chelation, relatively strain-free five-membered rings are formed involving PEI atoms. These rings typically consist of a metal ion, one nitrogen atom, two carbon atoms, and a neighbouring nitrogen atom from the polymer chain [53]. Due to the ability of nitrogen atoms to donate electron pairs, PEI can effectively chelate heavy metal ions. However, it has been suggested that PEI should be immobilised, as its high water solubility limits its direct application

in water treatment [58, 59]. PEI can be synthesised in linear or branched forms, the latter of which can develop into dendrimer-like architectures. The protonation of PEI depends on both the pH and the salt concentration of the solution. It is generally highly protonated under acidic conditions ($\text{pH} < 3$), while its degree of protonation decreases with an increasing pH, particularly above $\text{pH} = 7$ [60]. The chains modelled in the present work carry no additional charge on the nitrogen atoms, i.e., they are fully deprotonated, which corresponds to the form of PEI under alkaline conditions. Under these conditions, chelation becomes more effective due to reduced competition between metal ions and protons for coordination with nitrogen atoms [61]. For example, Kadioglu et al. [61] demonstrated that PEI is efficient in binding Cd^{2+} , Ni^{2+} , and Zn^{2+} ions at a high pH, and reported that the stability constants for the chelation of these cations are in the range of 7 to 11. This suggests that for other divalent metal ions, such as Hg^{2+} , the chelation efficiency should be comparably high.

Branched PEI is a polymer that is readily soluble in water at room temperature, whereas linear PEI is semicrystalline and dissolves easily in water only at higher temperatures [57]. PEI is commercially available in both linear and branched forms, but the chelation efficiency of (hyper)branched PEI has been reported to be up to 10 times higher than that of its linear counterpart [62, 63]. The reason for this difference in chelation efficiency has not yet been fully explained. One possible cause could be related to the presence of primary, secondary, and tertiary chelating amino groups in branched PEI, whereas its linear counterpart contains mainly secondary ones. An additional reason could be related to the stabilisation of the metal-ion complex by the presence of ramified conformations that are intrinsic to the branched PEI architecture, whereas the complexation of a metal ion with a linear chain entails a certain bending energy penalty. This explanation is also partially supported by the effectiveness of PEI-modified water purification systems [64–68], in which the arrangement of PEI fragments is regularised through their grafting onto specific types of surfaces.

Linear PEI has been utilised in the synthesis of hydrogels [57]. It has also

been employed as a building block in nanocomposites designed for the removal of heavy metals [69], including environmentally friendly chitosan/PEI-grafted magnetic gelatin [70], polystyrene-polyethyleneimine conjugates with high Pb adsorption capacity [59], and PEI-modified corn cob magnetic gel for the removal of Pb and Cu ions from water [58]. The behaviour of PEI has been investigated under various protonation levels using both atomistic [71–73] and coarse-grained [72, 74, 75] molecular modelling approaches, as well as experimental techniques such as NMR spectroscopy [76].

1.3.2. Ethylenediaminetetraacetic acid

Ethylenediaminetetraacetic acid (EDTA, Fig. 4.1) is a strong and effective hexadentate chelating agent that is widely used in waste management, chemical, pulp and paper, agricultural, healthcare, and other industries to bind metal ions. In particular, it is applied in wastewater treatment, soil washing, titrimetric analysis, and chelation therapy for the treatment of heavy metal poisoning and diseases related to xenobiotic metals [48], as well as in cleaning and food preservation products [77]. At high pH levels, EDTA is fully deprotonated, but as the acidity increases, it becomes progressively protonated, which reduces its chelation ability [54]. EDTA forms highly stable, water-soluble 1 : 1 complexes with a wide range of metal ions of valence > 1 . In contrast, its complexes with monovalent metal ions are significantly less stable, as evidenced by their lower stability constants [54].

EDTA typically forms complexes with a coordination number of 6, substituting water molecules in the first coordination shell of the metal ion with its 2 nitrogen and 4 oxygen donor atoms (Fig. 1.2) [55]. If the coordination number of a metal ion (M) is assumed to be 6, the corresponding water substitution reaction [53, 55] is $M^{2+}(H_2O)_6 + EDTA^{4-} \rightarrow (M EDTA)^{2-} + 6H_2O$.

The Coulombic attraction between metal cations and chelating anions increases with the charge of the cations and plays a predominant role in the chelation mechanism for elements in groups 1 and 2 [55]. An interesting observation made by

Mitchell et al. [55] is that for more polarising transition and post-transition metal cations, in addition to Coulombic interactions, a covalent contribution also plays a role, particularly in the case of bonding with the less electronegative nitrogen atoms of EDTA, as compared to its oxygen atoms. A strong electrostatic attraction also occurs between the metal cations and the 4 carboxylate oxygen atoms of EDTA [78].

Complexes of EDTA have been studied using computational techniques such as molecular modelling simulations, quantum mechanical/molecular mechanical (QM/MM) methods [79], and density functional theory (DFT), as well as experimental techniques including NMR spectroscopy, X-ray absorption spectroscopy [80], mass spectrometry [81], infrared (IR) and Raman spectroscopy, and extended X-ray absorption fine structure (EXAFS) and X-ray absorption near edge structure (XANES) spectroscopy. For example, the $\text{EDTA}^{3-}\text{-Hg}^{2+}$ complex has been studied using X-ray absorption spectroscopy to determine its structural properties [80], where the authors found that the Hg(II)-EDTA complex exhibits a distorted octahedral geometry, with the mercury ion coordinated to both nitrogen and oxygen atoms from EDTA, as well as to one water molecule. Complexes of EDTA with La^{3+} , Eu^{3+} , Gd^{3+} [82] and Eu^{3+} [83] in aqueous solution have also been investigated using density functional theory (DFT) and *ab initio* molecular dynamics (AIMD). This study shows that the coordination geometry (8- vs. 9-coordinate), ligand protonation state (pH-dependent), and temperature significantly influence the structural stability and binding strength of these complexes, helping to explain trends in lanthanide–EDTA binding across the periodic series.

EDTA is an important ligand used in complexometric titrations to determine metal ion concentrations in solutions [53]. To identify the point at which ion binding reaches equilibrium and to facilitate result analysis, chemical indicators (dyes) are often employed. These indicators change colour depending on whether they are in the bound or unbound state with a specific metal ion [53]. Since these indicator molecules are weaker ligands, they cannot effectively compete with the stronger chelator EDTA for metal ions. The resulting colour change signals the point at which the metal ions have been fully complexed by EDTA. Instrumental methods

such as potentiometry, visible (VIS) spectrophotometry, and conductometry can also be employed [53]. However, the pH of the titration mixture cannot be arbitrarily shifted into the alkaline range, as many metal ions form sparingly soluble metal hydroxides, thereby significantly reducing their side reaction coefficients. At a high pH, metal ions can form hydroxo complexes, which may slowly precipitate from a solution. To prevent this, an auxiliary ligand is used in such cases to bind metal ions [53]. EDTA then complexes the remaining free metal ions, as well as those that are displaced from the auxiliary ligand.

However, there are also negative sides to the strong chelating properties and for some applications such features as low selectivity and low biodegradability could be obstacles [77, 84]. For example, while EDTA is effective in neutralising heavy metals in the body and facilitate their natural removal, it may also cause the unintended removal of useful elements such as Ca, Cu, Mn as a side effect. However, at low doses, EDTA has been proven to be a safe and well-tolerated detoxification treatment, particularly effective in treating lead (Pb) poisoning, and also useful for removing cadmium (Cd) from the body [85]. An unfortunate consequence of wide and often unreasonable use of EDTA is the distribution of bound heavy metals and even radioactive elements in the environment, due to the high water solubility of EDTA and its complexes [77, 86]. This also complicates the application of otherwise more effective wastewater treatment techniques [87].

Procedures for recovering EDTA from treated water and minimising wastewater after soil washing have been under active development, with promising results that could help mitigate this problem [88–90]. EDTA can be used in chemical engineering to construct nanomaterials for wastewater purification and other purposes, such as the development of magnetic materials for enhanced heavy metal removal via magnetic separation. [52, 91, 92]. It also serves as a precursor for synthesising more complex EDTA-functionalised adsorbents [93–95], EDTA-functionalised silica gel [96], and covalent organic frameworks [97], among others. Such applications demonstrate the potential for more controllable and environmentally friendly alternatives to some conventional uses of the chelator.

1.3.3. Molecular modelling of heavy metal ions and their complexes in aqueous solution

Different factors such as temperature, pressure, solution composition, pH, charge transfer, and polarisation play a role in the behaviour of real systems involving an ion and a chelator in a solution [98]. When performing simulations, it is important to identify the most suitable model for studying the system properties of interest, given the available computational resources. In molecular dynamics (MD) simulations, the interaction between atoms or particles is typically described by classical force fields that include both bonded and non-bonded interactions [99–101]. Bonded interactions account for the internal structure of molecules and include bond stretching, angle bending, and dihedral torsions. Non-bonded interactions describe intermolecular and intramolecular forces between atoms which are not directly bonded, and are commonly modelled using the 12-6 Lennard-Jones potential for van der Waals forces and the Coulomb potential for electrostatic interactions, with atoms represented as fixed point charges. This general framework is adopted in widely used force fields such as AMBER [102], CHARMM [103], OPLS-AA [104], and GROMOS [105]. While they share the same physical principles, these force fields differ in their parameter sets and fitting strategies, which are optimised for different classes of molecules or simulation goals. They are regarded as fast and simple methods, available in most modelling software, but they cannot simultaneously reproduce all experimentally obtained properties of divalent transition metal ions, such as ion-oxygen distances (IODs), coordination numbers (CNs), and hydration free energy values (HFEs) [98]. For this reason, Li et al. proposed different parameter sets, each designed to reproduce specific experimental targets: IOD and HFE, for the IOD and HFE parameter sets, respectively. Moreover, these parameter sets differ across water models (TIP3P, SPC/E, TIP4P, and TIP4P_{EW}), as each model exhibits distinct properties [98]. The authors also proposed a parameter set called CM, intended as a compromise between coordination number (CN) and hydration free energy (HFE) experimental targets. The development and refinement of these parameters is of

great importance and has been carried out by several research groups. Babu and Lim developed Lennard-Jones parameters for divalent ions [106], using a single parameter set intended to sufficiently reproduce multiple experimental targets. In this study their parameters for mercury ions are used. The same force field parameters were applied by Anitha et al. [107] to investigate the adsorption behaviour of divalent heavy metal ions, Cd^{2+} , Cu^{2+} , Pb^{2+} , and Hg^{2+} , on single-walled carbon nanotubes (SWCNTs) functionalised with carboxyl ($-\text{COO}^-$), hydroxyl ($-\text{OH}$), and amide ($-\text{CONH}_2$) groups using molecular dynamics simulations. And de Araujo et al. developed new Lennard-Jones parameters for Pb^{2+} and Cd^{2+} ions to improve the accuracy of liquid-phase molecular simulations. These parameters were validated against experimental hydration free energy values and structural properties, showing good agreement and transferability across different water models [108].

Cations with charges greater than +2 are even more challenging to model accurately using relatively simple models. Alternative methods include hybrid quantum mechanical/molecular mechanical (QM/MM) approaches, bonded models, short-long range effective functions (SLEF), and polarisable force fields to account for polarisation and charge transfer. Similar to the TIP4P water model, which introduces a fourth dummy site to represent the charge distribution of a three-atom water molecule, there also exist dummy cationic models that use an additional site to carry the ionic charge, which can be positioned outside the metal ion itself. One such model for metal cations was developed by Jiang et al. [109]. Furthermore, there are models with the modified form of the Lennard-Jones potential, known as the 12-6-4 Lennard-Jones potential. This potential improves its predecessor by introducing an additional term proportional to r^{-4} (where r is the distance between atoms) to account for the ion-induced dipole interaction [110, 111]:

$$u_{LJ}(r) = -\frac{C}{r^4} + 4\epsilon \left[\left(\frac{\sigma}{r} \right)^{12} - \left(\frac{\sigma}{r} \right)^6 \right],$$

where the parameter C was originally estimated by Li et al. with ion-oxygen interactions in water in mind [110], but it can also be reparameterised to better model interactions with atoms of other molecules, including EDTA [111].

1.3.4. Hydration shells of Hg^{2+} , Cd^{2+} and Pb^{2+} ions

In one of the methods used to calculate the Gibbs free energy of complexation, it was necessary to resolve the first hydration shells of the metal ions. Typically, the coordination numbers and hydration shell structures obtained computationally depend on the method applied. Moreover, this issue remains an active area of investigation within the experimental research community.

It is generally accepted that Hg^{2+} and Cd^{2+} ions possess a six-coordinated first hydration shell with an octahedral geometry [98, 112], although there has been some debate regarding the possibility of seven-coordinated water molecules [113, 114].

For Pb^{2+} the situation is even more complex. There is ongoing debate not only regarding the number of water molecules coordinated to the ion and their respective distances [115], but also concerning the geometry of the coordination shell – whether it is holodirected (with water molecules distributed uniformly over the surface of a sphere around the ion) or hemidirected (with the ligands concentrated on one side, forming a hemispherical distribution) (see Fig. 1.3). Migliorati et al. [116], using *in silico* methods and X-ray spectroscopy, concluded that the hydration shell of Pb^{2+} is hemidirected. Le'on-Pimentel et al. [117] also supported the hemidirected structure based on *in silico* simulations, although they did not provide a definitive coordination number. Similarly, Pye et al. [118] identified the structure as hemidirected and additionally reported a hexacoordinate configuration.

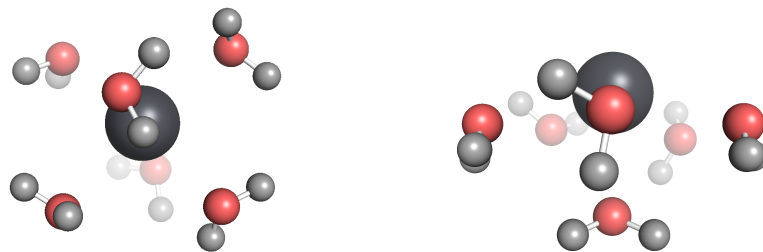


Figure 1.3. Holodirected (left) and hemidirected (right) types of geometries of first hydration shells. The large black spheres represent metal cations, while the red and small grey spheres represent O and H atoms of water, respectively.

1.4. Conclusions

Understanding the formation of molecular complexes in functional molecular systems is central to advancing applications in biotechnology, environmental remediation, and materials science. Two important and conceptually related problems involve, firstly, the self-assembly of polymers with enzymes, and secondly, the complexation of heavy metal ions with well-known chelating agents. In the former, recent modelling efforts have used coarse-grained and patchy particle approaches to investigate how polymer architecture and interaction specificity affect the complex formation of polymer-enzyme conjugates. In the latter, experimental studies and atomistic simulations have focused on the coordination binding between metal ions and such molecules as PEI or EDTA.

Both lines of research share a common conceptual framework rooted in associating fluids, anisotropic interactions, and valence-limited binding, studied by computer simulations. Together, they provide valuable insights into the design of functional hybrid materials, whether for biocatalysis and the immobilisation of enzymes or for selective removal and sensing of toxic metal ions.

CHAPTER 2

MODELLING OF POLYMER-ENZYME COMPLEXES

2.1. Introduction

The study presented in this chapter aims to deepen the understanding of enzyme complexation mechanisms mediated by polymer carriers, which enhance the efficiency of cellulose hydrolysis – a component of biofuel production processes. One experimentally known mechanism of complexation involves the association of enzymes with polymeric supports through the formation of non-covalent or covalent bonds between them. We propose a simplified model and, based on it, a theoretical approach for describing the process of polymer-enzyme self-assembly of large-scale systems of molecules. In this model a bottlebrush-like polymer scaffold is considered with the side chains treated implicitly, similarly to as in [119, 120], and a globular model of the enzyme possesses a single interaction site that is affine to the functional groups on the polymer (Fig. 2.1, right). The binding interaction between enzymes and polymers leads to the formation of complexes, commonly referred to as polymer-enzyme conjugates. Our aim is to find a method for the rapid theoretical prediction of the degree of binding, which would enable the reliable estimation of enzyme immobilization efficiency on polymer scaffolds under various conditions. For this, Wertheim’s thermodynamic perturbation theory of the first order was employed and detailed computer simulations were performed for the proposed model to assess the accuracy of the theoretical results. The model of polymers and enzymes is represented as a mixture of hard-sphere associative chains (polymers) and patchy monomers (enzymes). We demonstrate that the proposed approximation aligns well

with the Langevin dynamics computer simulations across a wide parameter range and significantly outperforms existing methods due to the considerable differences in interaction between the intermediate and terminal monomers of the polymer. An assessment of the degree of binding between polymers and enzymes was carried out depending on the number of functional groups on the polymer, under conditions where the total number of these groups equals the number of enzyme molecules in the solution and the packing fractions of the compared systems are equal.

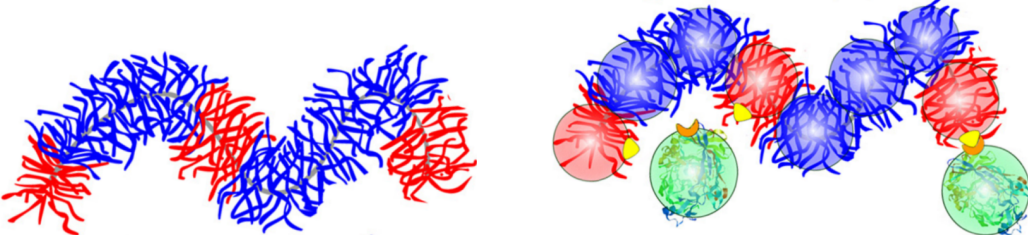


Figure 2.1. Schematic representation of a single bottlebrush block copolymer (left panel) and the same copolymer with enzyme molecules (shown in green), where the functional groups are denoted in yellow and orange (right panel). Reproduced from Ref. [3].

2.2. Theoretical model

This study is focused on developing and testing a coarse-grained (CG) model inspired by cellulosomes. The model describes a formation of polymer-enzyme conjugates in a mixture of enzyme globules and bottlebrush block copolymers with functional groups able to bind the globules. The bottlebrush polymer (Fig. 2.1, left) is modelled as a linear flexible chain of spheres representing each block, possibly bearing functional groups, while the enzyme globule is modelled as a single sphere, also carrying functional groups (Fig. 2.1, right). A functional group is described as an interacting site on a sphere that enables bond formation. The functional groups located on certain blocks of the bottlebrush polymer can bind specific groups of enzymes through affine interactions [121, 122]. This mechanism is represented by assigning two different types of interacting sites: one type for the polymer and the other for the enzyme. Sites of different types can form a bond upon close approach,

whereas no interaction occurs between sites of the same type.

The objective is to estimate the fraction of enzymes immobilized on the bottlebrush chains. Binding between polymers and enzymes results in the formation of polymer-enzyme complexes, where the degree of binding depends on the concentrations of both components and the system temperature.

We start our consideration from a general theoretic model proposed for a two-component mixture of flexible chains of two types, constructed from tangentially bonded monomers of different sizes bearing functional groups. In this model, for the description of the monomers, we use the concept of patchy particles. Therefore, each monomer is represented as a hard sphere with or without a patch. The interaction between patches is modelled via the attractive square-well potential acting only between patches of different types. The potential for pair interactions between the chain monomers is

$$U_{a_i b_j}(12) = U_{a_i b_j}^{(hs)}(r) + U_{a_i b_j}^{(sw)}(12), \quad (2.1)$$

where i , and j stand for the type of a monomer of the chain of type a and b , respectively,

$$U_{a_i b_j}^{(hs)}(r) = \begin{cases} \infty, & \text{for } r \leq \sigma_{a_i b_j} \\ 0, & \text{otherwise} \end{cases}, \quad (2.2)$$

$$U_{a_i b_j}^{(sw)}(12) \equiv U_{a_i b_j}^{(sw)}(z) = \begin{cases} -(1 - \delta_{ab})\epsilon_{a_i b_j}, & \text{for } z \leq \kappa \\ 0, & \text{otherwise} \end{cases}, \quad (2.3)$$

z is the distance between the square-well sites, $\sigma_{a_i b_j}$ is the diameter of a hard sphere, $\epsilon_{a_i b_j}$ is the square-well depth, κ is its width and δ_{ab} is the Kronecker delta. The indices $a, b = 1..2$, $i = 1, \dots, n_a$ and $j = 1, \dots, n_b$. Here n_a and n_b denote the numbers of monomers in the chains of types a , b , respectively. The number density in the system is $\rho = \rho_1 + \rho_2$, where ρ_1 and ρ_2 are the number densities of the chains of the types 1 and 2, respectively.

The polymer-enzyme mixture is treated as a special case of this two-component system, where the enzyme is represented as a chain consisting of a single monomer.

2.3. Computer simulation model

The Langevin dynamics (LD) method is applied in our study. This is a well-established and effective method for studying coarse-grained models of large macromolecules such as bottlebrush polymers, proteins, and enzymes. By incorporating both systematic forces and stochastic thermal fluctuations, this approach realistically captures the diffusive and dissipative nature of macromolecular motion in a solvent without explicitly modelling the solvent particles. This makes Langevin dynamics particularly suitable for large-scale simulations, where fully atomistic models would be computationally prohibitive. Moreover, the method allows efficient exploration of equilibrium and the dynamic properties of complex molecular systems, including conformational transitions, aggregation, and binding processes. Its flexibility and computational efficiency make it a powerful tool for investigating the mesoscale behaviour of biomolecular and polymeric assemblies.

The model introduced in the previous section is well-suited to be studied by the Wertheim's thermodynamic perturbation theory, which will be considered below. However, its application in LD simulations requires appropriate adjustments. This complication arises from the discontinuity functions presenting the hard-sphere (HS) and square-well (SW) pair interaction potentials, which do not yield continuous force expressions required for integrating the equations of motion in Langevin dynamics simulations. Therefore, for simulations we employ alternative pair potentials of analytical forms, i.e., they should possess continuous derivatives throughout, yet closely approximate the original interaction potentials.

For the interactions between monomers, we use the pair potential proposed by Jover et al. [123], which describes a strong repulsive interaction between two spheres:

$$u_{\text{phs}}(r) = \begin{cases} 50 \left(\frac{50}{49}\right)^{49} \epsilon_{\text{phs}} \left[\left(\frac{\sigma}{r}\right)^{50} - \left(\frac{\sigma}{r}\right)^{49} \right] + \epsilon_{\text{phs}}, & r < \frac{50}{49}\sigma \\ 0, & r \geq \frac{50}{49}\sigma \end{cases}, \quad (2.4)$$

where σ is the sum of the radii of the interacting sphere, and ϵ_{phs} is an adjustable energetical parameter.

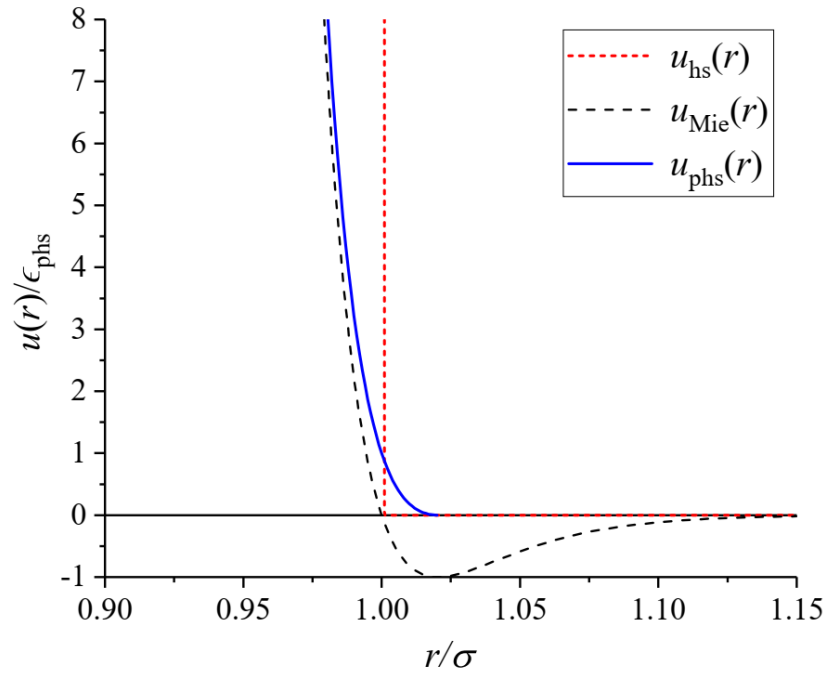


Figure 2.2. The pseudo-hard-sphere (PHS) pair potential (blue solid line), the hard-sphere (HS) potential (red dotted line), and the (50,49)-Mie pair potential (black dashed line).

The potential $u_{\text{phs}}(r)$ is a (50,49)-Mie-type pair potential that has been cut off and shifted at $r_{\text{min}} = \frac{50}{49}\sigma$, corresponding to its minimum value $u_{\text{phs}}(r_{\text{min}}) = -\epsilon_{\text{phs}}$, which is analogous to the Weeks-Chandler-Andersen (WCA) scheme. The WCA treatment is commonly used for the Lennard-Jones (LJ) potential to replace the full LJ interaction with its purely repulsive counterpart. However, since the Lennard-Jones (LJ) potential is too soft to adequately describe hard-sphere-type interactions in classical dynamics simulations, a (50,49)-Mie-type potential is used instead.

The pair potential $u_{\text{phs}}(r)$ was first introduced by Jover et al. in [123], where its applicability was demonstrated by accurately reproducing the structural, thermodynamic, and dynamic properties of the hard-sphere system. It was referred to as the pseudo-hard-sphere (PHS) pair potential. One can see that $u_{\text{phs}}(r)$ is very close to the conventional hard-sphere potential (see Fig. 2.2). Moreover, the PHS potential was also employed in a number of coarse-grained models, where a simple, close to hard-sphere repulsion interaction between particles is needed, including for the patchy particles model. Particularly, Espinosa et al. [124] proposed using the PHS

potential in a combination with the continuous square-well potential (CSW), which was introduced to replace the traditional square-well potential to model patchy particle interactions. The CSW patch-patch pair potential is a discontinuous function and it retains the key features of the square-well model (see Fig. 2.3), but it allows for more efficient molecular dynamics simulations:

$$u_{\text{CSW}}(r) = -\frac{1}{2}\epsilon_{\text{CSW}} \left[1 - \tanh \left(\frac{r - r_w}{\alpha} \right) \right], \quad (2.5)$$

where ϵ_{CSW} is an energy parameter, r_w is the width of the attractive well and α is the parameter to control the well steepness. In Fig. 2.3, one can compare the CSW potential with the conventional SW potential and see that they fit well to each other. It was shown that the combination of the PHS potential for monomer-monomer interactions and the CSW potential for patch-patch interactions is suitable for describing systems with directional, valence-limited interactions. This approach can be applied to globular proteins, biomolecular condensates, enzyme-polymer conjugates, patchy colloids, DNA-coated particles, and other soft matter systems where specific binding sites drive self-assembly and phase behaviour.

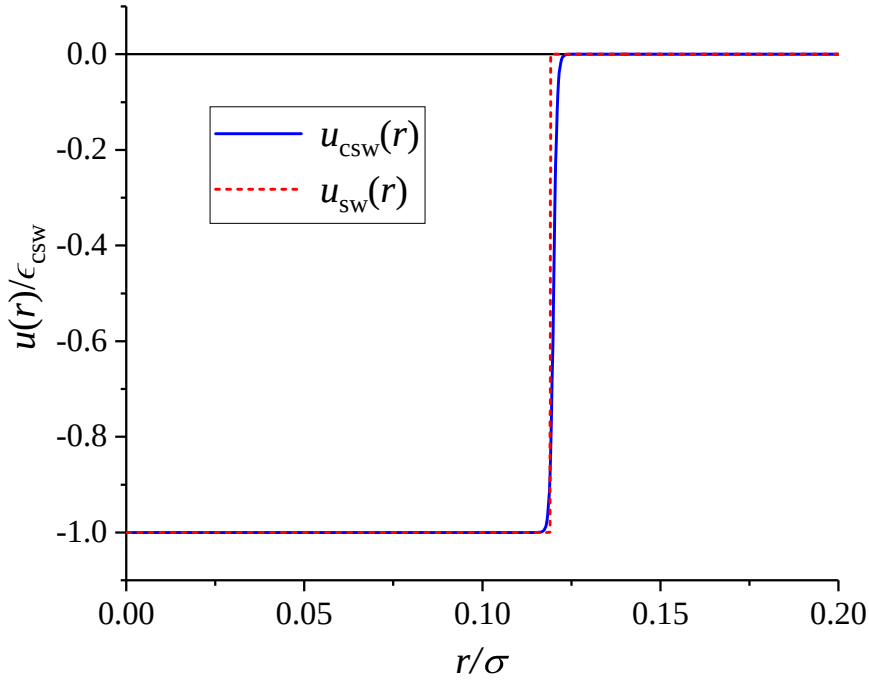


Figure 2.3. The continuous function of approximated square-well potential (2.5) and the conventional square-well potential.

2.4. Theoretical approach

Wertheim's multi-density integral-equation theory, combined with the first-order multi-density thermodynamic perturbation theory (TPT1), is a powerful tool for describing systems of patchy particles [125–127]. We use an extension of this theoretical approach to predict the binding between polymer and enzyme molecules according to the model presented above in Section 2.2. To evaluate the reliability of the theoretical approach, its predictions will later be compared with the results of computer simulations.

We begin our consideration with the general case of a two-component mixture of chain molecules composed of monomers of different sizes. According to Wertheim's theory [125, 126], we assume that the Helmholtz free energy A of the mixture can be written as the sum of the Helmholtz free energy of the reference system, A_{ref} , and the bonding contribution, ΔA_{bond} :

$$A = A_{\text{ref}} + \Delta A_{\text{bond}}, \quad (2.6)$$

where a two-component mixture of hard-sphere chains with $\epsilon_{a_i b_j} = 0$ is used as the reference system. Then, the bonding contribution to the free energy, ΔA_{bond} , is given by:

$$\beta \frac{\Delta A_{\text{bond}}}{V} = \sum_{a=1}^2 \rho_a \left[\sum_{i=1}^{n_a} \left(\ln X_{a_i} - \frac{1}{2} X_{a_i} \right) + \frac{1}{2} n_a \right], \quad (2.7)$$

where n_a denotes the number of square-well interaction sites on a chain molecule of type a , and X_{a_i} is the fraction of type- a molecules in which the square-well site on the i th monomer remains nonbonded. This fraction is determined by the mass action law (MAL):

$$X_{a_i} \sum_{b=1}^2 \rho_b \sum_{j=1}^{n_b} X_{b_j} K_{a_i b_j} + X_{a_i} - 1 = 0, \quad (2.8)$$

The equilibrium association constant $K_{a_i b_j}$ can be expressed as:

$$K_{a_i b_j} = 4\pi \int_{\sigma_{a_i b_j}}^{\sigma_{a_i b_j} + \kappa} r^2 \bar{f}_{a_i b_j}^{(sw)}(r) g_{a_i b_j}^{(ref)}(r) dr, \quad (2.9)$$

where $\bar{f}_{a_i b_j}^{(sw)}(r)$ is the monomer-monomer Mayer function averaged over all possible orientations,

$$\bar{f}_{a_i b_j}^{(sw)}(r) = \frac{(1 - \delta_{ab})F_{a_i b_j}^{(sw)}}{6\sigma_{a_i b_j}^2 r} (\kappa + \sigma_{a_i b_j} - r)^2 (2\kappa - \sigma_{a_i b_j} + r), \quad (2.10)$$

and $g_{a_i b_j}^{(ref)}(r)$ is the monomer-monomer pair correlation function (i.e., the radial distribution function). The Mayer function for the square-well potential is given by $F_{a_i b_j}^{(sw)} = e^{\beta \epsilon_{a_i b_j}} - 1$. This approximation follows from the short-range nature of the square-well potential $U_{a_i b_j}(12)$, since only configurations in which the sites (i, a) and (j, b) are separated by a distance $r \leq \kappa$ contribute significantly to the integral $K_{a_i b_j}$. Thus, the Mayer function can be assumed to be constant within this range, i.e., $\bar{f}_{a_i b_j}^{(sw)}(12) = F_{a_i b_j}^{(sw)}$, and $K_{a_i b_j}$ can be evaluated from (2.9). The validity of this approximation is confirmed by comparison with computer simulation results.

The thermodynamic properties of the reference system can be derived using Wertheim's first-order thermodynamic perturbation theory (TPT1). Specifically, for its Helmholtz free energy A_{ref} we have:

$$\beta \frac{A_{ref}}{V} = - \sum_{a=1}^2 \rho_a \sum_{i=1}^{n_a-1} \ln y_{a_i a_{i+1}}^{(hs)}(\sigma_{a_i a_{i+1}}), \quad (2.11)$$

where $y_{a_i b_j}^{(hs)}(r)$ is the hard-sphere cavity correlation function. Assuming that the value the integral for the equilibrium association constant $K_{a_i b_j}$ (2.9) is known, the fractions X_{a_i} can be calculated using the equation (2.8). Approximating the Mayer function $\bar{f}_{a_i b_j}^{(sw)}(r)$ (2.10) by the Dirac delta function $\delta(r)$ simplifies the evaluation of the integral (2.9):

$$\bar{f}_{a_i b_j}^{(sw)}(r) = T_{a_i b_j} \delta(r - \sigma_{a_i b_j}), \quad (2.12)$$

where

$$T_{a_i b_j} = \sigma_{a_i b_j}^{-2} \int_{\sigma_{a_i b_j}}^{\sigma_{a_i b_j} + \kappa} r^2 \bar{f}_{a_i b_j}^{(sw)}(r) dr. \quad (2.13)$$

Thus, $K_{a_i b_j}$ can be expressed as

$$K_{a_i b_j} = 4\pi \sigma_{a_i b_j}^2 T_{a_i b_j} g_{a_i b_j}^{(ref)}(\sigma_{a_i b_j}), \quad (2.14)$$

where $g_{a_i b_j}^{(ref)}(\sigma_{a_i b_j})$ are the contact values of the pair correlation functions. These contact values can be obtained using the analytical solution of the integral equation according to the polymer Percus-Yevick ideal chain approximation for heteronuclear hard-sphere chain fluids [128–130]:

$$\begin{aligned} \sigma_{a_i b_j} g_{a_i b_j}^{(ref)}(\sigma_{a_i b_j}) &= \frac{\sigma_{a_i b_j}}{1 - \eta} + \frac{1}{4} \frac{\rho s_n \sigma_{a_i} \sigma_{b_j}}{(1 - \eta)^2} \\ &+ \frac{1}{4(\eta - 1)} \sigma_{b_i} \left[(1 - \delta_{j1}) \frac{\sigma_{b_{j-1}}}{\sigma_{b_j b_{j-1}}} + (1 - \delta_{jn_b}) \frac{\sigma_{b_{j+1}}}{\sigma_{b_j b_{j+1}}} \right] \\ &+ \frac{1}{4(\eta - 1)} \sigma_{a_i} \left[(1 - \delta_{i1}) \frac{\sigma_{a_{i-1}}}{\sigma_{a_i a_{i-1}}} + (1 - \delta_{in_a}) \frac{\sigma_{a_{i+1}}}{\sigma_{a_i a_{i+1}}} \right] \\ &+ \frac{\delta_{ab}}{8\pi\rho_a} \left[\frac{(1 - \delta_{1i})(1 - \delta_{2i})}{\sigma_{a_i a_{i-1}} \sigma_{a_{i-1} a_{i-2}}} \delta_{ij+2} + \frac{(1 - \delta_{in_a})(1 - \delta_{in_a-1})}{\sigma_{a_i a_{i+1}} \sigma_{a_{i+1} a_{i+2}}} \delta_{ij-2} \right], \end{aligned} \quad (2.15)$$

where $\eta = \pi/6 \sum_a \rho_a \sum_i \sigma_{a_i}^3$ and $s_n = \pi/\rho \sum_a \rho_a \sum_i \sigma_{a_i}^2$.

2.5. Computer simulation details

The simulation model introduced in Section 2.3 is studied by the Langevin dynamics (LD), where the polymer-enzyme conjugation process is considered in the NVT ensemble [100] using the LAMMPS software package (<https://www.lammps.org>, version 30Nov2020) [131]. Since the CSW pair potential (2.3) is not supported in LAMMPS, its source code had to be modified accordingly. Unlike in Ref. [124], a smaller value of $\alpha = 0.001\sigma$ in eq. 2.5 is used to make the square-well potential steeper and more closely resemble the conventional square-well potential (see Fig. 2.3). The width of the attractive well is set to $r_w = 0.12\sigma$, the cutoff radius is 0.17σ , and the depth of the attractive well, ϵ_{CSW} , is taken as the unit of energy. All patches are fixed on the corresponding monomers of the polymers and on the enzymes at a distance of $\sigma/2$ from the monomer centres using the SHAKE algorithm, with a tolerance parameter of 0.0001. So, these monomers with the patches move as single entities. The diameters of all monomers in the system were equal and set to σ , which serves as the unit length in the computer simulations.

The hard-sphere pair potential (2.2) its PHS counterpart is used with the pa-

rameters suggested in in [123, 124]. Since the systems are studied at different temperatures, the parameter ϵ_{phs} of the PHS potential was rescaled as $\epsilon_{\text{phs}} = \epsilon_{\text{csw}} T^*/1.5$, where $T^* = k_B T / \epsilon_{\text{csw}}$ is the temperature expressed in reduced units. For the intramolecular interactions within the polymer molecules, harmonic bonds between chain monomers were used, described by the potential $u_{\text{bond}}(r) = K(r - r_0)^2$, with the rigidity constant $K = 160.0 \epsilon_{\text{csw}} / \sigma^2$ and the equilibrium bond length $r_0 = \sigma$. For two monomers belonging to the same molecule, the non-bonded pairwise interactions $u_{\text{csw}}(r)$ and $u_{\text{phs}}(r)$ were applied only if the monomers did not share a common bond.

The advantage of LD is that it allows avoiding explicitly modelling a solvent, which helps to save computational resources especially for large systems such as with polymers and enzymes, what is important even at a coarse-grained level. The solvent can be modelled implicitly through the friction force $\mathbf{F}_f$ and the random forces $\mathbf{F}_r$ acting on monomers [132] as

$$\mathbf{F}_f = -m\mathbf{v}/\tau_d, \quad \mathbf{F}_r \propto \sqrt{\frac{k_B T m}{\tau_d \delta t}}. \quad (2.16)$$

These two forces are connected through the fluctuation-dissipation theorem and are governed by the damping time τ_d , which is used to control the temperature during the simulations via the Langevin thermostat [132]. In contrast, molecular dynamics (MD) simulations performed without explicit solvent particles, but using the given pair potentials may become unstable, as particles can experience excessively large forces and velocities. Introducing small solvent particles together with the coarse-grained monomers and/or reducing the simulation time step could stabilise the system, but would substantially increase the computational cost of MD. Therefore, the LD method is more efficient and better suited to the type of system considered in this study.

Newton's equations of motion were integrated using the velocity-Verlet algorithm implemented in the LAMMPS simulation package. The timestep for the algorithm was set as $\delta t = 0.0001\tau$ and the LD damping parameter was chosen as $\tau_d = 0.1\tau$, where $\tau = (m\sigma^2\epsilon_{\text{csw}}^{-1})^{1/2}$ is the time unit. The time step δt was chosen

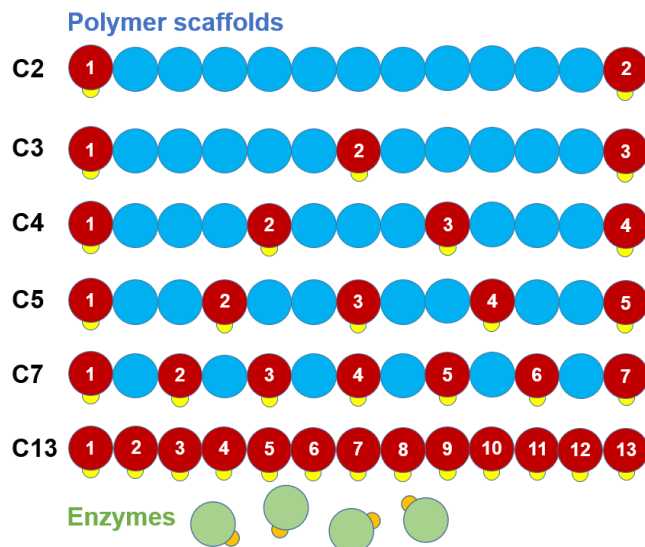


Figure 2.4. Block copolymer scaffold models used to construct systems C2..C13, and enzyme models. The systems Ck are mixtures of enzymes (green beads with orange patches) with polymers which have k patches (yellow spots on red beads) distributed on it according to the given scheme. Blue beads do not participate in complexation with enzymes. Reproduced from Ref. [3].

to be sufficiently small to ensure system stability, given the steep pair potentials and the very short interaction range r_w . A cubic simulation box with side length $L = 50.0\sigma$ and periodic boundary conditions in all directions was used for all systems studied (for each set of parameters). The number of particles varied from system to system (see Fig. 2.4) based on the number densities of polymers and enzymes (see Table 2.1). In the case of the Model C13 the number of enzymes reached its maximum (about 15000) and the number of polymer chains reached its minimum (1154). In the case of Model C2 there was the largest number of polymer chains, reaching 2000. A simulation of every system was a 3-stage process with the following steps:

1. The system was prepared by constructing patchy polymers according to the required functional group pattern (see Fig. 2.4), attaching a patch to each enzyme sphere, and packing the polymers and enzymes into the simulation box. Since the initial packing was not optimal, the system was relaxed for 10^6 simulation steps with the square-well potential temporarily disabled to prevent

enzyme-polymer bond formation via their binding sites.

2. At this stage, the patches were activated and the system was equilibrated for approximately 2×10^8 simulation steps to allow the number of bonds to converge to its equilibrium value.
3. The final stage was used for data accumulation and lasted for approximately 3×10^8 simulation steps, with data recorded every 10000 steps.

After this, the collected data was averaged, and the corresponding analysis of the obtained results was performed.

Table 2.1. Number densities (ρ_p) of polymer chains per model. Reproduced from Ref. [3].

C2	C3	C4	C5	C7	C13
0.016	0.015	0.014	0.013	0.012	0.009

2.6. Results and discussion

To demonstrate the application of the developed theoretical approach within the framework of TPT1 and analyze its accuracy in detail, we considered 6 types of polymer scaffolds with varying numbers of functional groups distributed along the chain (one per monomer), labeled accordingly as Model C2, C3, C4, C5, C7, and C13 (see Fig. 2.4). Each chain represents a polymer scaffold consisting of 13 hard-sphere monomers with identical diameters σ . The number in the name of each model corresponds to the quantity of monomers bearing a functional group. Such monomers were uniformly and symmetrically distributed along the polymer chain. Enzyme molecules were represented by individual monomers in the form of hard spheres of the same diameter σ , which, like polymer monomers, each possess one functional group capable of conjugating with the functional group on the polymer via a square-well pair potential (2.3). The parameters for the width and depth of the square-well potential between functional groups (associative sites) were chosen

as $\kappa = 0.119\sigma$ and $\varepsilon_{ij} = \varepsilon$. In this study, two reduced temperature values were considered: $T^* = T/\varepsilon = 0.09$ and 0.12 .

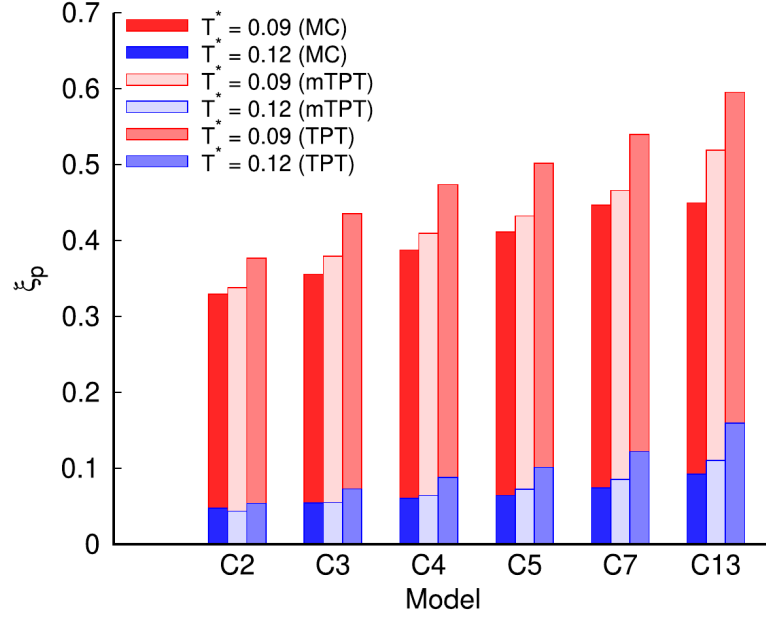


Figure 2.5. The average number of bonds per enzyme monomer for the Models C2, C3, C4, C5, C7 and C13 (described in Fig. 2.4) at the fixed packing fraction $\eta = 0.126$ so that the total number of polymer patches in the system is equal to the number of enzymes: $n_e = n_p k_p$ (k_p – number of functional groups per polymer chain, n_e and n_p – number of enzyme and polymer molecules, respectively). Red and blue correspond to the temperatures $T^* = 0.09$ and $T^* = 0.12$, respectively. Reproduced from Ref. [3].

Before proceeding to compare the theoretical results with computer simulations, we conducted a general analysis of the system behaviour based exclusively on computer simulations. Fig. 2.5 shows the simulation results for the average number of bonds formed between enzymes and polymers, normalized by the number of enzymes in the system, for the six types of polymer chains. The total packing fraction of all monomers in a system was fixed at $\eta = \frac{\pi}{6}(\rho_e + 13\rho_p)\sigma^3 = 0.126$, and the density ratio was fixed as $\rho_e/\rho_p = k_p$, where k_p is the number of monomers with functional groups per polymer chain. With this choice of densities, the number of enzyme molecules is equal to the total number of polymer monomers bearing functional groups. It is observed that at a lower temperature ($T^* = 0.09$), there is a noticeable increase in the fraction of bound enzymes as the number of functionalized monomers

per polymer chain, k_p , increases. At a higher temperature ($T^* = 0.12$), this increase is also present but less pronounced. At $k_p = 7$ (Model C7), the situation changes qualitatively. Specifically, at a higher temperature, there is a further increase in the fraction of bound enzymes. On the other hand, at a lower temperature, the increase significantly slows down and remains practically unchanged, as observed at $k_p = 13$ (Model C13). One can conclude that at lower temperatures, enzymes already bound to the polymer can block binding sites for other enzymes if the density of the functional groups is sufficiently high. At higher temperatures, this phenomenon is not observed due to the relatively low average number of enzyme-polymer bonds.

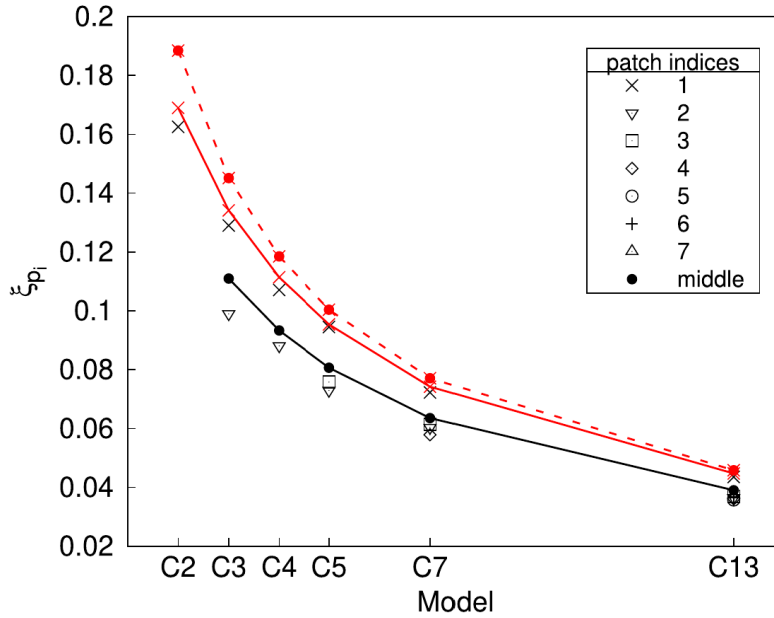


Figure 2.6. The average number of bonds with the specific polymer patches per number of enzyme molecules (for $n_e = n_p k_p$) at temperature $T^* = 0.09$, where each patch index refers to its index at the polymer molecule (see Fig. 2.4). The data is generalized for all patches located in the middle in a polymer chain. The red crosses and red filled circles, connected by the dashed line correspond to the results of the TPT, red crosses connected by a red solid line and black filled circles connected by a solid black line represent results of the mTPT. Other symbols correspond to the computer simulation results. Reproduced from Ref. [3].

We analysed the differences in enzyme association with polymer monomers depending on their position along the polymer chain. The proposed theory assumes that the probability of enzyme association with particular functionalized monomers

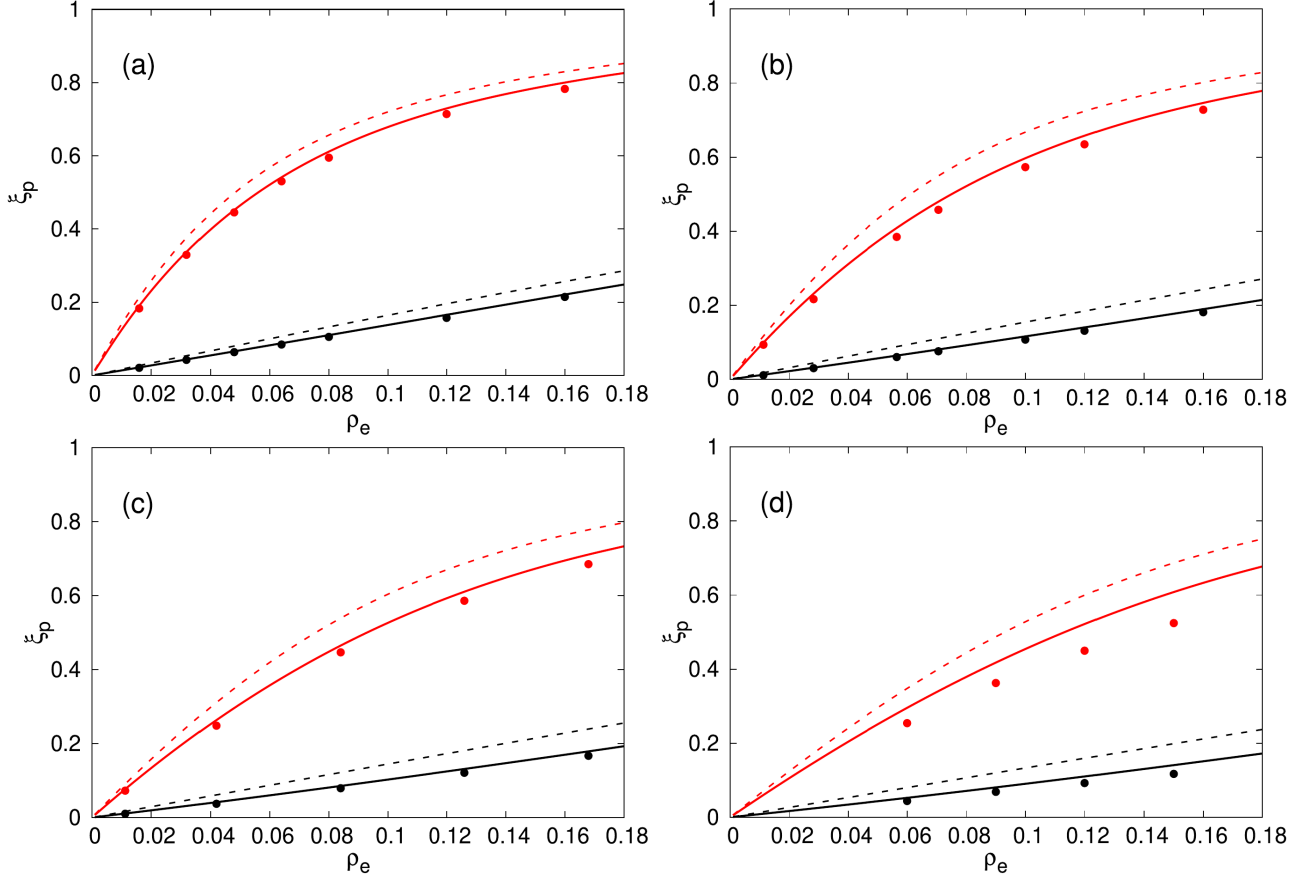


Figure 2.7. The number of bonds in the system per polymer patch ξ_p vs enzyme number density ρ_e . Panels (a), (b), (c) and (d) correspond to the models C2, C4, C7 and C13, respectively. Theoretical results: mTPT1 and TPT1, are shown by solid and dashed lines, respectively. Symbols mark the computer simulation results. Results corresponding to the $T^* = 0.09$ are shown in red and for $T^* = 0.12$ black is used. Reproduced from Ref. [3].

of the polymer depends twofold on their positions along the chain: (i) the monomer is located at the chain end (first or last); (ii) the monomer is located inside the chain (at any position other than the first and last). It should be noted that previous theoretical TPT1 approaches do not distinguish these positions, leading to poor quantitative descriptions of the connectivity. In our case, we specifically investigated how much the binding degree differs for internal polymer monomers, determining whether there is a significant difference depending on whether the monomer is closer or further away from the chain ends. Fig. 2.6 demonstrates that such a difference is negligible, and the most important distinction exists only between terminal and internal monomers. This further confirms the applicability of our proposed theory.

We now proceed to compare the results of computer simulations with the modified TPT1 theory, which we propose to denote as mTPT1. The previous version of the generalized theory is referred to as TPT1. Fixing the polymer density $\rho_1 = \rho_p$, we analyze the dependence of the fraction of bound enzymes on their density $\rho_2 = \rho_e$ in the range 0-0.18 at both lower and higher temperatures $T^* = 0.09$ and 0.12, for different model types. The polymer density $\rho_1 = \rho_p$ for each model type was taken according to Table 2.1. The resulting dependencies are shown in Fig. 2.7, where one can observe very good quantitative agreement of mTPT1 with the data from computer simulations. On the other hand, the previous TPT1 approach provides significantly worse predictions.

2.7. Conclusions

A computer simulation study was carried out to investigate the formation of polymer-enzyme complexes or conjugates using Langevin dynamics. The system was modelled as a two-component mixture of hard-sphere associative chain molecules, where the first component represents linear polymer scaffolds composed of monomers bearing functional groups, and the second component consists of enzyme monomers with a single specific site capable of binding to these groups. The simulations enabled a detailed analysis of the conjugation process under various model parameters.

The results demonstrated that the binding degree of polymers and enzymes (defined as the number of bonds formed per enzyme molecule) increases with the number of functional groups per polymer chain, under the condition that the total number of functional groups equals the number of enzyme molecules in the system. For a fixed total number of monomers, it was observed that the degree of polymer-enzyme conjugation increased with the number of functional groups on the polymer. However, when nearly all polymer monomers are functionalised, this increase slows down rapidly. This is due to steric effects, where enzymes bound to closely spaced sites block the binding of additional enzymes. This phenomenon, clearly captured by Langevin dynamics simulations, highlights the importance of spatial constraints

in limiting conjugate formation.

To complement the simulation results, a theoretical description based on Wertheim’s first-order thermodynamic perturbation theory (TPT1) was developed. The theory was adapted to account for the distinction between terminal and inner monomers along the polymer chain, resulting in improved agreement with the simulation data over a broad range of parameters. However, the current formulation of TPT1 does not incorporate the steric exclusion effects observed in simulations at high functionalisation levels and thus overpredicts the binding degree and polymer-enzyme complex formation in this regime. To address this limitation and improve the theoretical description, a transition to the second-order thermodynamic perturbation theory (TPT2) is necessary.

CHAPTER 3

CHELATION OF MERCURY IONS BY POLYETHYLENEIMINE

3.1. Introduction

In this chapter the use of linear polyethyleneimine (PEI) is investigated as a chelating agent, focusing on its interaction with mercury ions (Hg^{2+}) in an aqueous solution. Atomistic molecular dynamics (MD) simulations, using the OPLS/AA force field, are employed to study the system. The microscopic configuration of the complexes formed between PEI chains and Hg^{2+} ions is analysed, considering different PEI chain lengths. The results show that a single linear PEI chain with ten amino groups can bind up to four Hg^{2+} ions. The stability of these complexes is further supported by density functional theory (DFT) calculations.

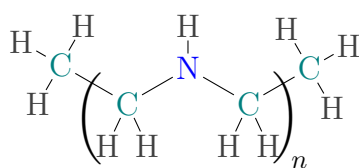


Figure 3.1. Linear PEI, in the brackets the monomer chunk is shown, on the left - the terminal group, n – degree of polymerization.

3.2. Atomistic model for linear PEI chains based on the OPLS/AA force field

All-atom MD simulations were performed for systems containing complexes of a PEI molecule with mercury ions in water. The linear PEI polymers (denoted

as PEI- n) considered here are illustrated in Fig. 3.1, with $n = 4, 5$ and 10. Each polymer chain terminates with two methyl groups. Each simulation system consisted of one PEI- n molecule, one mercury ion for PEI-4 and PEI-5, and from 1 to 4 mercury ions for PEI-10, along with two counterions per mercury ion to compensate for their charge. Thus, one PEI chain was allowed to bind multiple cations, whereas each cation was restricted to binding to a single PEI molecule. However, this latter condition does not always hold in real systems [61].

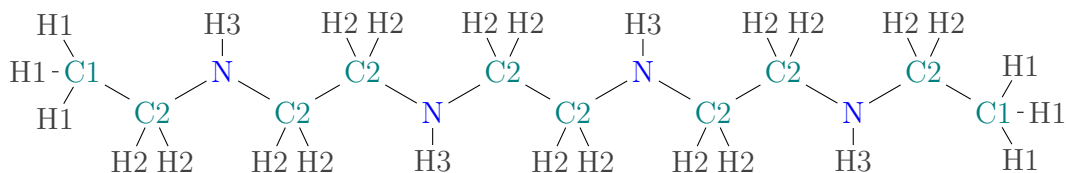


Figure 3.2. PEI molecule with 4 nitrogen atoms (PEI-4). The notation of atom names corresponds to the atom types in Table 3.1.

Table 3.1. OPLS-AA FF parameters for PEI and LJ parameters for mercury ion [106]. The atom types of PEI molecule are visualized in Fig. 3.2

Type	Charge	Mass	$\epsilon, \frac{\text{kcal}}{\text{mol}}$	$\sigma, \text{\AA}$	Bond	$K, \frac{\text{kcal}}{\text{mol}\cdot\text{\AA}^2}$	$r_0, \text{\AA}$
N	-0.78	14.007	0.17	3.3	C-C	268	1.529
C1	-0.18	12.011	0.066	3.5	C-N	382	1.448
C2	0.08	12.011	0.066	3.5	C-H	340	1.09
H1	0.06	1.008	0.03	2.5	N-H	434	1.01
H2	0.06	1.008	0.015	2.5			
H3	0.38	1.008	0	0			
Hg ²⁺	2	200.592	0.0409	2.36			

Angle	$K, \frac{\text{kcal}}{\text{mol}\cdot\text{rad}^2}$	θ_0°	Dihedral	$K_1, \frac{\text{kcal}}{\text{mol}}$	$K_2, \frac{\text{kcal}}{\text{mol}}$	$K_3, \frac{\text{kcal}}{\text{mol}}$	$K_4, \frac{\text{kcal}}{\text{mol}}$
C-C-N	56.2	109.47	N-C-C-N	11.035	-0.968	0.27	0
H-C-H	33.0	107.8	N-C-C-H	-1.013	-0.709	0.473	0
N-C-H	35.0	109.5	H-C-C-H	0	0	0.3	0
C-C-H	37.5	110.7	C-C-N-C	0.416	-0.128	0.695	0
C-N-C	51.8	107.2	C-C-N-H	-0.19	-0.417	0.418	0
C-N-H	35.0	109.5	H-C-N-C	0	0	0.56	0
			H-C-N-H	0	0	0.4	0

PEI was modelled using the OPLS/AA force field (FF) [133]. The relevant parameters, along with the non-bonded Lennard-Jones (LJ) parameters for Hg²⁺, are listed in Table 3.1, and the atom types are illustrated using the example of PEI-

4 in Fig. 3.2. The rigid SPC/E model [134] was applied for water molecules. The Lennard-Jones parameters for mercury were chosen to ensure compatibility with the selected force fields for both the polymer and water [107, 135, 136].

For bonded interactions within the molecules, the following potentials were used: a harmonic bond potential $E = K(r - r_0)^2$ for two bonded atoms, where r_0 is the equilibrium bond length and K is the spring constant; a harmonic angle potential $E = K(\theta - \theta_0)^2$ for three atoms, where θ_0 is the equilibrium angle; and a dihedral potential $E = \frac{1}{2}K_1[1 + \cos(\phi)] + \frac{1}{2}K_2[1 - \cos(2\phi)] + \frac{1}{2}K_3[1 + \cos(3\phi)] + \frac{1}{2}K_4[1 - \cos(4\phi)]$ for four-atom torsional interactions, where ϕ is the dihedral angle. Non-bonded pairwise interactions within the same molecule were excluded for atom pairs separated by fewer than three bonds, while for 1–4 interactions (i.e., atoms separated by exactly three bonds), the non-bonded interaction was included but scaled by a factor of 0.5. The LJ potential, $E = 4\epsilon \left[\left(\frac{\sigma}{r} \right)^{12} - \left(\frac{\sigma}{r} \right)^6 \right]$ and electrostatic interaction potential $E = \frac{q_i q_j}{r}$ were used for non-bonding pairwise interactions. For the atoms of different types the LJ interaction parameters were determined based on the interaction parameters for the interaction of similar atoms using the geometrical combining rules $\epsilon_{ij} = \sqrt{\epsilon_i \epsilon_j}$ and $\sigma_{ij} = \sqrt{\sigma_i \sigma_j}$. The simulation box (Fig. 3.3) was rectangular, and periodic boundary conditions were applied in all three spatial directions. To account for the long-range Coulombic interactions the particle-particle-particle-mesh (PPPM) solver with 0.0001 accuracy and 12.0 Å cutoff radius was used. The cutoff of the LJ interactions was set to 10 Å. The SHAKE algorithm was used to constrain the bond lengths and angles of the SPC/E water model.

Each simulation consisted of four stages:

1. system initialization and PEI-Hg²⁺ complex formation without water molecules;
2. introduction of water molecules and relaxation of the system in ambient conditions;
3. system equilibration;
4. final simulation of the equilibrated system and accumulation of data for further analysis.

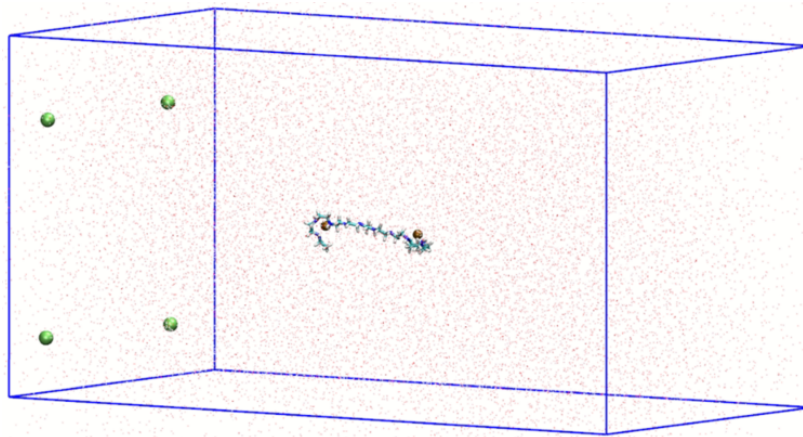


Figure 3.3. A simulation box containing PEI-10 + 2 Hg^{2+} , 4 counterions (in green) and water molecules which are pictured as small red points.

The purpose of stage 1 was to form an initial PEI-mercury complex for subsequent verification of its stability. The absence of water molecules facilitated rapid complex formation. This process was performed in the NVT ensemble with the temperature set to 298.15 K. To maintain the system at the desired temperature, a Nosé-Hoover thermostat with a relaxation parameter of 10 fs was employed. Initially, the simulation box (Fig. 3.3) had dimensions of $150 \text{ \AA} \times 75 \text{ \AA} \times 75 \text{ \AA}$, extended in the X -axis to place the counterions at a sufficient distance (approximately $70 \text{ \AA}$) from the complexes. The counterions and mercury ions were fixed in place to prevent mutual coordination, allowing only the PEI molecule to move. In the absence of water screening at this stage, the electrostatic attraction between cations and counterions could otherwise significantly influence the initial complex formation, even at large separations. The duration of this stage was 1 ns.

In the second stage, 20000 water molecules were introduced into the system at arbitrary positions, ensuring that the distance between any two molecules was greater than $1.5 \text{ \AA}$. Mercury cations were allowed to move at this point in time, but not the counterions. Then after a short energy minimization of the system the simulations were continued in the NPT ensemble at ambient conditions ($T=298.15 \text{ K}$, $P = 1 \text{ atm}$). The Nosé-Hoover thermostat-barostat was employed in this case with the time of relaxation parameters 100 fs for temperature and 1000 fs for pressure. As a result, the box size decreased to an equilibrium value of approximately

134 Å × 67 Å × 67 Å within 100 ps, and the density of the water molecules reached the typical bulk value for SPC/E water, $\rho = 0.99$ g/cm³. This box size was sufficient to keep the ions and counterions at a distance of no less than 50 Å, preventing them from approaching too closely. Consequently, the influence of counterions on the dynamics of the PEI-mercury complex could be neglected, assuming that the long-range Coulombic interactions were substantially screened by the presence of water molecules.

The remaining two stages were intended to verify the stability of the complex. The parameters at these stages remained the same as for the previous stage. In stage 3 an equilibration of the system was performed for 3 ns. Stage 4 involved the actual simulation and subsequent data collection. The simulation was conducted for 4 ns, and system snapshots were recorded every 1 ps. It was monitored whether the complex approached the counterions too closely. In general, the complex moved randomly without a preferential direction toward the counterions, occasionally approaching them but subsequently moving away. This suggests that the interaction between the ions and counterions can indeed be neglected. If the complex could eventually approach a counterion too closely, which was observed in some parallel simulations, they were discarded.

3.3. Molecular dynamics results for complexation between linear PEI chains and Hg²⁺ ions

The simulation procedure described in Section 3.2 was applied to three different lengths of the PEI molecule, and the following complexes were investigated: PEI-4+Hg²⁺, PEI-5+Hg²⁺, PEI-10+Hg²⁺, as well as PEI-10 complexes with 2 to 5 Hg²⁺ ions. Representative examples are shown in Fig. 3.4. The data stored in the final stage of these simulations was analysed. In particular, radial distribution functions (RDFs), $g(r)$, and running coordination numbers (CNs), $n(r)$, were calculated between nitrogen atoms of PEI and the mercury ion, between the nitrogen atoms within PEI, as well as between the mercury ion and oxygen atoms of water

molecules. The relevant plots for the systems are shown in Figs. 3.5-3.11, with the exception of the PEI-10+5 Hg^{2+} complex was found to be unstable, as the electrostatic repulsion between the mercury ions caused the complex to lose one or more of them over time.

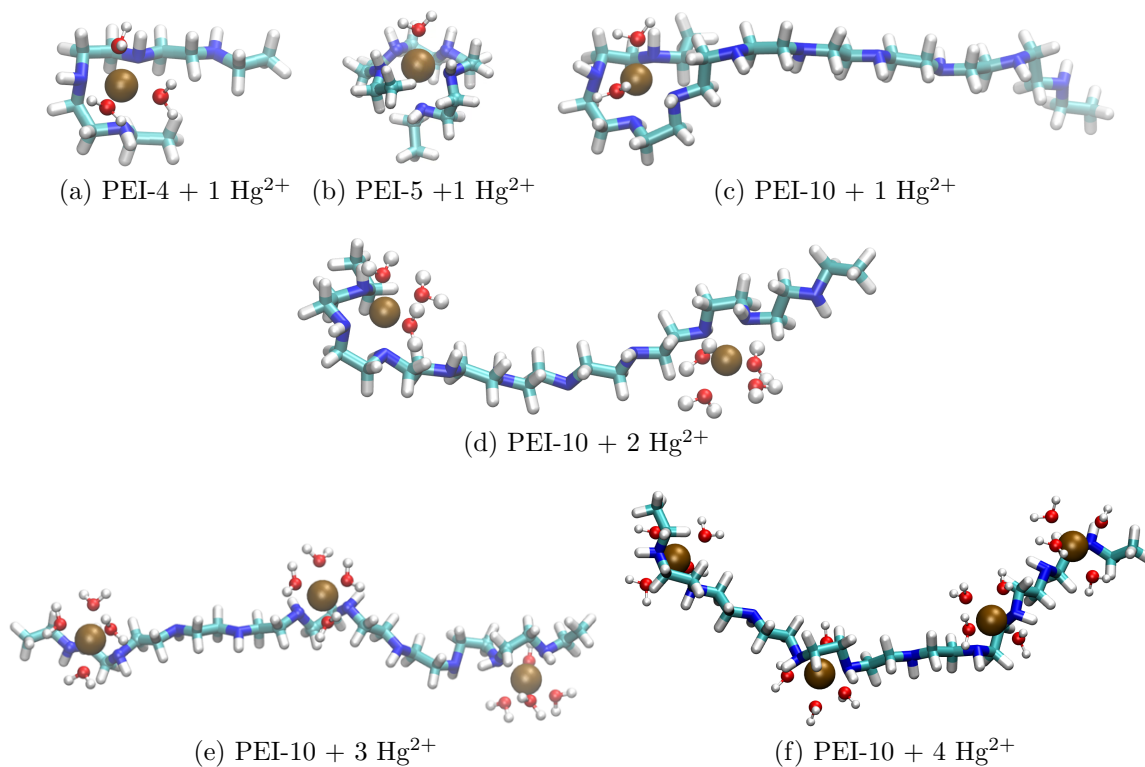


Figure 3.4. Snapshots of PEI- Hg^{2+} complex configurations from the MD simulations, including water molecules in the first coordination shells of mercury ions. The C, N, O, H and Hg atoms are coloured in cyan, blue, red, light-grey, brown, respectively.

As shown in Fig. 3.4, both PEI-4 and PEI-5 coordinate efficiently with a single Hg^{2+} ion. All five nitrogen atoms of PEI-5 participate in chelation, whereas three out of four nitrogen atoms in PEI-4 are involved (see $n(r)$ functions in Fig. 3.5). According to the radial distribution functions, the typical distance between the Hg^{2+} ion and nitrogen atoms in the first coordination shell is approximately 2.15 Å in both complexes (see $g(r)$ functions in Fig. 3.5). Additionally, the first coordination shell of Hg^{2+} accommodates approximately three and one water molecules at a distance of ~ 2.1 Å from the oxygen atom O_w in the PEI-4+ Hg^{2+} and PEI-5- Hg^{2+} complexes, respectively (Fig. 3.6). The PEI-5 complex with one mercury ion (Fig. 3.4b) forms

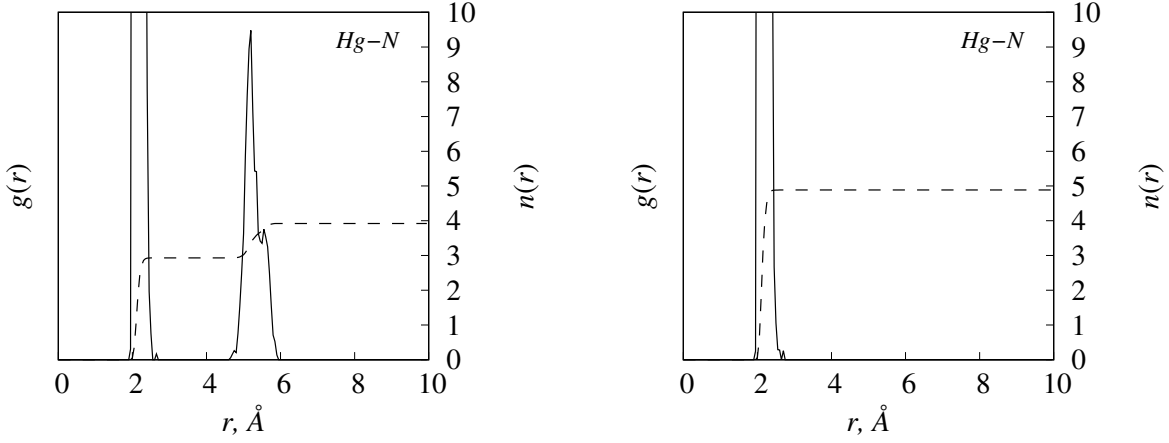


Figure 3.5. Radial distribution function, $g(r)$ (solid line), and running coordination number, $n(r)$ (dashed line), for Hg–N pairs, obtained from the MD simulations of the PEI-4+1 Hg^{2+} (left) and PEI-5+1 Hg^{2+} (right) complexes.

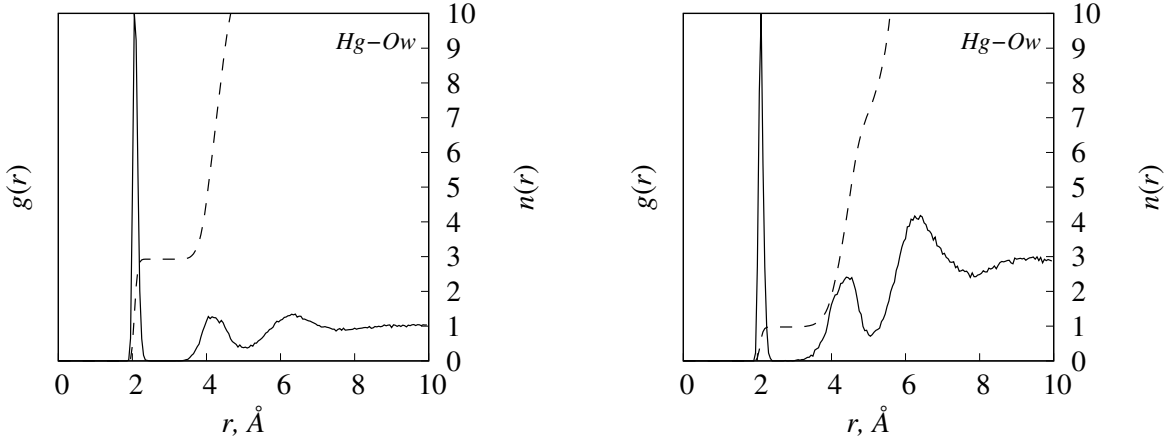


Figure 3.6. Radial distribution function, $g(r)$ (solid line), and running coordination number, $n(r)$ (dashed line), for Hg–Ow pairs, obtained from the MD simulations of the PEI-4+1 Hg^{2+} (left) and PEI-5+1 Hg^{2+} (right) complexes.

a globular conformation, with the largest distance between nitrogen atoms reaching approximately 4.2 Å (as shown by the $g(r)$ function in the right panel of Fig. 3.7). In the case of the PEI-4+ Hg^{2+} complex (Fig. 3.4a), the N–N distances range from approximately 2.55 to 6.4 Å (Fig. 3.7).

The MD simulation results show that 4 out of 10 nitrogen atoms of the PEI-10 chain participate in the chelation of a single mercury ion (Fig. 3.8), while staying at a distance of 2.1 Å from the ion. In addition, two water molecules are present in the first hydration shell of the mercury ion (Fig. 3.10). The ion is located near the

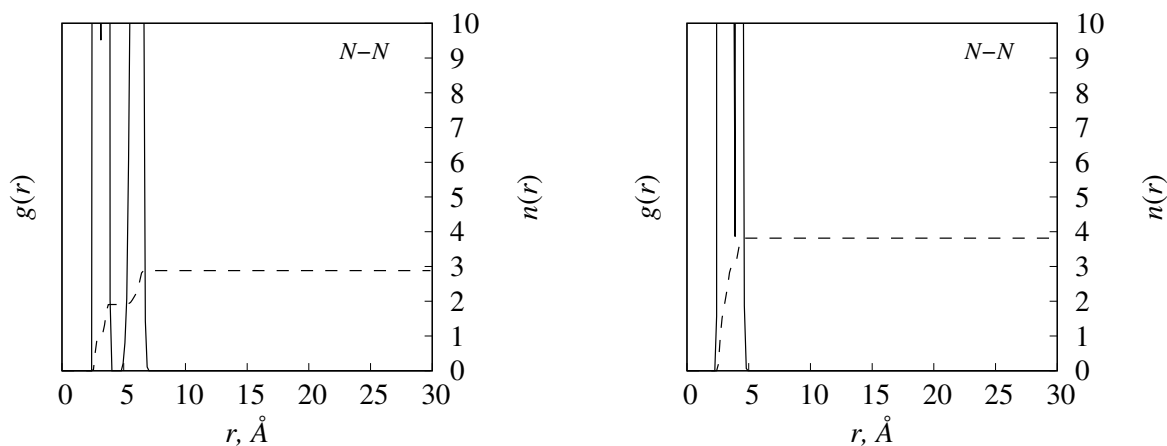


Figure 3.7. Radial distribution function, $g(r)$ (solid line), and running coordination number, $n(r)$ (dashed line), for N–N pairs, obtained from the MD simulations of the PEI-4+1 Hg^{2+} (left) and PEI-5+1 Hg^{2+} (right) complexes.

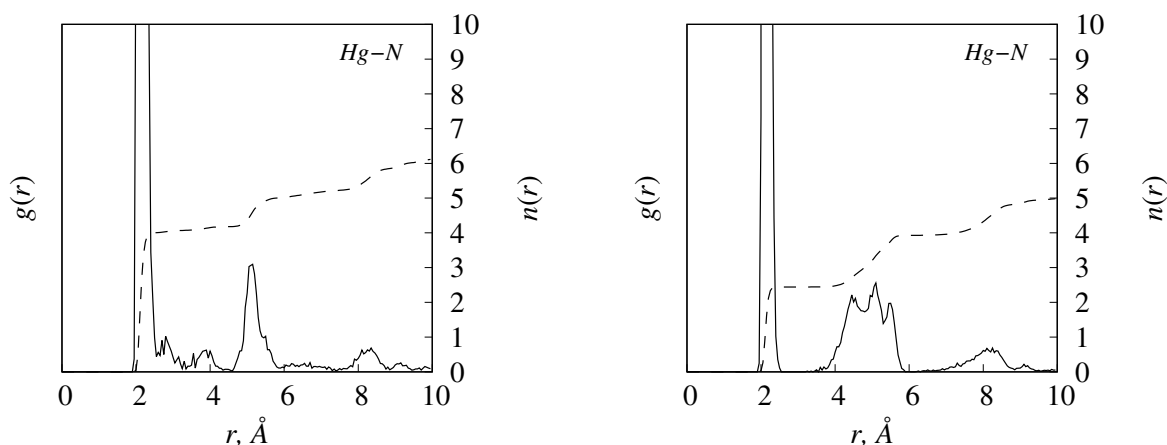


Figure 3.8. Radial distribution function, $g(r)$ (solid line), and running coordination number, $n(r)$ (dashed line), for Hg–N pairs, obtained from the MD simulations of the PEI-10+1 Hg^{2+} (left) and PEI-10+2 Hg^{2+} (right) complexes.

end of the chain, leaving a dangling tail that extends into the surrounding water. In the complex with two Hg^{2+} ions, one ion is coordinated with three nitrogen atoms at one end of the chain, while the other is coordinated with two nitrogen atoms closer to the opposite end (Fig. 3.4d), resulting in an average coordination number of 2.5 (Fig. 3.8). Simultaneously, 3 and 4 water molecules are present in the first coordination shells of these ions, with the average coordination number of 3.5 (Fig. 3.10). In the cases with a larger number of ions (three or four), the Hg^{2+} ions are arranged along the PEI-10 chain, each coordinated by two nitrogen

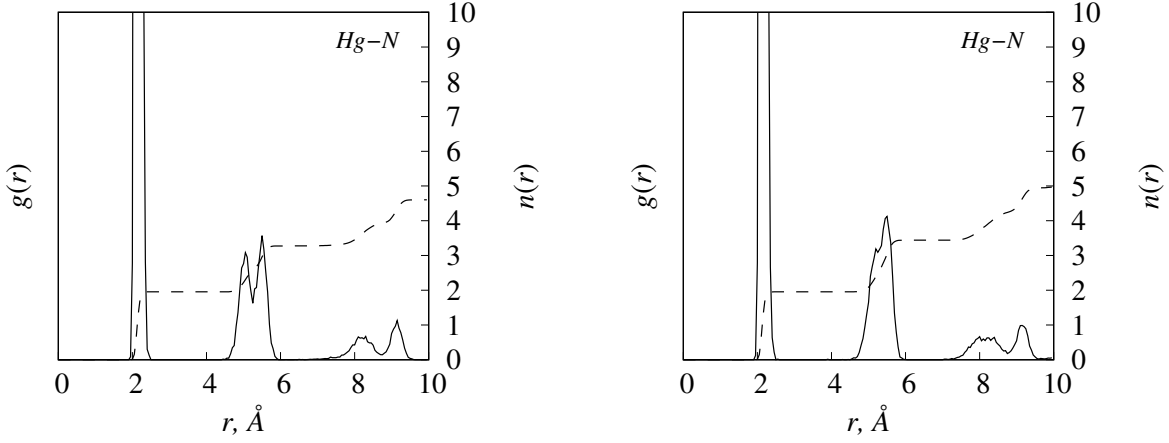


Figure 3.9. Radial distribution function, $g(r)$ (solid line), and running coordination number, $n(r)$ (dashed line), for Hg–N pairs, obtained from the MD simulations of the PEI-10+3 Hg^{2+} (left) and PEI-10+4 Hg^{2+} (right) complexes.

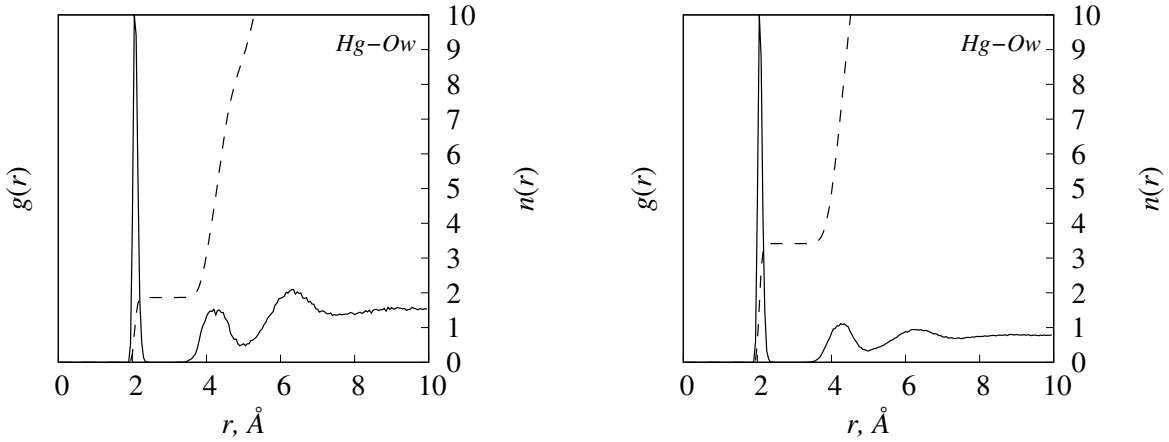


Figure 3.10. Radial distribution function, $g(r)$ (solid line), and running coordination number, $n(r)$ (dashed line), for Hg–Ow pairs, obtained from the MD simulations of the PEI-10+1 Hg^{2+} (left) and PEI-10+2 Hg^{2+} (right) complexes.

atoms (Fig. 3.9) and accompanied by four water molecules in their hydration shells (Fig. 3.11). The loss of one coordinated nitrogen atom leads to its substitution by one water molecule, maintaining a total of six coordinating atoms (nitrogen atoms and water oxygen atoms) around the Hg^{2+} ion. This corresponds to the typical coordination number observed in the octahedral hydration shell of a free mercury ion, as reported in the literature [98, 112]. Oliynyk et al. also concluded that the coordination numbers following divalent ion complexation with PEI [137], though in

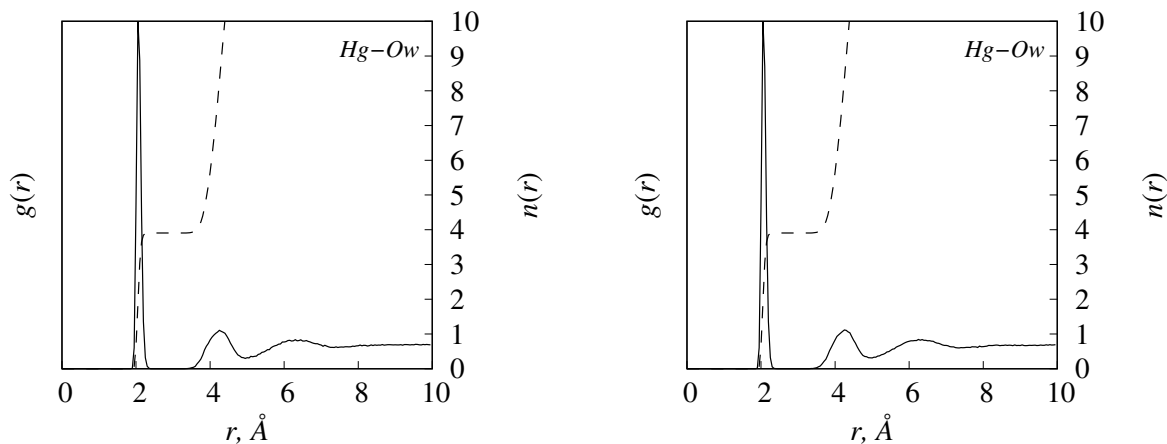


Figure 3.11. Radial distribution function, $g(r)$ (solid line), and running coordination number, $n(r)$ (dashed line), for Hg–Ow pairs, obtained from MD simulations of the PEI-10+3 Hg^{2+} (left) and PEI-10+4 Hg^{2+} (right) complexes.

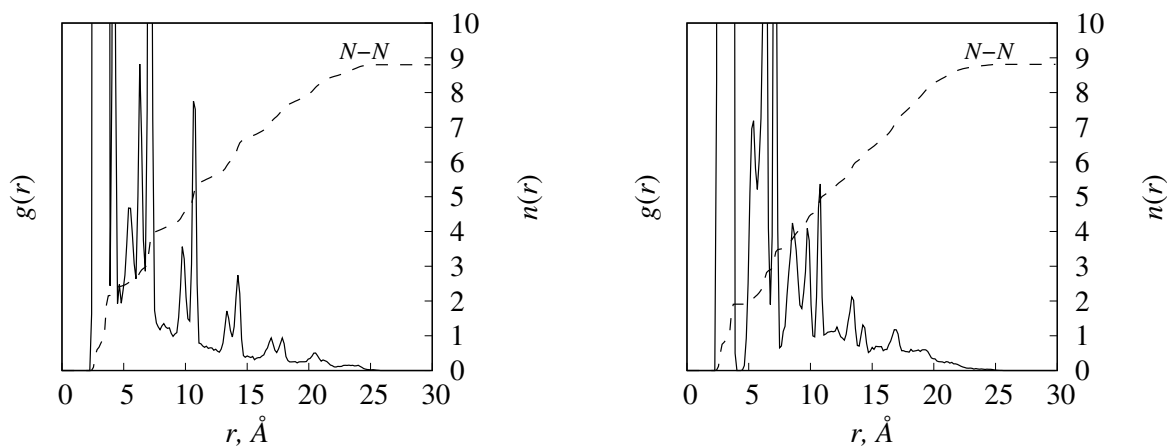


Figure 3.12. Radial distribution function, $g(r)$ (solid line), and running coordination number, $n(r)$ (dashed line), for N–N pairs, obtained from the MD simulations of the PEI-10+1 Hg^{2+} (left) and PEI-10+2 Hg^{2+} (right) complexes.

branched form due to its high molecular weight [61], were lower (typically 2 or 4) for cases where the concentration of nitrogen atoms per metal ion was low. Under such conditions, an ion is likely to be coordinated by two neighbouring nitrogen atoms of the PEI chain [137]. For higher concentrations of N per Me^{2+} ion, the coordination numbers reported in the literature were 5 or 6 [138], and in this study a similar trend was observed.

In all 4 cases (of chelation of 1 to 4 Hg^{2+} ions), the polymer chains remained

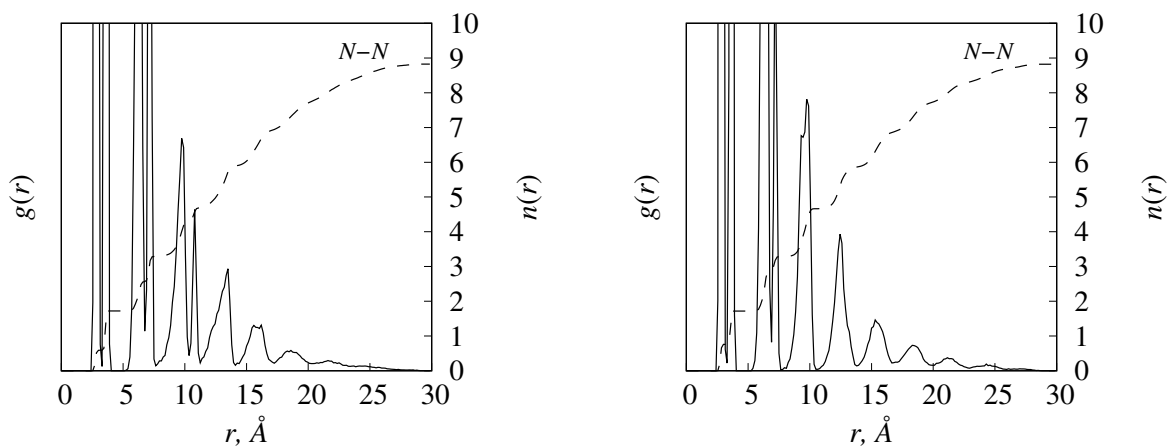


Figure 3.13. Radial distribution function, $g(r)$ (solid line), and running coordination number, $n(r)$ (dashed line), for N–N pairs, obtained from the MD simulations of the PEI-10+3 Hg^{2+} (left) and PEI-10+4 Hg^{2+} (right) complexes.

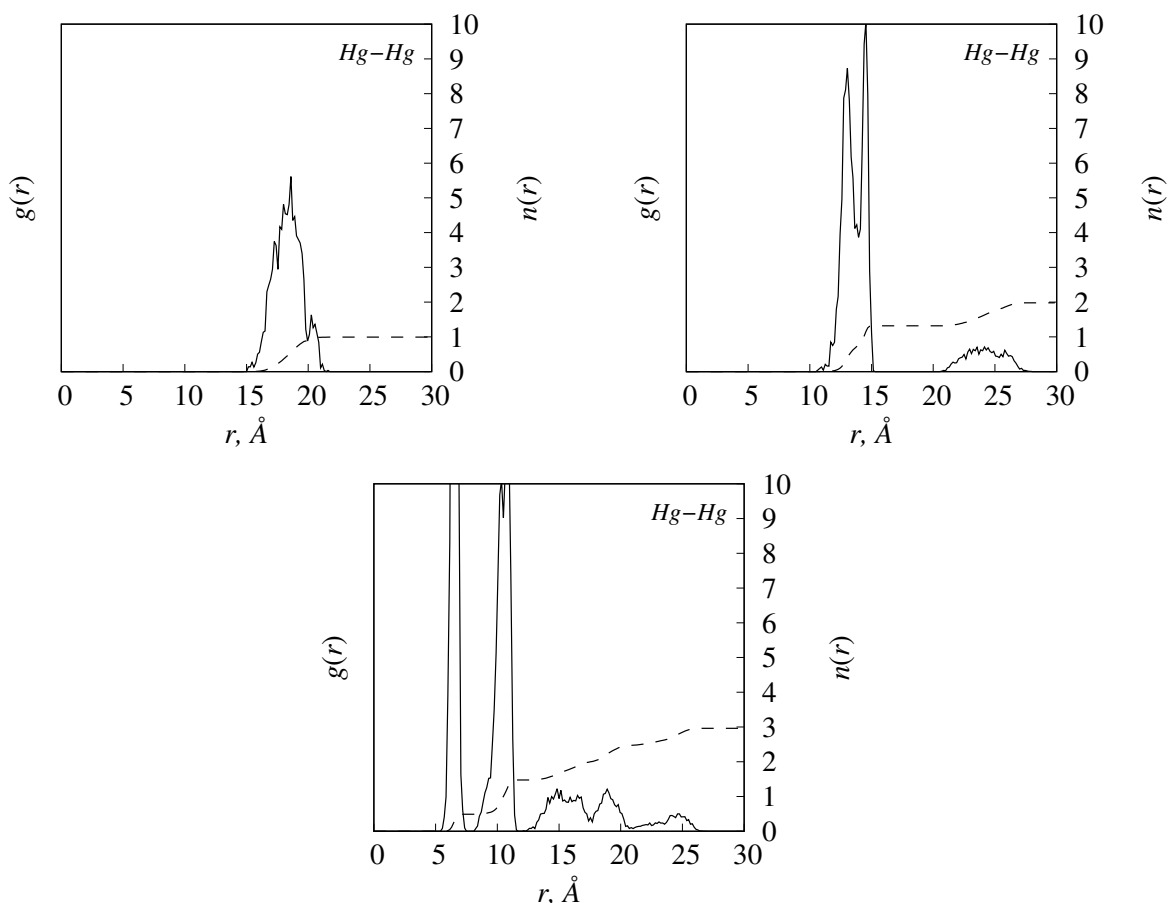


Figure 3.14. Radial distribution function, $g(r)$ (solid line), and running coordination number, $n(r)$ (dashed line), for Hg–Hg pairs, obtained from the MD simulations of the PEI-10+2 Hg^{2+} (top left), PEI-10+3 Hg^{2+} (top right), and PEI-10+4 Hg^{2+} (bottom) complexes.

relatively elongated, as evidenced by the N–N distances corresponding to the peaks in the RDFs (Figs. 3.12–3.13). This behaviour can be attributed to electrostatic repulsion between the Hg^{2+} ions and the intrinsic water solubility of PEI. The minimum distance between Hg^{2+} ions was approximately 5.55 Å for the PEI-10 complex with four Hg^{2+} ions, and this minimum distance increased by about 10 Å as the number of Hg^{2+} ions decreased (Fig. 3.14).

3.4. Adsorption energy

Adsorption energy in chelation refers to the energy change that occurs when a metal ion binds to a chelating molecule, forming a stable complex. It is typically calculated as the difference between the energy of the complex and the sum of the energy values of the free chelating agent and the unbound metal ion. More negative adsorption energy indicates stronger and more thermodynamically favourable binding, making it a useful measure of complex stability and chelation efficiency. The adsorption energy calculated from potential energy differences approximates the enthalpic component of the Gibbs adsorption energy for chelation of polymer-ion complexes.

The adsorption energy values were calculated for the systems according to the expression $E_{\text{cmpx}} = E(\text{PEI} + n\text{Hg}^{2+}) - E(\text{PEI}) - nE(\text{Hg}^{2+})$, where $E(\text{PEI} + n\text{Hg}^{2+})$, $E(\text{PEI})$, and $E(\text{Hg}^{2+})$ denote the potential energy of the PEI- $n\text{Hg}^{2+}$ complex, the potential energy of PEI in water, and the potential energy of unbound (dechelated) Hg^{2+} in water, respectively, n is the number of mercury ions. To obtain $E(\text{PEI})$ and $E(\text{Hg}^{2+})$, additional MD simulations were performed for free PEI polymers (PEI-4, PEI-5, and PEI-10) and the unbound Hg^{2+} ion in an aqueous environment, following the MD protocol described in Section 3.2. The results indicate favourable complexation (Table 3.2), with the strongest mercury ion binding observed for the PEI-5 polymer, followed by PEI-10 and PEI-4 chelating a single ion, and then by PEI-10 chelating 2, 3, and 4 ions.

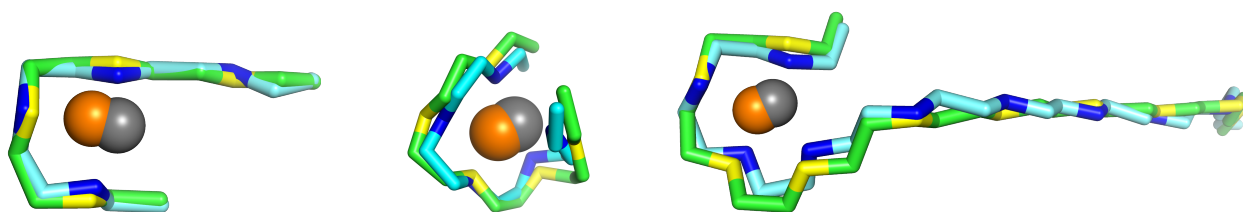


Figure 3.15. A comparison of DFT and MD conformations of PEI-4, PEI-5 and PEI-10 complexes with 1 Hg^{2+} cation optimized at the M06-2X/LanL2DZ/6-31+g(d,p) level (MD: carbon atoms are shown in cyan, nitrogen atoms – in blue, mercury ion – in orange, DFT: Carbon atoms are shown in green, nitrogen atoms – in yellow, mercury ion – in grey, the pictures were generated using PyMol [139]). Only the backbones of PEI were taken into account during alignment (H atoms were discarded).

3.5. Validation of obtained structures via density functional theory

Samples of the complexes, with and without explicit water molecules in the first coordination shell of mercury, were extracted from the MD simulations for further analysis (Fig. 3.4). It was observed that longer PEI chains, particularly in the PEI-10 complex with one mercury ion-where the dangling segment exhibits significant motion (Fig. 3.4c)-show greater conformational variability. However, a single conformation was used as a representative sample for each system, as the first coordination shells of mercury remained largely unchanged throughout most of the simulation time. The stability of the complex samples was evaluated using DFT calculations performed with the Gaussian 16, Revision C.01 software. Specifically, the complexes underwent geometry optimizations using various levels of theory (M05-2X/LanL2DZ, M06-2X/LanL2DZ, and M06-2X/LanL2DZ/6-31+G(d,p)), and the Gibbs free energy of complexation values were calculated to assess whether the complexes were thermodynamically favourable (Table 3.2). The Minnesota functional M06-2X and, for comparison, its earlier version M05-2X [140] were used in the calculations, as both are known for their reliable performance and sufficient accuracy in systems containing transition metals. For mercury ions, the LANL2DZ relativistic pseudopotential [141] was used as the basis set, as it is known to perform well for

transition metal complexes [142]. For the other atoms (in PEI and water), the 6-31+G(d,p) basis set was used, although in some calculations the LANL2DZ basis set was applied to all atoms. A similar combination of methods (M06-2X/LANL2DZ/6-31+G(d,p)) has been successfully used as a DFT level of theory, for example by Costa et al. [143]. The aqueous environment was modelled using the solvent-consistent reaction field (SCRF) approach with the SMD solvent model.

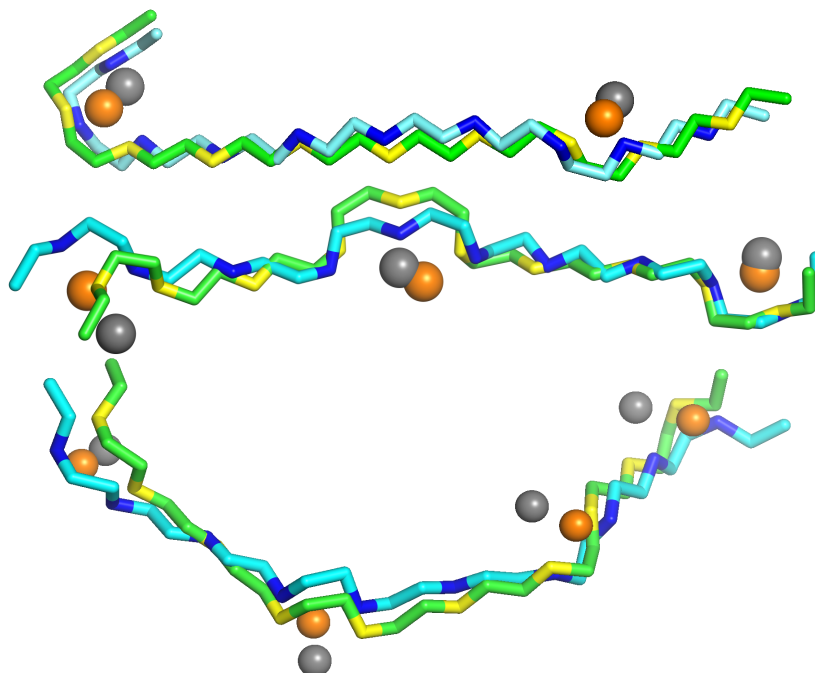


Figure 3.16. A comparison of DFT and MD conformations of PEI-10 with 2, 3 and 4 Hg^{2+} ions (same colours as in Fig. 3.15).

Table 3.2. G_{cmpx} (kJ mol^{-1}) per Hg^{2+} ion for the PEI-mercury complexes. Water is taken into account implicitly.

System		MD	Binding energy, MD	M05-2X/ LanL2DZ	M06-2X/ LanL2DZ	M06-2X/ LanL2DZ/ 6-31+g(d,p)
PEI-4	+ 1 Hg^{2+}	-58	-395 ± 15	-154	-131	-47
PEI-5	+ 1 Hg^{2+}	-112	-670 ± 21	-188	-174	-70
PEI-10	+ 1 Hg^{2+}	-91	-541 ± 58	-193	-169	-61
PEI-10	+ 2 Hg^{2+}	-51	-333 ± 12	-117	-97	-38
PEI-10	+ 3 Hg^{2+}	-40	-258 ± 8	-99	-82	-30
PEI-10	+ 4 Hg^{2+}	-29	-256 ± 8	-80	-65	-21

The alignment of DFT-optimized structures with the initial MD geometries is shown in Figs. 3.15–3.16. While the DFT geometry optimizations refined the structures, they did not alter the coordination numbers of the complexes. The distances in the optimized structures containing water molecules in the first hydration shells of mercury, computed at the M06-2X/LANL2DZ/6-31+G(d,p) level, are summarized in Table 3.3. The optimization resulted in an increase in ion-nitrogen distances compared to those observed in MD, from approximately 2.14 Å to 2.5 Å, and in ion-oxygen (water) distances from around 2.1 Å to 2.55 Å.

Table 3.3. The Hg-N and Hg-O distances (Å) in the PEI-mercury-water complexes optimized at the M06-2X/LANL2DZ/6-31+G(d,p) level, where the oxygen atoms belong to water molecules in the first hydration shell of mercury.

Complex	Hg ²⁺	N1	N2	N3	N4	N5	O ₁	O ₂	O ₃	O ₄
PEI-4 + 1 Hg ²⁺	1	2.49	2.51	2.57			2.55	2.66	2.7	
PEI-5 + 1 Hg ²⁺	1	2.48	2.51	2.53	2.56	2.65	3.19			
PEI-10 + 1 Hg ²⁺	1	2.47	2.49	2.55	2.57		2.6	2.73		
PEI-10 + 2 Hg ²⁺	1	2.49	2.49	2.57			2.59	2.6	2.65	
	2	2.48	2.51				2.53	2.54	2.55	2.56
PEI-10 + 3 Hg ²⁺	1	2.5	2.54				2.53	2.54	2.56	2.56
	2	2.49	2.57				2.5	2.55	2.56	2.56
	3	2.52	2.53				2.51	2.54	2.56	2.59
PEI-10 + 4 Hg ²⁺	1	2.51	2.55				2.48	2.49	2.5	2.63
	2	2.5	2.52				2.48	2.54	2.54	2.62
	3	2.49	2.54				2.51	2.55	2.55	2.72
	4	2.49	2.52				2.55	2.59	2.59	2.61

For all systems and levels of theory used, the Gibbs free energy of complexation values were calculated using the expression $G_{\text{cmpx}} = G(\text{PEI} + n\text{Hg}^{2+}) - G(\text{PEI}) - nG(\text{Hg}^{2+})$, where G is the sum of electronic and thermal free energy values at $T = 298.15$ K, n is the number of mercury ions. For the calculation of $G(\text{PEI})$, the geometry of the PEI molecule was first optimized using the corresponding level of theory. The initial PEI structure for optimization was taken from an additional MD simulation of a free PEI polymer. Negative values of G_{cmpx} indicate that com-

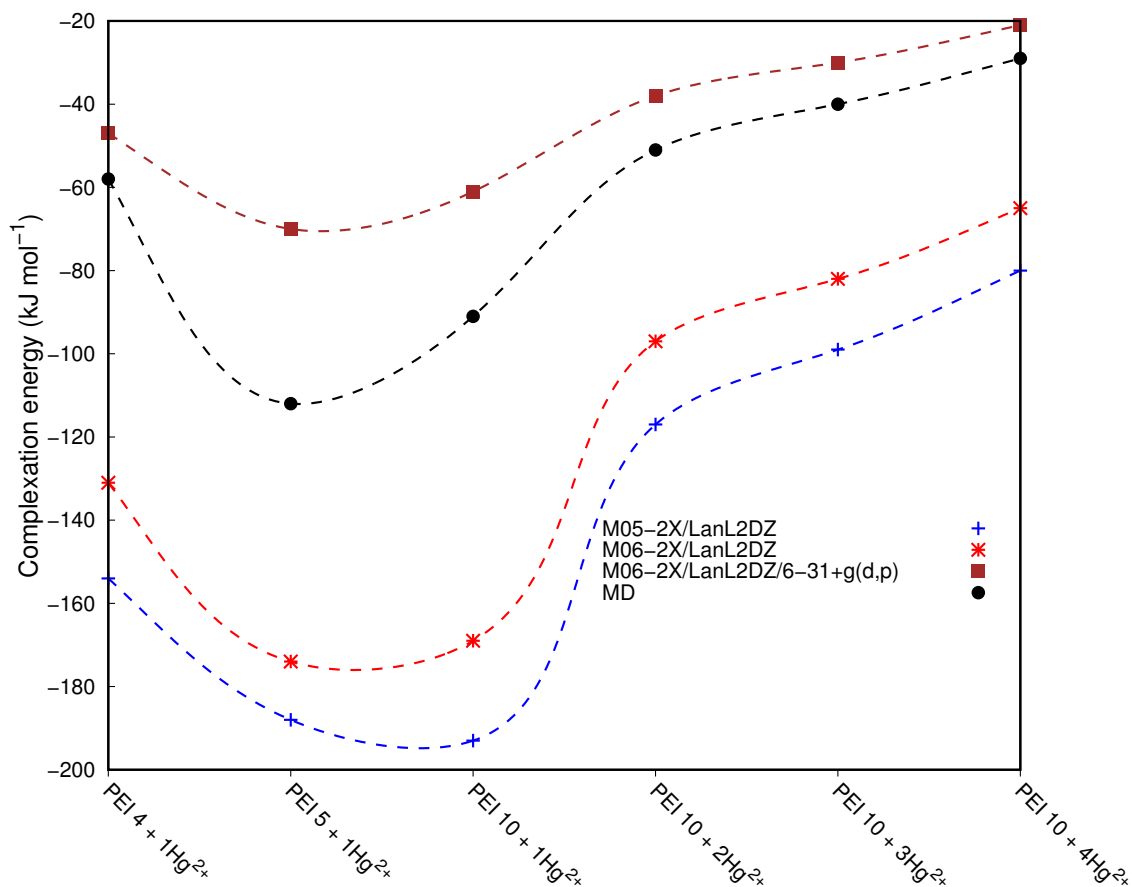


Figure 3.17. Complexation energy values per Hg^{2+} ion (see Table 3.2). Water is taken into account implicitly. The dashed lines are visual guides.

plexation was thermodynamically favourable for all studied cases (Table 3.2). The results show stronger binding of a single ion in the case of PEI-5 compared to PEI-4 for all computational levels (Fig. 3.17), and compared to all other samples when using computational levels based on the M06-2X functional. This can be explained by the highest Hg-N coordination number for the Hg^{2+} -PEI-5 complex. With an increasing number of mercury ions in the system, the complexation energy becomes less negative, indicating weaker binding.

3.6. Conclusions

The chelation of divalent mercury ions (Hg^{2+}) by polyethyleneimine (PEI) chains of varying lengths (PEI-4, PEI-5, and PEI-10) was investigated using a combined approach of classical molecular dynamics (MD) simulations and quantum

chemical calculations based on density functional theory (DFT). The MD simulations revealed that the number of nitrogen atoms involved in chelation increases with the polymer length. However, the coordination number tends to saturate due to steric effects and chain flexibility. Among the systems studied, PEI-5 consistently exhibited the most efficient chelation of a single Hg^{2+} ion, forming a compact and stable complex structure.

Water molecules were observed to coexist with polymer nitrogen atoms in the first coordination shell of Hg^{2+} . As more nitrogen atoms participated in the complexation, the number of coordinated water molecules decreased, indicating a competitive relationship between the polymer and solvent molecules for binding to the metal ion. This highlights the significance of solvent effects when evaluating chelating performance in aqueous environments.

A thermodynamic analysis based on adsorption energy values computed from MD simulations indicated favourable complexation in all cases, with PEI-5 again showing the strongest interaction. An increase in the number of coordinated Hg^{2+} ions led to a gradual decrease in the binding strength per ion, which was attributed to electrostatic repulsion between the ions and reduced flexibility of the polymer backbone.

These trends were further confirmed by DFT calculations. Geometry optimisations and free energy analyses at various levels of theory showed that the coordination environment remained largely unchanged compared to the MD snapshots. All complexes exhibited negative Gibbs free energy of complexation values, supporting their thermodynamic stability. Longer chains such as PEI-10 could coordinate multiple ions, but the individual ion binding was weaker, and the polymer remained in an extended conformation due to ion-ion repulsion and the hydrophilic character of PEI.

The obtained results provide molecular-level insights into the structural and energetic aspects of mercury chelation by PEI. These findings are valuable for guiding the rational design of polymer-based chelating agents intended for heavy metal capture and environmental remediation applications.

CHAPTER 4

CHELATION OF HEAVY METAL IONS BY EDTA

4.1. Role of EDTA protonation in chelate complex formation with mercury ions

In this chapter, different protonation states of ethylenediaminetetraacetic acid (EDTA), corresponding to various pH levels, are studied with respect to their influence on the chelation strength of mercury ions. The fully deprotonated form of EDTA (EDTA^{4-}) was proven to have the strongest binding ability. In addition to Hg^{2+} , ions of other divalent heavy metals (Cd^{2+} and Pb^{2+}) complexed with EDTA^{4-} are also investigated. To verify the stability of the complexes, all-atom molecular dynamics (MD) simulations were conducted in explicit aqueous solutions, while density functional theory (DFT) calculations were performed using an implicit solvent model. Adsorption energy values were calculated to confirm the stability of the complexes and to rank them accordingly. To better understand the binding mechanisms, the charges of the metal and EDTA atoms were analysed based on the charge models employed in the DFT calculations.

4.2. Chelation of mercury ion by EDTA

4.2.1. Chelation of Hg^{2+} ions by EDTA at different protonation states in aqueous solution

The protonation state of EDTA strongly depends on the pH of the solution and plays a crucial role in its metal-binding ability. With increasing solution acidity, from

pH 14 to pH 0, a dissolved EDTA molecule undergoes protonation, transforming from its fully deprotonated form (EDTA^{4-}) to the fully protonated form (EDTA^{2+}) (Fig. 4.1) [54]. Protonated forms of EDTA containing $-\text{COOH}$ carboxyl groups ($\text{EDTA}^{[1-, \dots, 2+]}$) are predominant at very low pH values ($\text{pH} < 3$) [54], and are not considered for ion removal in this work. However, to specifically study the chelating effect of EDTA resulting from the protonation of COO^- groups, we include additional theoretical EDTA forms with unprotonated nitrogen atoms (Fig. 4.2).

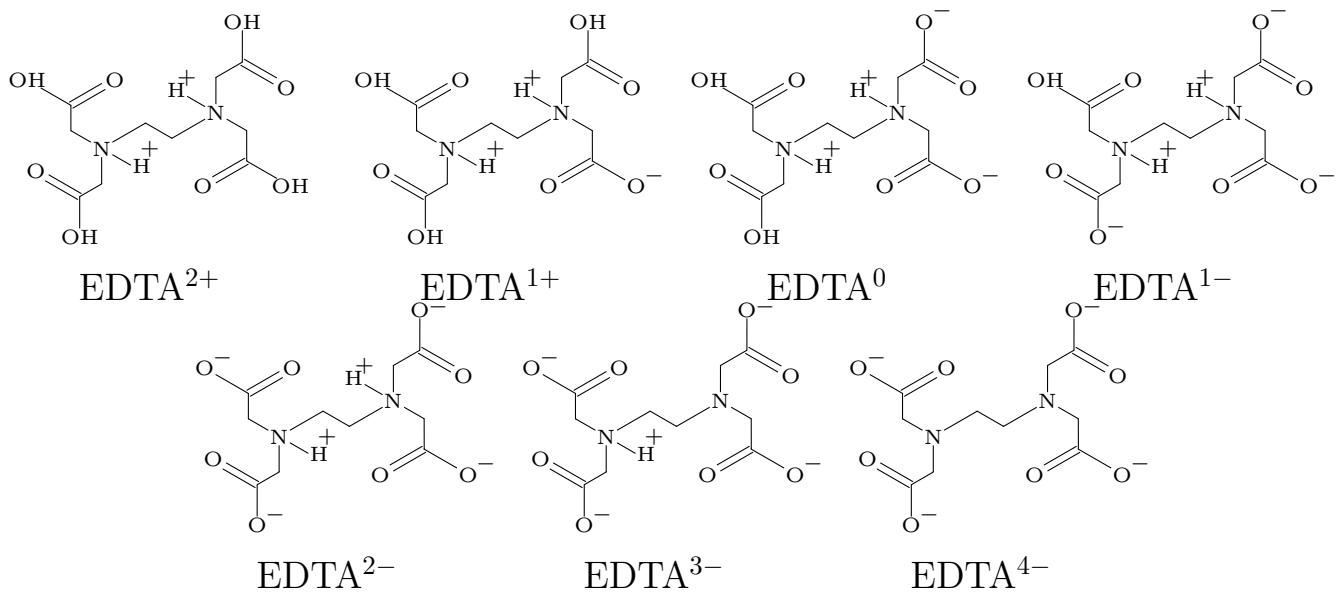


Figure 4.1. Protonation states of EDTA in an aqueous solution, ranging from the fully protonated form EDTA^{2+} to the fully deprotonated form EDTA^{4-} . Reproduced from Ref. [1] with permission from the PCCP Owner Societies.

4.2.2. Molecular dynamics simulation

To obtain the structures of EDTA-Hg^{2+} complexes with different EDTA protonation forms in water and to evaluate their stability, a series of MD simulations were performed. The OPLS/AA force field [133] and the rigid SPC/E water model [134] were used to describe the EDTA and water molecules, respectively. To model the mercury ion, Lennard-Jones parameters proposed by Babu et al. [106] were applied. The charge of the mercury ion was set to 2+, while the charges for EDTA and water molecules were assigned according to the OPLS/AA and SPC/E force field schemes,

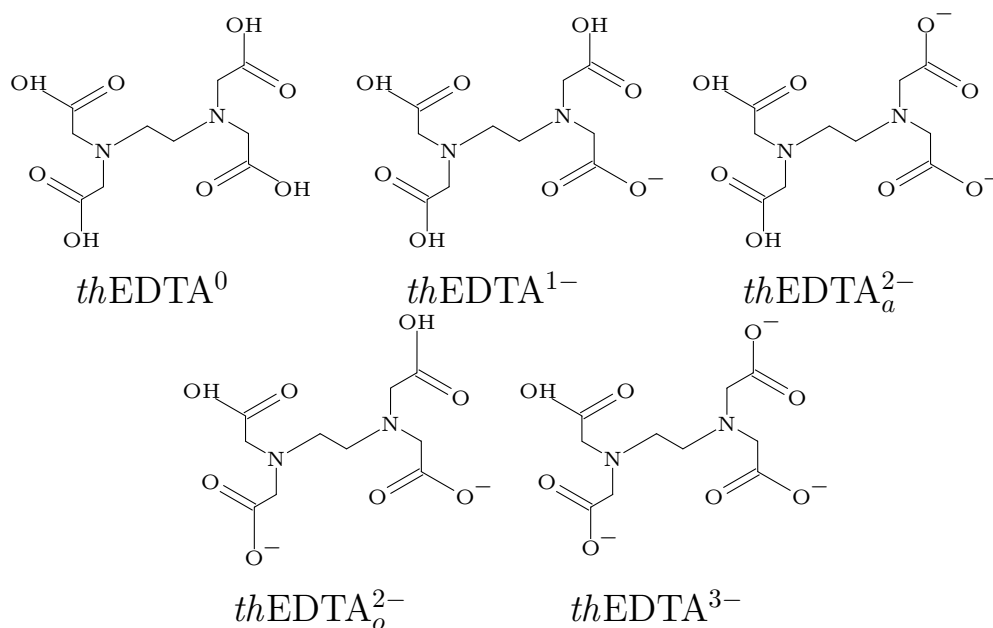


Figure 4.2. The theoretical protonation states of EDTA ($thEDTA^q$) used to isolate the effect of carboxyl group protonation while keeping the nitrogen atoms unprotonated. Reproduced from Ref. [1] with permission from the PCCP Owner Societies.

respectively, and remained fixed throughout the simulation. The OPLS/AA scheme defines partial charges based on chemical groups and atom types. For example, the partial charge of a nitrogen atom in an unprotonated tertiary amine group is -0.63 , and protonation increases it to -0.10 , thereby reducing the Coulombic interaction with Hg^{2+} . A similar effect is observed for carboxyl groups: the partial charge on the oxygen atom of a deprotonated carboxylate group (COO^-) is approximately -0.8 , whereas protonation to form a neutral carboxylic acid ($COOH$) raises this value to about -0.53 or even higher, depending on the force field. This protonation significantly reduces the ability of groups to coordinate with cations. In general, protonation of donor atoms (N and O) reduces their electron density and weakens their electrostatic attraction to metal ions, thereby diminishing the chelating capacity of the EDTA molecule. The partial charges of the EDTA molecules according to the OPLS/AA model are listed in Appendix A.

Each MD simulation involved the following steps:

1. Preparation of the initial geometry of the complex in the absence of water molecules, performed in the NVT ensemble. The Hg^{2+} ion was initially posi-

tioned near the central binding site of the EDTA molecule to facilitate complex formation.

2. Addition of water molecules and relaxation of the system in the NPT ensemble for 100 ps using a time step of 1 fs. The relaxation was carried out at 298.15 K and 1 atm. A density of 0.99 g/cm³ was achieved, which closely matches the expected density of SPC/E water under these conditions.
3. System equilibration for 3 ns under the same thermodynamic conditions.
4. Final production run for data collection, lasting 5 ns.

To neutralize each system, two additional counterions were placed at a distance from the complexes. For this purpose, an elongated simulation box of dimensions 75 Å × 75 Å × 150 Å was used (Fig. 4.3). The counterions were permanently immobilized by setting the forces acting on them to zero and were positioned such that the complex remained at a distance of approximately 60 Å from them. Additionally, the position of the Hg²⁺ ion was fixed prior to the equilibration stage. After unfixing the Hg²⁺ ion, the simulation trajectories were monitored to ensure that the complex remained at a distance of approximately 50 Å from the counterions, which is sufficient to neglect any interference from them. These counterions were not modelled after any specific real ions, their sole purpose was to compensate for the non-zero total charge of the complex.

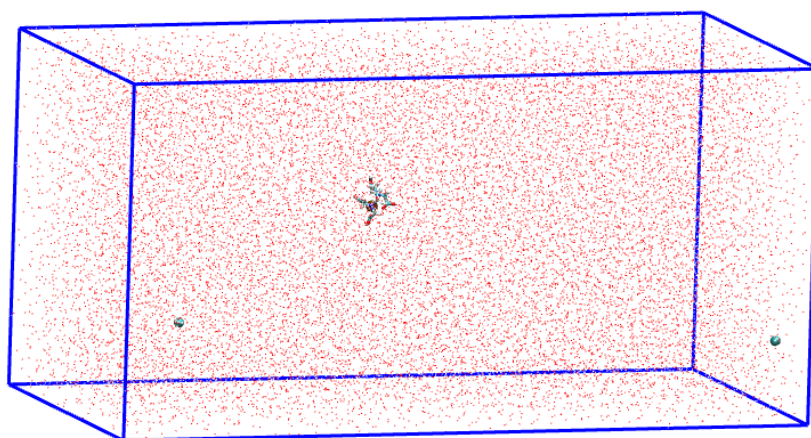


Figure 4.3. Simulation box used in molecular dynamics simulations of EDTA-Hg²⁺ complexes.

The MD simulation protocol employed for EDTA+Hg²⁺ complexes closely followed that described in Chapter 3, which focused on PEI+Hg²⁺ complexes. The simulated systems were periodic in all directions. To maintain the temperature during simulations in the NVT ensemble, the Nosé-Hoover thermostat with a relaxation parameter of 10 fs was applied. For the final stages in the NPT ensemble, the Nosé-Hoover thermostat-barostat was used, with relaxation parameters of 100 fs and 1 ps to maintain constant temperature and pressure, respectively. The remaining simulation settings were the same as those described in Section 3.2 of Chapter 3.

The simulations were carried out using the LAMMPS software package (version 2 Aug 2023) [144], and the Moltemplate package [145] was used to prepare the input files.

4.2.3. DFT calculations

In order to verify the stability of the MD-generated structures and to compute Gibbs free energy of complexation, DFT-based geometry optimizations and energy analyses were conducted. For this purpose, DFT calculations using several functionals and basis sets were performed for the EDTA-mercury complexes, with the input geometries of samples taken from tMD simulations, as well as for the individual molecules involved in the complexes. All DFT calculations were carried out using the Gaussian 16 software package (Revision C.01). Geometry optimizations were performed to obtain the lowest-energy conformers from the initial structures. To verify the quality of the optimized geometries, frequency calculations were conducted to ensure the absence of imaginary frequencies. Among the resulting vibrational frequencies there were no negative values, confirming that the geometry optimization successfully reached a true energy minimum. The singlet spin state and closed-shell configuration were chosen, which resulted in stable wavefunction convergence during the calculations. The quadratically convergent Hartree-Fock (QC-SCF) method was employed as the self-consistent field (SCF) algorithm [146]. For the thermochemical analysis, the default temperature of 298.15 K and pressure of 1 atm were used,

corresponding to the same conditions applied in the MD simulations.

The selection of functionals was guided by their established use in previous studies [84, 147–152]. The following density functionals were considered: B3LYP [153, 154], M05-2X [140], PBE [155], PBE0 [156], and ω B97X-D [157]. In addition, the hybrid functional HSE06 [158], known for its good accuracy, was also included. For the basis sets applicable to mercury and supported by Gaussian, Def2-TZVP [159, 160], Def2-TZVPP [159, 160], and LanL2DZ [161, 162] were used. For the basis sets applicable to mercury and available in the Gaussian software, the following were used: Def2-TZVP [159, 160], Def2-TZVPP [159, 160], and LanL2DZ [161, 162].

Because of their use in similar works [84, 147–152] the functionals considered were the following: including B3LYP [153, 154], M05-2X [140], PBE [155], PBE0 [156], and ω B97X-D [157], additionally the widely used for its accuracy hybrid HSE06 [158] functional was considered. These basis sets that are applicable to the mercury element and provided by the Gaussian program were used: Def2-TZVP [159, 160], Def2-TZVPP [159, 160], and LanL2DZ [161, 162]. To represent the aqueous environment implicitly, the standard solvent models SMD, CPCM, and PCM were applied.

Among the tested combinations of functionals, basis sets, and solvent models, those that provided the best geometry optimization of the experimentally determined $[\text{Hg}(\text{H}_2\text{O})\text{EDTA}]^{2-}$ structure [80] were selected. The selection criterion was the lowest root mean square displacement (RMSD) between the geometries of the optimized and the experimental structures, excluding hydrogen atoms from the comparison. These comparisons were performed using ChemCraft 1.8 software [163]. As a result, the M05-2X/LanL2DZ/SMD computational level was selected as the primary method, yielding a root mean square displacement (RMSD) value of 0.694 Å. Additional details are provided in the supplementary material of [1]. However, we do not limit our focus exclusively to this experimental structure, as it may not be the only configuration possible under the experimental pH conditions. Moreover, the $\text{Hg}(\text{H}_2\text{O})\text{EDTA}^{2-}$ complex may exhibit a coordination number of 6, rather than

the commonly reported 5 for Hg-EDTA chelation, and it is possible that the water molecule is not part of the first coordination shell of the mercury ion.

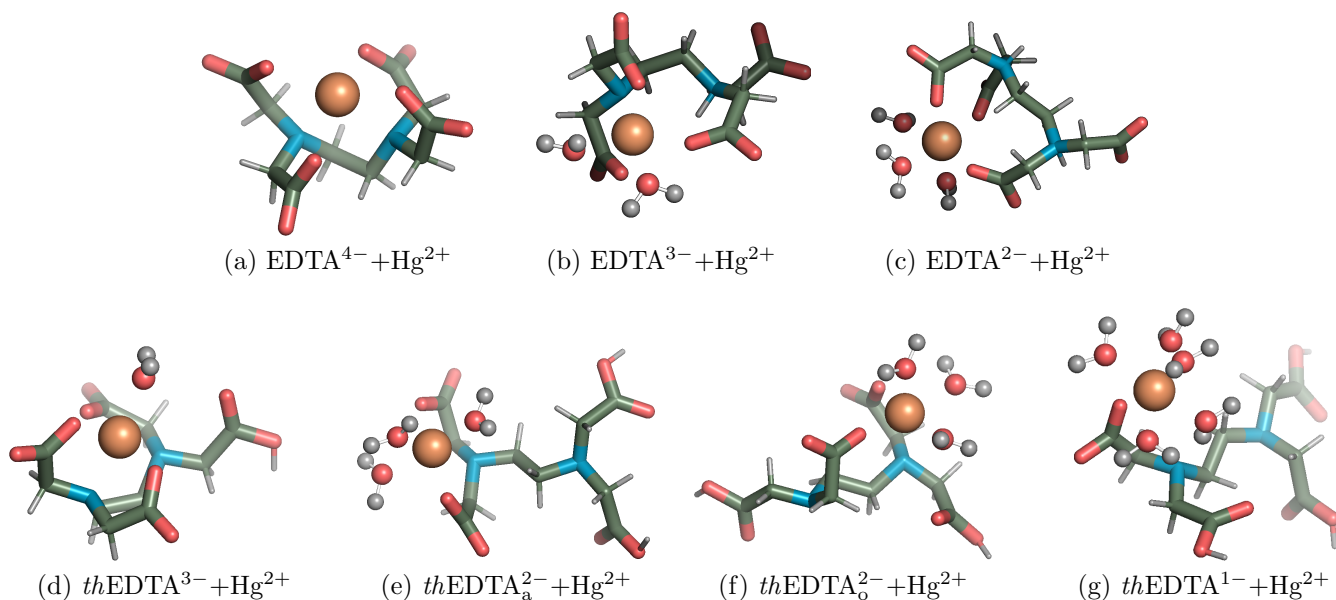


Figure 4.4. Samples of EDTA- Hg^{2+} complexes configurations from the MD simulations, including water molecules in the first coordination shells of mercury ions. The C, N, O, H and Hg atoms are coloured in green, blue, red, grey and orange, in the given order. Reproduced from Ref. [1] with permission from the PCCP Owner Societies.

4.3. EDTA+ Hg^{2+} complex formation

4.3.1. Structure

The mercury ion was considered in complex with EDTA in the following forms: (i) EDTA with unprotonated carboxylate groups, i.e., EDTA^{4-} , EDTA^{3-} , and EDTA^{2-} (Fig. 4.1); and (ii) EDTA with protonated carboxyl groups, i.e., thEDTA^0 , thEDTA^{1-} , thEDTA_a^{2-} , thEDTA_o^{2-} , and thEDTA^{3-} (Fig. 4.2). In the notation thEDTA_a^{2-} , the subscript *a* refers to a configuration in which both carboxyl groups are adjacent to a single nitrogen atom, whereas in thEDTA_o^{2-} , the subscript *o* indicates that the carboxyl groups are bonded to carbon atoms located opposite each other with respect to the two nitrogen atoms. Fig. 4.4 shows representative

snapshots from the MD simulations of these complex structures.

After the MD simulations, the radial distribution functions (RDFs), $g(r)$, and the running coordination numbers (CNs), $n(r)$, were plotted using trajectories from the final stages of the respective simulations (Figs. 4.5–4.7). In the plots, N denotes a nitrogen atom, Np indicates a protonated nitrogen atom, Oc refers to an oxygen atom from a carboxylate group, and Ow denotes an oxygen atom from a water molecule. The RDFs reveal the most probable distances between atoms in the structures, while the CN values indicate the most likely number of atoms in the first coordination shell of the Hg^{2+} ions.

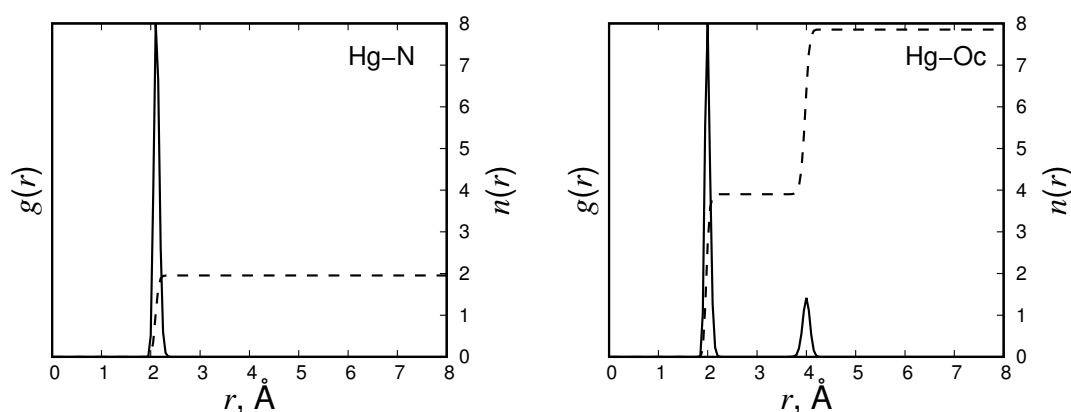


Figure 4.5. $g(r)$ (solid line) – RDFs, $n(r)$ (dashed line) – CNs obtained from the MD for the $\text{EDTA}^{4-} + \text{Hg}^{2+}$ complex. Reproduced from Ref. [1] with permission from the PCCP Owner Societies.

As shown by the coordination numbers in Fig. 4.5, the first coordination shell of mercury in the $\text{EDTA}^{4-} - \text{Hg}^{2+}$ complex consists of two nitrogen atoms and four oxygen atoms from the carboxylate groups, with no water molecules present. This coordination geometry remained stable throughout the MD simulation, as indicated by the sharp first peaks in RDFs. The typical Hg-N distance in this complex is approximately 2.1 Å, and the typical Hg-Oc distance is about 2.0 Å.

In the $\text{EDTA}^{3-} - \text{Hg}^{2+}$ complex, where one nitrogen atom is protonated, only the unprotonated nitrogen remains in the first coordination shell of mercury at a distance of approximately 2.1 Å (Fig. 4.6). This observation is consistent with findings for the protonated amine group in the $\text{EDTA}^{3-} - \text{Ln}^{3+}$ complex [82]. Only three oxygen atoms from the carboxylate groups are coordinated to the Hg^{2+} ion in

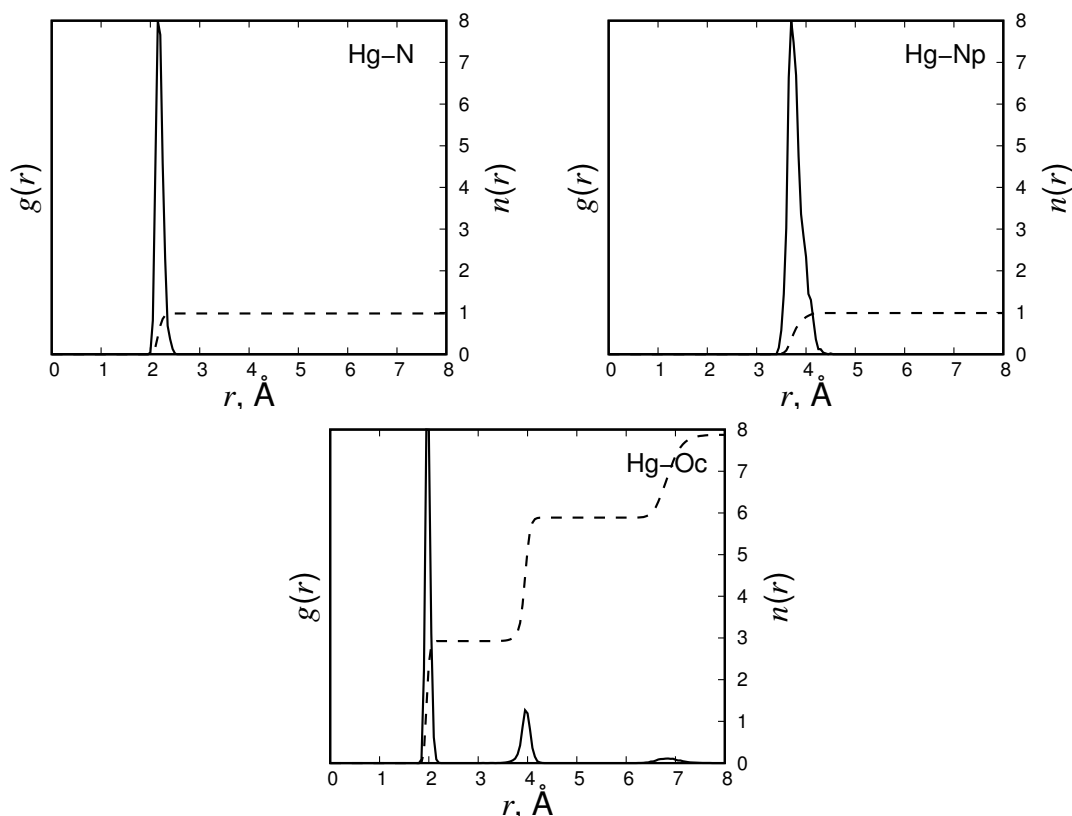


Figure 4.6. $g(r)$ (solid line) – RDFs, $n(r)$ (dashed line) – CNs obtained from the MD for the $\text{EDTA}^{3-}+\text{Hg}^{2+}$ complex. Reproduced from Ref. [1] with permission from the PCCP Owner Societies.

this case, which allows space for two water molecules to enter the first coordination shell (Fig. 4.8a).

If both nitrogen atoms are protonated, they shift out of the coordination shell, and the Hg^{2+} ion becomes coordinated by only three oxygen atoms (Fig. 4.7). As a result, three water molecules enter the first coordination shell of Hg^{2+} (Fig. 4.8b). In both cases, protonation of the nitrogen atoms reduces the ability of the EDTA molecule to coordinate the mercury ion. Regarding the $\text{thEDTA}^{1-}-\text{Hg}^{2+}$ complex, only the oxygen atom of the unprotonated COO^- group remained in the first coordination shell of the mercury ion. Although the complex was stable during the MD simulations and was later shown to be favourable according to DFT calculations, it does not contain a ring structure and therefore should not formally be classified as a chelated complex. The $\text{thEDTA}^0-\text{Hg}^{2+}$ complex, initially prepared at an early stage of the simulation, was found to be unstable and ceased to exist in the later stages.

Thus, the highest coordination number is observed in the $\text{EDTA}^{4-}\text{-Hg}^{2+}$ complex, and it decreases with increasing protonation of the EDTA molecule (of N and Oc atoms), shifting the coordination environment towards dechelation and increased hydration of mercury, as seen in the case of the $\text{thEDTA}^0\text{-Hg}^{2+}$ complex.

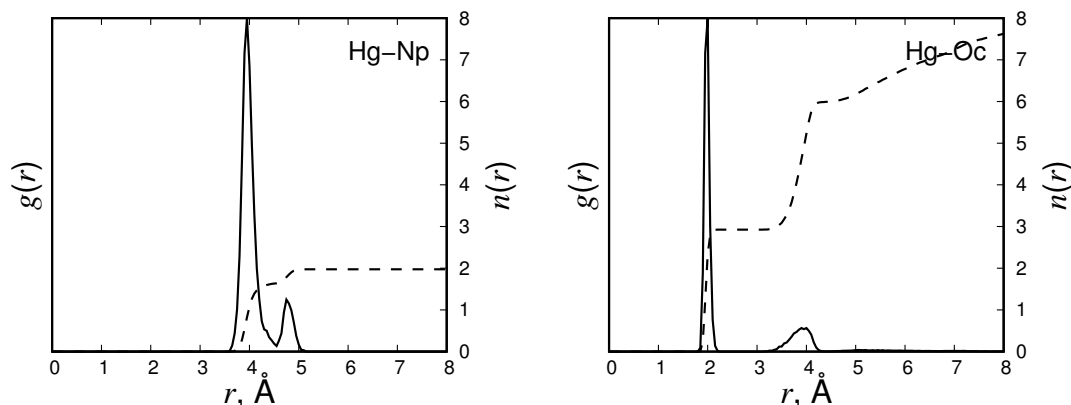


Figure 4.7. $g(r)$ (solid line) – RDFs, $n(r)$ (dashed line) – CNs obtained from the MD for the $\text{EDTA}^{2-}\text{-Hg}^{2+}$ complex. Reproduced from Ref. [1] with permission from the PCCP Owner Societies.

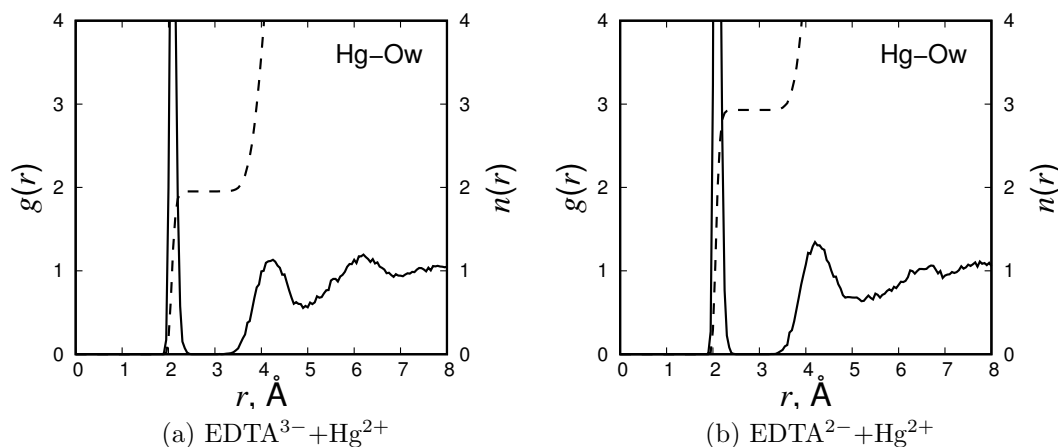


Figure 4.8. $g(r)$ (solid line) – RDFs, $n(r)$ (dashed line) – CNs obtained from the MD for the mercury ion and oxygen ions of the water molecules. Reproduced from Ref. [1] with permission from the PCCP Owner Societies.

4.3.2. Energy

It is of particular interest to examine the Gibbs free energy of complexation values for different protonation states of EDTA and varying hydration levels of the

mercury ion, and to compare the resulting energy trends with the adsorption energy values obtained from MD simulations. DFT offers a more fine-grained representation of molecular systems compared to MD and thus serves as a valuable tool for refining the geometries of structures obtained from MD simulations (Fig. 4.4). It is sufficient to investigate only the selected sample structures, as the conformational variety observed during the MD simulations was not significant enough to justify considering multiple samples for the same complex type. This is supported by the sharp peaks in the RDFs (Figs. 4.5-4.8) and the consistent coordination numbers observed in the final stages of the simulations. Complex formation was considered as a process of producing a (hydrated) product, $\text{EDTA}^{2-}\text{-Hg}^{2+}\text{-}n\text{H}_2\text{O}$, from the following input reactants: EDTA^{4-} , Hg^{2+} , and n water molecules, where n corresponds to the number of water molecules in the cation's first hydration shell observed in the MD simulations. The geometries of both the resulting complex and the individual molecules were first optimized in an implicit water environment, with the n water molecules treated as separate molecules rather than as a single n -molecule cluster. Then, the validity of the results was verified, and the zero-point and thermal energy sums were extracted. Based on these values, the Gibbs free energy of complexation values (in implicit solvent), G_{cmpx} , were calculated as the difference between the energy of the product and the sum of the energy values of the molecules: $G_{\text{cmpx}} = G(\text{EDTA}^{2-} + \text{Hg}^{2+} + n\text{H}_2\text{O}) - G(\text{EDTA}^{4-}) - G(\text{Hg}^{2+}) - nG(\text{H}_2\text{O})$. This scheme is similar to the thermodynamic integration approach for a given ligand and varying Ln^{3+} ions as described by O'Brien et al. [82]. The values of G_{cmpx} for the different computational levels are listed in Table 4.1.

To compare the stability of complexes with EDTA at different protonation levels in the scope of MD, the adsorption energy values were calculated from MD simulations (Table 4.1). To obtain these values, the potential energy of the dechelated Hg^{2+} ion was subtracted from the potential energy of the chelated Hg^{2+} . The energy of the dechelated Hg^{2+} ion was obtained from a separate MD simulation, following the procedure described above. In MD, the potential energy of an ion includes both Lennard-Jones (LJ) and Coulombic interaction contributions.

As shown by the DFT calculations, for both the real and theoretical protonation schemes and across all considered computational levels, the Gibbs free energy of complexation values increase with the number of hydrogen atoms in the EDTA structure (Table 4.1). The minimum values of complexation free energy correspond to the fully deprotonated form, confirming that EDTA^{4-} is the most effective protonation state for mercury ion chelation, and that the $\text{EDTA}^{4-}\text{-Hg}^{2+}$ complex is the most stable in an aqueous solution. This finding is in good agreement with other results demonstrating the high stability of deprotonated EDTA complexes with various metal ions such as Ce, Nd, La, and Pr [164], and lanthanides in general [82].

Complexes with an adjacent protonation type ($\text{thEDTA}_a^{2-}\text{-Hg}^{2+}$) exhibit slightly lower G_{cmpx} values than those with the opposite protonation type ($\text{thEDTA}_o^{2-}\text{-Hg}^{2+}$). Except for the theoretical $\text{thEDTA}^{1-} + \text{Hg}^{2+}$ complex analysed using the $\omega\text{B97X-D/Def2-TZVPP}$ level of theory, all complexations were thermodynamically favourable in water.

While the partial charges of the EDTA molecule species and the mercury ion remained fixed during the MD simulations, in reality, spatial configurations significantly affect the charge distribution. To investigate how the protonation state of EDTA and its complexation with Hg^{2+} influence this distribution, an alternative charge scheme was applied within the framework of DFT. Specifically, the atomic polar tensor (APT) charge model was used to calculate the partial charges of mercury, EDTA, and water molecules both within and outside the complexes. As can be seen from the charge distributions of uncomplexed and complexed EDTA with the mercury ion (Appendix B), both protonation and complexation significantly influence the atomic partial charges. The extent of this influence on individual atoms depends on the molecular conformation and the distance between the atom and the newly introduced charge, either a proton or a chelated metal ion. The charge redistribution is particularly pronounced for the Hg atom and the oxygen and nitrogen atoms of the EDTA molecule before and after chelation occurs. The negatively charged oxygen atoms in the deprotonated carboxylate groups (COO^-) exhibit a greater magnitude of negative charge compared to those in the protonated

carboxylic acid groups (COOH). This stronger negative charge enhances their ability to bind the oppositely charged mercury cation, whereas the presence of a proton in the carboxyl group reduces this binding affinity by diminishing the negative character of the oxygen atoms. Nitrogen atoms also carry negative charges, which are stronger in the absence of a bound proton. As a result, nitrogen atoms coordinate with the cation in the deprotonated state, whereas protonation causes them to move out of the cation's first coordination shell. In the absence of protonation, 4 oxygen atoms and 2 nitrogen atoms can form a 6-coordinated complex with the mercury ion, as confirmed by both DFT optimizations and MD simulations.

In the case of protonated nitrogen in EDTA^{3-} , the absolute value of its negative charge is reduced, and the proton effectively screens the Coulombic attraction to the mercury ion, leading to the absence of bonding between the ion and the nitrogen atom. This dissociation was observed during the MD simulations, along with the detachment of one oxygen atom from the original four possible coordination sites. The OPLS model correctly accounted for the charge redistribution due to nitrogen protonation, and the resulting structure was validated through DFT calculations. According to the APT charge model, the mercury cation gains an additional positive charge upon complexation, while the interacting atoms in its first coordination shell become more negatively charged. Although charge models are typically not directly comparable or transferable, they are employed here primarily for relative comparisons within the same model. Kovács et al. observed a reduction in the positive charge of metal cations due to partial electron transfer from the coordinating atoms of EDTA [78]. Additionally, charge transfer occurs between the oxygen atoms of EDTA and the hydrogen atoms of water molecules involved in hydrogen bonding, contributing to charge redistribution during complexation.

A similar picture is observed for the EDTA^{2+} complex with the mercury ion, where both protonated nitrogen atoms are unable to participate in coordination with mercury. As the distance between the cation and the nitrogen atoms increases, the presence of 4 coordinating oxygen atoms would impose considerable strain on the complex. As a result, some oxygen atoms are forced to leave the coordination shell.

After DFT geometry optimization, as many as three oxygen atoms were excluded from the first coordination shell, whereas during the MD simulations only one oxygen atom dissociated, with water molecules entering the shell instead.

Speaking of the carboxyl groups in the theoretical EDTA series, the protonated oxygen atoms significantly lose their negative charge, along with their ability to bind the metal cation. Additionally, the positive charge on the carbon atom bonded to the oxygen slightly decreases, while the negative charge on the adjacent nitrogen atom also becomes weaker. As a result, the cation is less attracted to the EDTA chelation centres, the coordination number decreases, and the chelation stability diminishes with each successive protonation step.

To conclude, the charge analysis of EDTA protonation states provides valuable insight into why chelation is more effective in the case of deprotonated EDTA. For both nitrogen and oxygen binding sites of the chelating agent, deprotonation results in stronger negative charges, thereby enhancing electrostatic interactions with the positively charged ion.

Table 4.1. Adsorption energy values for Hg^{2+} -EDTA complexes at different states of EDTA protonation, in kJ mol^{-1} . Reproduced from Ref. [1] with permission from the PCCP Owner Societies.

Complex	G_{cmpx} calculated at different levels of theory			
	MD	B3LYP/ LanL2DZ	M05-2X/ LanL2DZ	ω B97X-D/ Def2-TZVPP
$\text{EDTA}^{4-} + \text{Hg}^{2+}$	-181.7 ± 33.2	-197.2	-223.6	-247.5
$\text{EDTA}^{3-} + \text{Hg}^{2+}$	-112.1 ± 33.7	-72.8	-100.4	-108.9
$\text{EDTA}^{2-} + \text{Hg}^{2+}$	-141.9 ± 35.8	-45.3	-92.6	-70.5
$\text{thEDTA}^{3-} + \text{Hg}^{2+}$	-115.4 ± 32.8	-143.5	-185.6	-191.1
$\text{thEDTA}_{\text{a}}^{2-} + \text{Hg}^{2+}$	-82.4 ± 33.1	-81.0	-82.5	-89.6
$\text{thEDTA}_{\text{o}}^{2-} + \text{Hg}^{2+}$	-77.6 ± 35.5	-60.3	-61.6	-88.3
$\text{thEDTA}^{1-} + \text{Hg}^{2+}$	-63.4 ± 35.3	-10.0	-18.6	26.6

4.4. Chelate complexes of fully deprotonated EDTA with heavy metal ions

In addition to mercury, other divalent heavy metals such as lead (Pb^{2+}) and cadmium (Cd^{2+}) are of significant environmental and biological concern due to their high toxicity [49]. This section focuses on the chelation of Pb^{2+} and Cd^{2+} ions by EDTA, providing a comparative analysis of their binding behaviour and complex stability.

From pH 5 to 12, nearly all Cd-EDTA complexes exist in the form CdEDTA^{2-} [89], and similarly, HgEDTA^{2-} is the predominant species between pH 5 and 9 [80], while PbEDTA^{2-} dominates in the pH range from 5 to 13 [90]. As the pH decreases below these ranges, the predominant complex species begin to protonate, forming species such as MH_3EDTA^+ (e.g., $\text{PbH}_3\text{EDTA}^+$), and eventually dissociate around pH 2. Conversely, at higher pH values, the MEDTA^{2-} complexes begin to bind hydroxide ions from the strongly alkaline solution, forming MOHEDTA^{3-} complexes [80, 89, 90]. The ionic form of metals is not the only possible one. Depending on factors such as the pH and temperature, other forms like hydroxides or oxides may also be present [54]. Only complexes with fully deprotonated EDTA (EDTA^{4-}) were considered in this section, since this form is known to exhibit the highest chelation efficiency and stability due to its maximal number of available coordination sites (see Fig. 4.9).

4.4.1. Molecular dynamics simulation and structure of complexes

As in the simulations described in Subsection 4.2.2, a similar MD simulation protocol was employed for this case. The main differences lie in the choice of Lennard-Jones (LJ) parameters and the application of arithmetic mixing rules: $\epsilon_{ij} = \sqrt{\epsilon_i \epsilon_j}$ and $\sigma_{ij} = \frac{1}{2}(\sigma_i + \sigma_j)$, as proposed by Li et al. [98]. The sets of LJ parameters developed in that work were used for the metal ions under study. Specifically, three parameter sets were considered: the IOD set (designed to reproduce experimental ion-oxygen distances), the HFE set (calibrated to match hydration free en-

ergy values), and the CM set (optimized to simultaneously reproduce coordination numbers and hydration free energy values for divalent ions in aqueous solutions) [98]. To obtain data on the geometry of the complexes, molecular dynamics (MD) simulations of EDTA complexes with Cd^{2+} and Hg^{2+} ions were performed using the IOD parameter set. Since the parameters for Pb^{2+} were not available in the IOD set, the CM parameter set was used for Pb^{2+} instead. To obtain energy-related data, simulations for the metal ions were carried out using the HFE parameter set. In all cases, the LJ parameters were chosen to be compatible with the SPC/E water model.

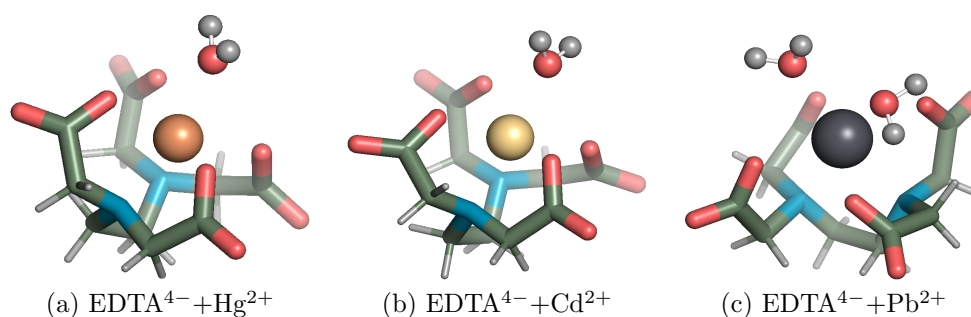


Figure 4.9. Sample configurations of $\text{EDTA}^{4-}\text{-M}^{2+}$ complexes from the MD simulations, including water molecules in the first coordination shells of the metal ions (M^{2+}). The C, N, O, H, Hg, Cd and Pb atoms are coloured green, blue, red, grey, orange, yellow, and black, respectively. The IOD parameter set was used for Cd^{2+} and Hg^{2+} , while the CM set was applied for Pb^{2+} .

After the MD simulations, radial distribution functions, $g(r)$ (RDFs), and running coordination numbers, $n(r)$ (CNs), were plotted using the trajectories from the final stages of the respective simulations (Figs. 4.10-4.12). As the coordination numbers show, the first coordination shells of all metal ions in the complexes contain two nitrogen atoms and four oxygen atoms. While the $\text{EDTA}^{4-}\text{-Hg}^{2+}$ complex in the previous subsection contained no water molecules, a single water molecule is coordinated to mercury at a distance of approximately 2.33 Å when using the IOD Lennard-Jones (LJ) parameters for Hg^{2+} described above (see Fig. 4.9a and the Hg-Ow coordination number in Fig. 4.10). Previously, in the same complex, the typical Hg-N and Hg-Oc distances were ~ 2.1 Å and ~ 2.0 Å, respectively, while they now

increase to 2.37 Å and ~ 2.25 Å. This change can be explained by the larger size of Hg ion defined by the LJ parameter set by Li et al. [98] (~ 3.15 Å) compared to the one proposed by Babu et al. [106] (~ 2.65 Å).

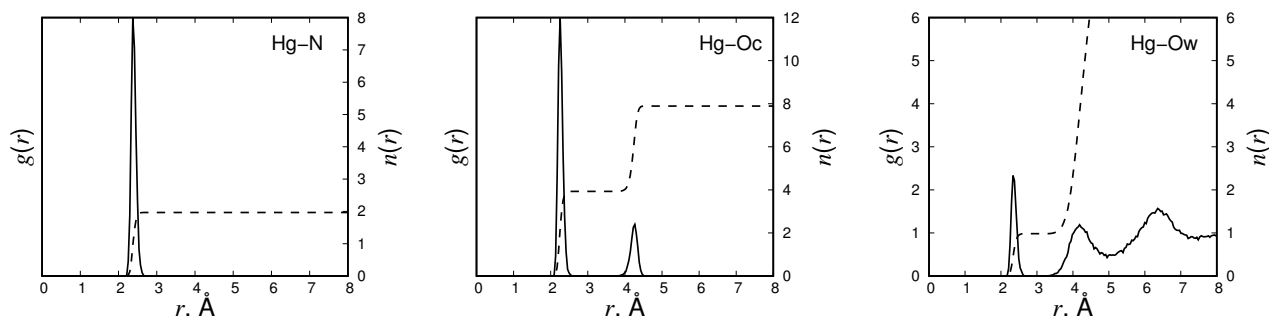


Figure 4.10. $g(r)$ (solid line) – RDFs, $n(r)$ (dashed line) – CNs obtained from the MD for the $\text{EDTA}^{4-} + \text{Hg}^{2+}$ complex. IOD parameters set was used for the MD.

Similarly, in the simulation using IOD parameters, the Cd atom in the $\text{EDTA}^{4-} - \text{Cd}^{2+}$ complex is coordinated by a single water molecule at a distance of approximately 2.29 Å (see Fig. 4.9b and the Cd-Ow coordination number in Fig. 4.11), two nitrogen atoms at a distance of ~ 2.33 Å, and four oxygen atoms at ~ 2.17 Å (Fig. 4.11).

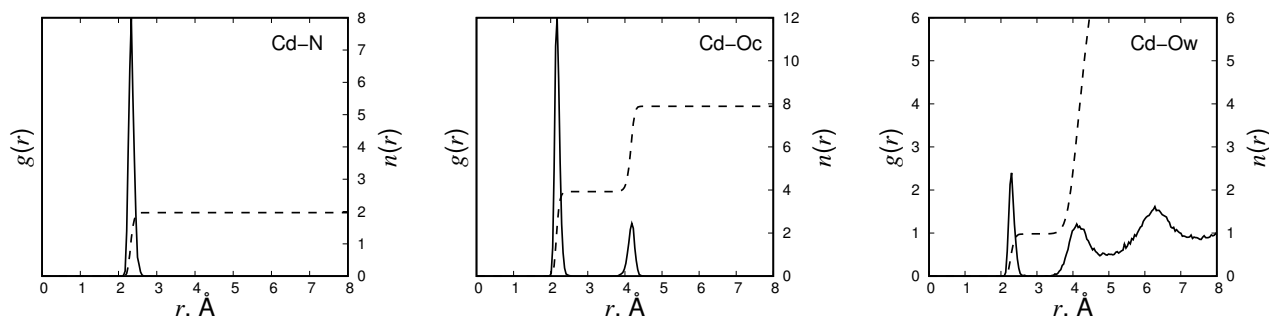


Figure 4.11. $g(r)$ (solid line) – RDFs, $n(r)$ (dashed line) – CNs obtained from the MD for the $\text{EDTA}^{4-} + \text{Cd}^{2+}$ complex. IOD parameters set was used for the MD.

In the $\text{EDTA}^{4-} - \text{Pb}^{2+}$ complex, the distances between the Pb atom and the nitrogen atoms are approximately 2.58 Å, and the Pb-O distances are ~ 2.46 Å (see Fig. 4.9c). However, for the $\text{EDTA}^{4-} - \text{Pb}^{2+}$ chelate studied using the CM parameter set, nearly two water molecules enter the first coordination shell of Pb^{2+} , located at ~ 2.57 Å from the ion (see Fig. 4.9c and the Pb-Ow coordination number in

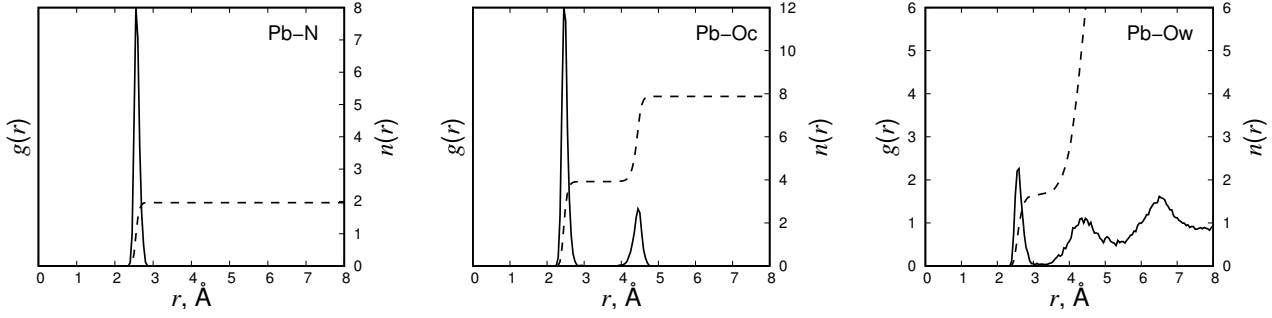


Figure 4.12. $g(r)$ (solid line) – RDFs, $n(r)$ (dashed line) – CNs obtained from the MD for the $\text{EDTA}^{4-} + \text{Pb}^{2+}$ complex. CM parameters set was used for the MD.

Fig. 4.12). It is worth noting, however, that when using the HFE parameter set for Pb^{2+} , the Pb-Ow coordination number is also 1, which can be attributed to the smaller Pb-Pb equilibrium distance in the HFE set compared to the CM set (3.22 Å vs. 3.46 Å). It is common for one or even more water molecules to enter the first coordination shell of a fully coordinated EDTA complex, especially for larger metal ions (see, e.g., [54]).

4.4.2. Adsorption energy

To determine which of the aforementioned heavy metals is most strongly bound by deprotonated EDTA, experimental data can be used. Additionally, it is of interest to rank the metals according to their adsorption energy values and Gibbs free energy of complexation values (G_{cmpx}) obtained from the MD simulations and DFT calculations, respectively.

The strength of chelation of a divalent metal ion M by EDTA is characterised by the (absolute) formation or stability constant K , defined as $K = \frac{[\text{MEDTA}^{2-}]}{[\text{M}^{2+}][\text{EDTA}^{4-}]}$, where $[\text{MEDTA}^{2-}]$, $[\text{M}^{2+}]$, and $[\text{EDTA}^{4-}]$ denote the concentrations of the EDTA^{4-} - M^{2+} complex, the unbound metal ion, and the free EDTA^{4-} ligand in a solution, respectively [54].

The Van't Hoff equation relates the stability constant to the Gibbs free energy: $\Delta G = -RT \ln 10 \cdot \log K$, where R , T , and K denote the universal gas constant, temperature, and the formation constant, respectively. The experimental stability

constants [54] for the complexes of interest, along with the corresponding G_{cmpx} values, are listed in Table 4.2.

Table 4.2. The experimental stability constants for $\text{EDTA}^{4-} + \text{M}^{2+}$ [54], and the corresponding values of G_{cmpx} derived from them, at a temperature of $T = 298.15$ K and an ionic strength of 0.1 M.

Complex	Experimental G_{cmpx}	Experimental $\lg\beta$
$\text{Hg}^{2+} + \text{EDTA}^{4-}$	-122.7	21.5
$\text{Cd}^{2+} + \text{EDTA}^{4-}$	-94.1	16.5
$\text{Pb}^{2+} + \text{EDTA}^{4-}$	-102.7	18.0

To calculate the adsorption energy values, the differences between the potential energy values of the metal ions in their complexed and uncomplexed states were evaluated. The results are presented in Table 4.3. The adsorption energy values for Hg and Cd are similar, while the value for Pb is significantly higher. These values show a clear correlation with the hydration free energy values (HFEs) of the respective ions (-420.7 kcal/mol for Hg^{2+} , -419.5 kcal/mol for Cd^{2+} , and -340.6 kcal/mol for Pb^{2+}) [98], which is not surprising, as the LJ parameters employed were developed specifically to reproduce these HFEs. Without considering additional factors, lower (i.e., more negative) hydration free energy would suggest that the water substitution reaction proceeds more smoothly. However, the experimental stability constants (Table 4.2) follow a different trend, indicating that other factors influence complex formation. This discrepancy highlights the need for further investigation using complementary approaches.

Table 4.3. Adsorption free energy values obtained from the MD and G_{cmpx} from DFT and the experimental data, kJ/mol.

Complex	MD	PBE1PBE/def2tzvp	water shell	Experimentally deduced
$\text{Hg}^{2+} + \text{EDTA}^{4-}$	-195	-251	-285	-122.7
$\text{Cd}^{2+} + \text{EDTA}^{4-}$	-200	-230.6	-257	-94.1
$\text{Pb}^{2+} + \text{EDTA}^{4-}$	-98	-327.9	-328.4	-102.7

DFT is capable of describing single molecules and complexes more accurately than MD. However, the accuracy of DFT calculations strongly depends on the cho-

sen level of theory. It is essential to identify a computational level that is applicable to complexes involving all studied metal ions, particularly considering the complications introduced by relativistic effects. In Subsection 4.2.3, the main computational level employed for calculations involving EDTA–mercury complexes was M05-2X/LanL2DZ/SMD. However, the LanL2DZ basis set proved to be unsuitable for Pb, as the computed energy of a single Pb^{2+} ion was anomalously high in comparison to other heavy metal ions and even to Be^{2+} , which is the lightest among divalent ions. Specifically, at the M05-2X/LanL2DZ/SMD level, the sum of electronic and zero-point energy values for Pb^{2+} was -2.19 Hartree, whereas for Hg^{2+} , Cd^{2+} , and Be^{2+} the respective values were -42.31 Hartree, -47.63 Hartree, and -14.38 Hartree. Changing the functional to M06-2X, B3LYP, or performing the calculations in the gas phase did not resolve this discrepancy. Hence, the LanL2DZ basis set was discarded for Pb. The Def2-TZVP [159, 160] basis set was found to be suitable and the employed DFT level was PBE1PBE/Def2-TZVP/SMD with the GD3 empirical dispersion, with the temperature set to 298.15 K. Singlet spin state and closed-shell options were used in all calculations.

First of all, the geometries of the products and reactants involved in the chelation reactions were optimized using the computational level described above. A notable deviation from the MD-obtained geometry was observed in the case of lead (Pb). The structure of the $\text{Pb}^{2+} + \text{EDTA}^{4-}$ complex taken after MD simulations with the CM parameter set contained two water molecules in the first coordination shell, while the one obtained using the HFE parameter set contained one water molecule. However, during DFT geometry optimizations, these water molecules were pushed significantly farther from the Pb atom, reaching distances of approximately $3.74 \text{ \AA}$, which is substantially greater than the corresponding distances observed for other metals. Since the water molecules were displaced far from the Pb^{2+} ion during geometry optimization, they were excluded from the final structure. The $\text{Pb}^{2+} + \text{EDTA}^{4-}$ complex was re-optimised without water for subsequent calculations. In contrast, one water molecule remained coordinated to each of the other metal ions in their respective optimized complexes. The corresponding atomic coordinates are pro-

vided in Appendix B, and the distances between the metal ions and the nitrogen and oxygen atoms of EDTA, as well as the oxygen atom of the first-shell water molecule, are listed in Table 4.4. The sums of electronic and thermal free energy values obtained from these optimized structures were then used to calculate the Gibbs free energy of complexation, G_{cmpx} . Two possible calculation schemes can be employed to evaluate the Gibbs free energy of complexation, G_{cmpx} [84]. The first approach calculates G_{cmpx} as the difference between the Gibbs free energy of the $\text{M}^{2+} + \text{EDTA}^{4-}$ complex (optionally including any coordinated water molecules) and the sum of the Gibbs free energy values of the individual reactants: M^{2+} , EDTA^{4-} , and water if applicable. This scheme was used in Subsection 4.2.3. Another scheme explicitly treats the chelation process as a substitution reaction, in which water molecules from the first hydration shell of the metal ion are replaced by donor atoms of the EDTA ligand. In some cases, the second hydration shell is also considered [84]. Assuming that a divalent metal ion in an aqueous solution is initially coordinated by 6 water molecules, the corresponding substitution reaction can be written as [55, 84]: $\text{M}^{2+}(\text{H}_2\text{O})_6 + \text{EDTA}^{4-} \rightarrow (\text{M} + \text{EDTA})^{2-} + 6\text{H}_2\text{O}$. In cases where the resulting complex retains one coordinated water molecule, the reaction becomes: $\text{M}^{2+}(\text{H}_2\text{O})_6 + \text{EDTA}^{4-} \rightarrow (\text{M} + \text{EDTA} + \text{H}_2\text{O})^{2-} + 5\text{H}_2\text{O}$. To perform this calculation, it was necessary to additionally optimize the geometries of the first hydration shells of the metal ions. However, convergence issues arose during optimizations in implicit solvent, as the hydration shells are more labile compared to the more rigid chelate complexes [53]. Similar difficulties were reported by Chen et al. [84], indicating that the problem of modelling hydration shell structures is non-trivial. Nevertheless, by applying the NoSymmetry keyword in Gaussian to prevent reorientation of the input coordinates, the optimizations were successfully completed, and no imaginary frequencies were observed in the vibrational analyses. The G_{cmpx} values obtained using both calculation schemes are summarised in Table 4.3.

Table 4.4. Optimised distances (in Å) between metal ions and coordinating atoms (N and O) in the EDTA-metal ion-water complexes.

Complex	N1	N2	O ₁	O ₂	O ₃	O ₄	O _w
Hg	2.4	2.43	2.51	2.54	2.55	2.66	2.47
Cd	2.43	2.43	2.37	2.4	2.42	2.45	2.41
Pb	2.57	3.35	2.51	2.51	2.65	2.65	-

4.4.3. Structures of the first hydration shells of Hg²⁺, Cd²⁺, Pb²⁺

In one of the methods used for calculating the Gibbs free energy of complexation values, it was necessary to optimise the first hydration shells of the heavy metal ions: Hg²⁺, Cd²⁺, and Pb²⁺. For all three cases, the geometries of the ions coordinated with 6 water molecules were optimised using the selected DFT level in an implicit water solvent, as suggested in [55, 84]. The resulting structures are shown in Fig. 4.13. After optimisation, all complexes remained hexacoordinated. For Hg²⁺ and Cd²⁺, the hydration shells adopt approximately octahedral geometries (Figs. 4.13a and 4.13b), whereas for Pb²⁺, the structure is hemidirected (Fig. 4.13c). The corresponding Cartesian coordinates of the obtained configurations are provided in Appendix B.

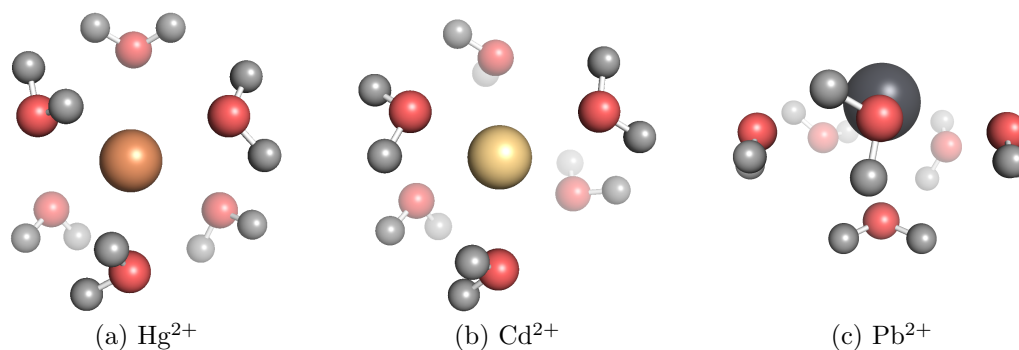


Figure 4.13. The first hydration shells of Hg²⁺, Cd²⁺, Pb²⁺ cations. The O, H, Hg, Cd and Pb atoms are shown in red, grey, orange, yellow and black, respectively.

4.4.4. Comparison of complexation energy values with the experimental data

According to the experimental stability constants at $T = 298.15$ K and an ionic strength of 0.1 M (Table 4.2), the mercury ion forms the most stable complex with fully deprotonated EDTA, followed by lead and cadmium ions, i.e., $\text{Hg} > \text{Pb} > \text{Cd}$. However, it should be noted that experimental stability constant measurements depend on factors such as the temperature and ionic strength. At a lower ionic strength of 0.01 M, alternative experimental data are available for the stability constants of Cd ($\log K = 18.2$) and Pb ($\log K = 18.0$) [165], suggesting a different order of stability: $\text{Cd} > \text{Pb}$. Unfortunately, the details regarding the temperature in this alternative dataset were not specified, and no easily accessible information on the stability constant of Hg^{2+} at this ionic strength was found. However, given the consistently high reported stability of mercury-EDTA complexes at higher ionic strengths (Table 4.2), it is reasonable to assume that the alternative ranking may follow the order: $\text{Hg} > \text{Cd} > \text{Pb}$. Quantitative agreement with the computational results remains challenging due to the inherent approximations of DFT calculations and the difficulty in accounting for experimental conditions such as the ionic strength and temperature. MD results show that lead forms the least stable complexes with EDTA, while the adsorption energy values for Hg and Cd complexes are relatively close, with cadmium showing slightly stronger adsorption. DFT calculations using both complexation schemes yield a different trend, predicting the following order of chelation strength: $\text{Pb} > \text{Hg} > \text{Cd}$. While the relative stability of the Hg and Cd complexes agrees with experimental observations, the computed stability of the Pb complex is significantly lower than that of the other metals. This discrepancy may be attributed to the unusual geometry of the Pb^{2+} first hydration shell. It is likely that inclusion of the second hydration shell and additional explicit water molecules around the complex would improve the accuracy of the Pb^{2+} calculations. However, such additions often lead to convergence issues and increased the basis set sensitivity in DFT. To overcome these limitations, periodic DFT simulations with

plane-wave basis sets, such as those implemented in VASP, could provide a more reliable estimate of the complexation energetics.

The optimized M^{2+} +EDTA geometries were used for natural bond orbital (NBO) calculations. Prior to complexation, all cations were considered to carry a charge of +2. The results reveal that, among the studied metals, mercury exhibits the most significant charge transfer contribution to complex stability, followed by Cd and Pb: $Hg > Cd > Pb$ (see Table 4.5). This trend aligns more closely with the ranking based on experimental stability constants. For Pb, the charge transfer is the least pronounced, yet the Gibbs free energy of complexation was the lowest among all complexes studied. This suggests that other interactions, such as electrostatics, play a substantial role in stabilising Pb^{2+} -EDTA complexes, as has been reported in other Pb coordination systems [166]. Kovács et al. [78] also observed the highest charge transfer in the case of Hg^{2+} -EDTA complexation, but reported a different order for Cd and Pb, with Cd showing a slightly higher transferred charge (1.52) than Pb (1.37).

Table 4.5. Charges of Hg, Cd, Pb cations chelated by EDTA obtained after NBO calculations.

Element	Hg	Cd	Pb
	1.299	1.423	1.476

Conclusions

In this chapter, the chelation of divalent heavy metal ions, Hg^{2+} , Cd^{2+} , and Pb^{2+} , with ethylenediaminetetraacetic acid (EDTA) was investigated using a combination of molecular dynamics (MD) simulations and density functional theory (DFT) calculations. Particular attention was paid to the influence of the EDTA protonation state on complex stability.

It was found that a mercury ion forms the most stable complex with fully deprotonated EDTA ($EDTA^{4-}$), both according to the MD and DFT data, in agreement with experimental stability constants. Increasing the protonation of EDTA weakens complexation due to the occupation of coordination sites by hydrogen atoms

and a reduction in the negative charge on donor atoms. The first coordination shell of Hg^{2+} in the EDTA^{4-} complex typically contains two nitrogen and four oxygen atoms, forming a stable six-coordinated structure. For partially protonated forms of EDTA, the number of coordinating donor atoms decreases, and water molecules enter the coordination shell, resulting in reduced stability.

As well as with Hg^{2+} , with Cd^{2+} and Pb^{2+} the fully deprotonated EDTA forms stable six-coordinated complexes. MD simulations reveal the possibility of inclusion of one or two (in case of Pb^{2+}) water molecules in the first hydration shell of the metal ions, depending on the ionic radii and energy parameters used. DFT calculations confirm that one water molecule can stay in the coordination shell of the chelated Hg^{2+} and Cd^{2+} ions but not of Pb^{2+} .

Gibbs free energy values from DFT support the experimental trend that Hg^{2+} complexes are the most stable, followed by Cd^{2+} , though Pb^{2+} shows inconsistencies which are likely to be due to challenges in modelling its hemidirected hydration shell geometry. Two schemes for DFT-based calculation of Gibbs free energy of complexation values were evaluated. The hydration substitution scheme, although more physically realistic, introduced convergence difficulties, especially for Pb^{2+} . Nevertheless, both schemes confirmed the favourable energetics of complex formation. Adsorption energy values from MD also suggest that the complexes are stable and partially support the experimental trend.

Charge distribution analysis using the atomic polar tensor (APT) model and natural bond orbital (NBO) calculations indicate that charge transfer plays a significant role in complex stability. Hg^{2+} exhibits the highest degree of charge transfer upon complexation, which correlates with its high stability.

In general, the combined computational approach provides valuable insights into the coordination chemistry and stability of heavy metal-EDTA complexes and highlights the crucial role of the protonation state, metal ion features, and accurate modelling parameters in determining chelation effectiveness. The findings of this study may contribute to the development of effective chelation-dechelation protocols for the removal of heavy metal ions from aqueous environments [88, 89].

CONCLUSIONS

This thesis has presented an investigation of molecular complexation phenomena involving polymer-enzyme conjugates and heavy metal chelation by both polymeric and small-molecule ligands. By employing a combination of advanced molecular simulation techniques, such as Langevin dynamics, classical molecular dynamics (MD), and quantum chemical calculations based on density functional theory (DFT), the study provides detailed insights into the structural and thermodynamic aspects of binding interactions. The general conclusions drawn from each chapter are summarised below.

Chapter 2 represents a detailed computational investigation of polymer-enzyme systems and their complexation using Langevin dynamics simulations and thermodynamic theory. The results demonstrate that the binding degree between polymer and enzymes is highly sensitive to the number and distribution of functional groups on the polymer chains, with steric constraints limiting the binding efficiency at high functionalisation levels. A theoretical approach based on Wertheim’s first-order perturbation theory (TPT1) was successfully adapted to describe the binding process, although it tends to overestimate complexation in densely functionalised regimes, as steric effects are not yet fully captured in the current formulation. The study highlights the interplay between molecular design and spatial constraints in controlling complex formation and provides a foundation for developing more accurate theoretical treatments using higher-order perturbation theories.

Through a combined MD and DFT approach, *Chapter 3* elucidates the molecular mechanisms underlying the chelation of Hg^{2+} ions by polyethyleneimine (PEI) chains of different lengths. Simulations revealed that PEI-5 offers optimal chelation efficiency for a single ion, achieving a balance between nitrogen availability and

steric accessibility. The competitive binding between polymer nitrogen atoms and water molecules in the coordination shell was shown to influence complex structure and stability. It was shown that longer chains, such as PEI-10, are capable of coordinating up to four Hg^{2+} ions. However, the binding strength per ion gradually decreases with an increasing number of coordinated ions. This effect was attributed to electrostatic repulsion between closely spaced metal ions and the reduced flexibility of the polymer backbone in extended conformations. Thermodynamic analyses confirmed the favourable nature of these interactions, while DFT calculations supported the structural consistency and stability of the resulting complexes. These findings provide a rational basis for tailoring PEI-based ligands for selective and efficient mercury capture.

Chapter 4 extends the computational study of heavy metal chelation to EDTA complexes with Hg^{2+} , Cd^{2+} , and Pb^{2+} , with a focus on the role of ligand protonation. Both MD and DFT results confirmed the superior stability of fully deprotonated EDTA complexes, particularly with Hg^{2+} , due to enhanced charge transfer and complete coordination. Partial protonation weakens complexation by reducing available donor sites and promoting competition from water molecules. Differences in ion-specific hydration behaviour, particularly the hemidirected shell of Pb^{2+} , introduced modelling challenges but were partially resolved through multiple computational protocols. The obtained results provide valuable insight into the chemical principles governing EDTA chelation and demonstrate the importance of protonation control and accurate modelling for designing efficient chelators for heavy metal remediation.

To summarise, this study advances the ability to predict binding characteristics in polymer-enzyme complexes and enhances the understanding of the processes governing heavy metal ion chelation in aqueous solutions. The obtained insights offer valuable perspectives for fundamental research and practical applications, such as improving biocatalytic efficiency in biofuel production from cellulosic biomass and facilitating the removal of hazardous elements from water.

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APPENDIX A

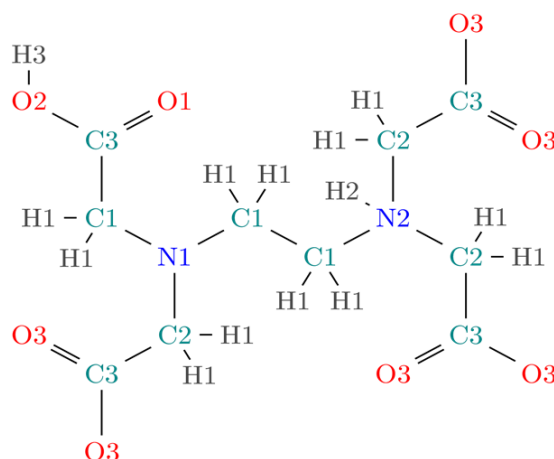


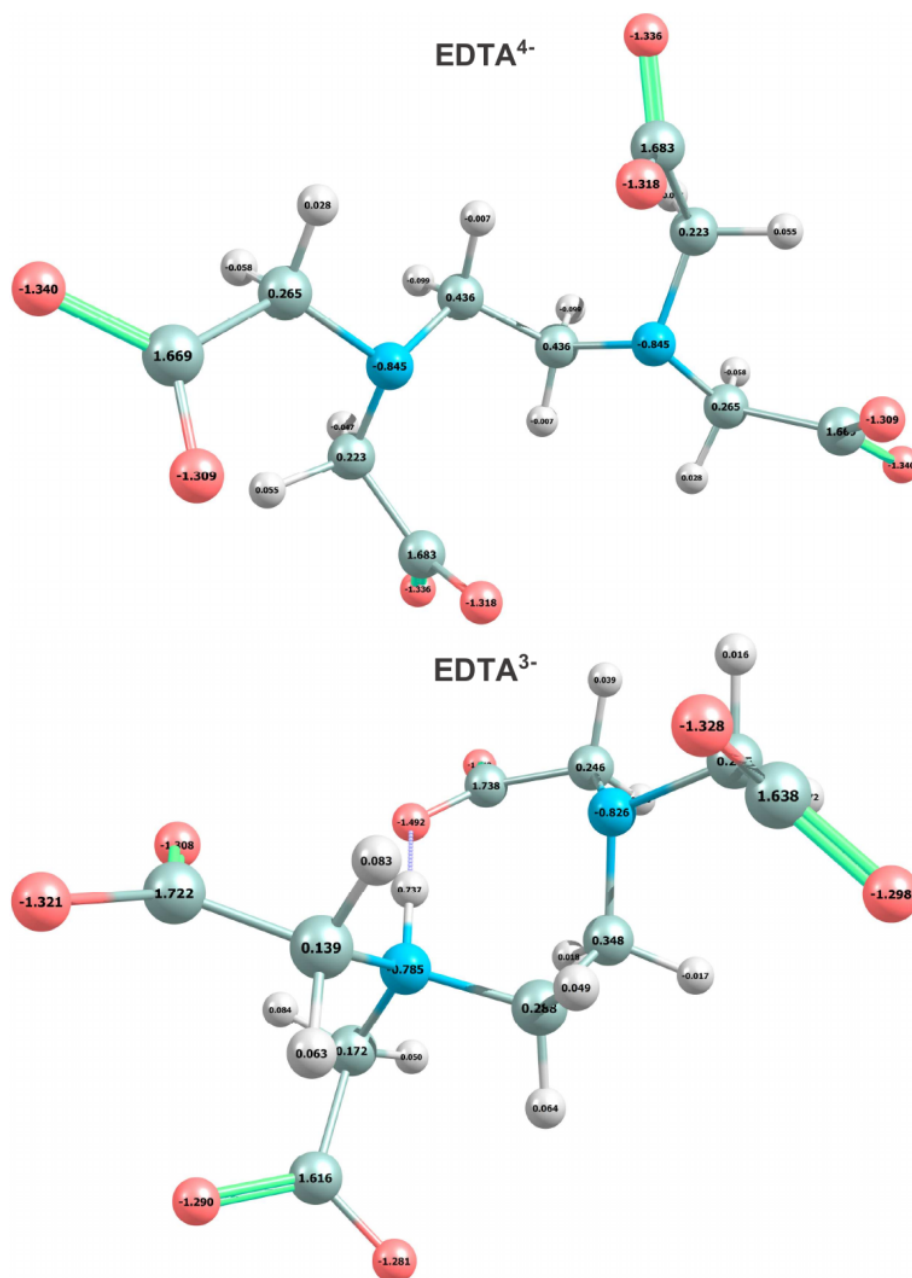
Figure A.1. An atomistic model of EDTA²⁻ described using the OPLS/AA force field. The non-bonding OPLS/AA force field parameters are reported in Table A.1. Reproduced from Ref. [1] with permission from the PCCP Owner Societies.

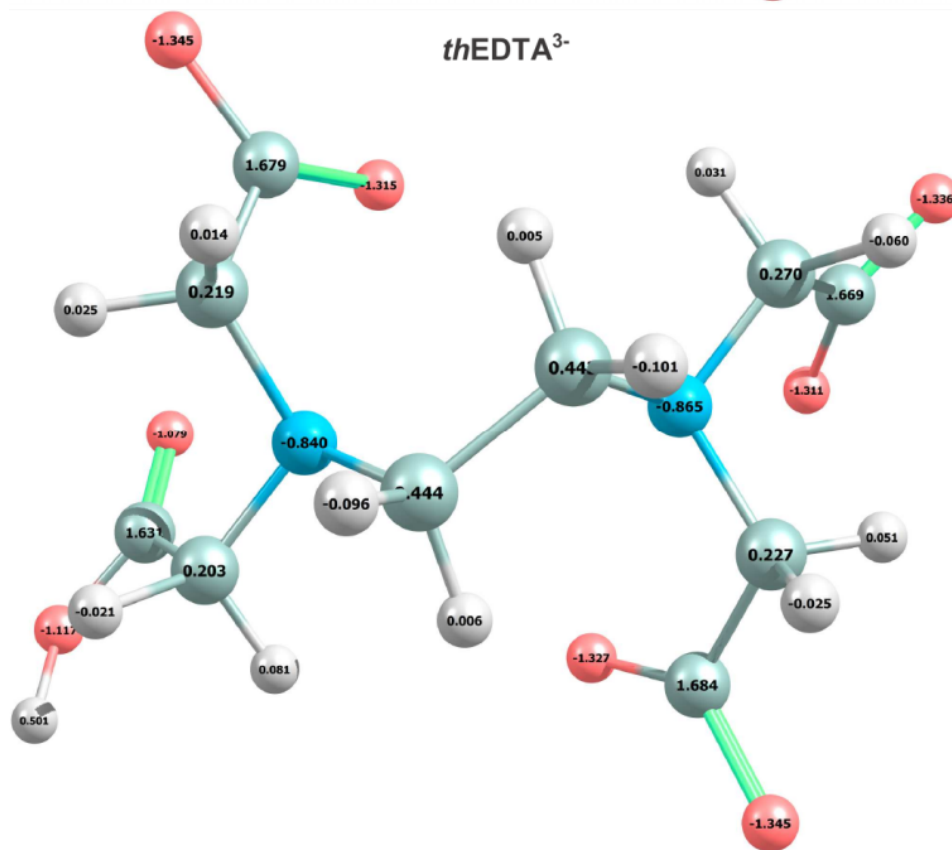
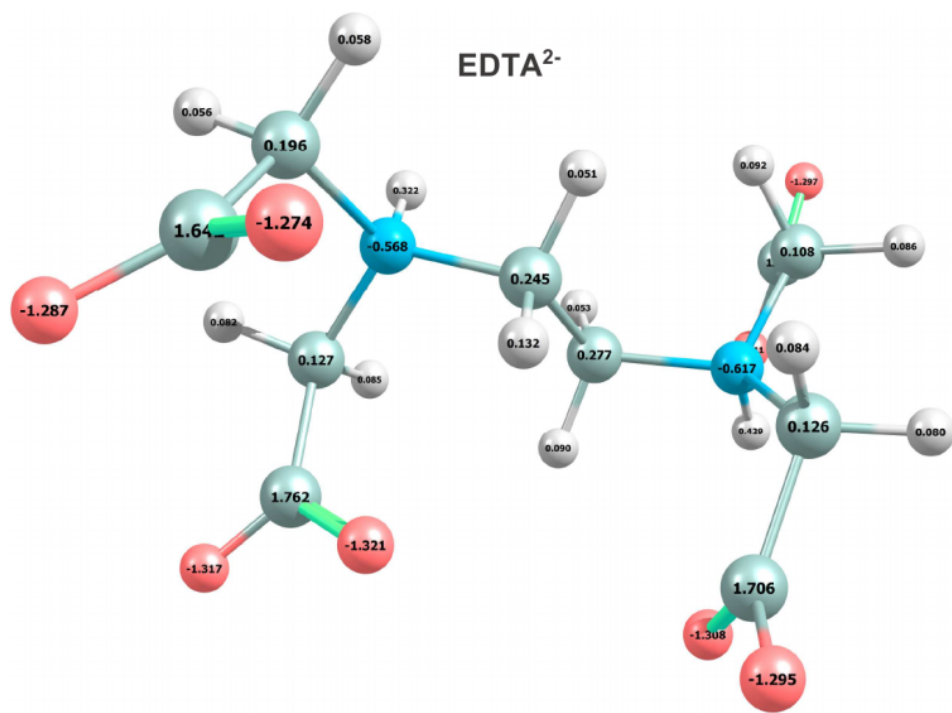
Table A.1. The non-bonding OPLS/AA parameters for EDTA and the Hg²⁺ ion. Notations for the EDTA atoms correspond to those used in Figure A.1. The parameters are used with the Lennard-Jones potential $U_{\text{LJ}}(r) = 4\epsilon \left[\left(\frac{\sigma}{r}\right)^{12} - \left(\frac{\sigma}{r}\right)^6 \right]$ and the Coulomb potential $U_{\text{coul}}(r) = \frac{q_i q_j}{\epsilon r}$. Reproduced from Ref. [1] with permission from the PCCP Owner Societies.

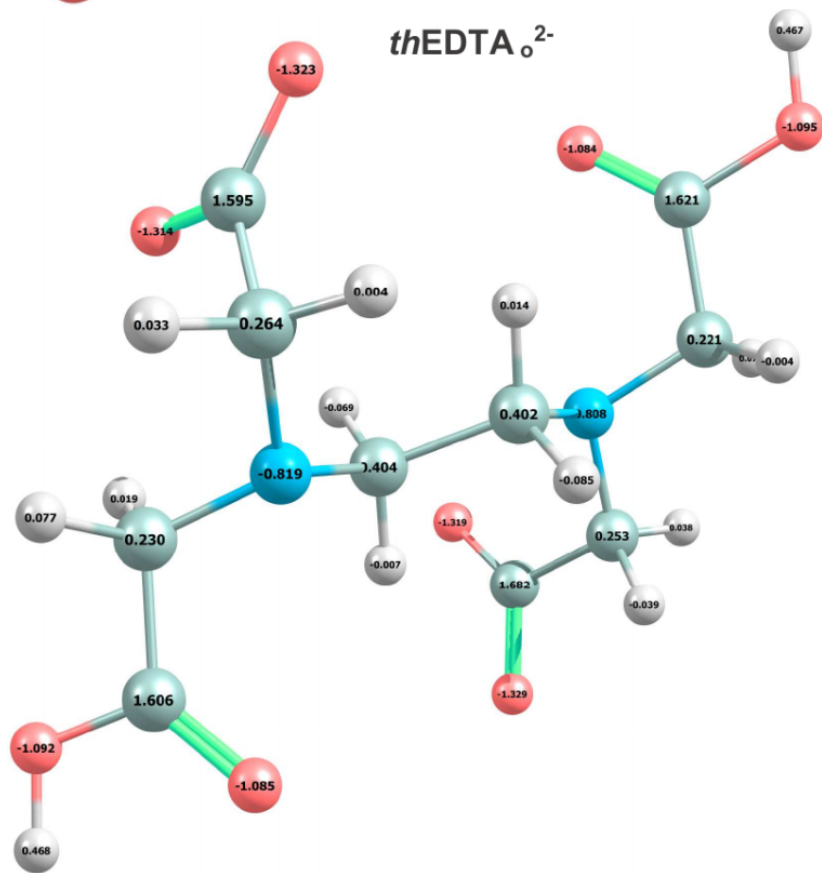
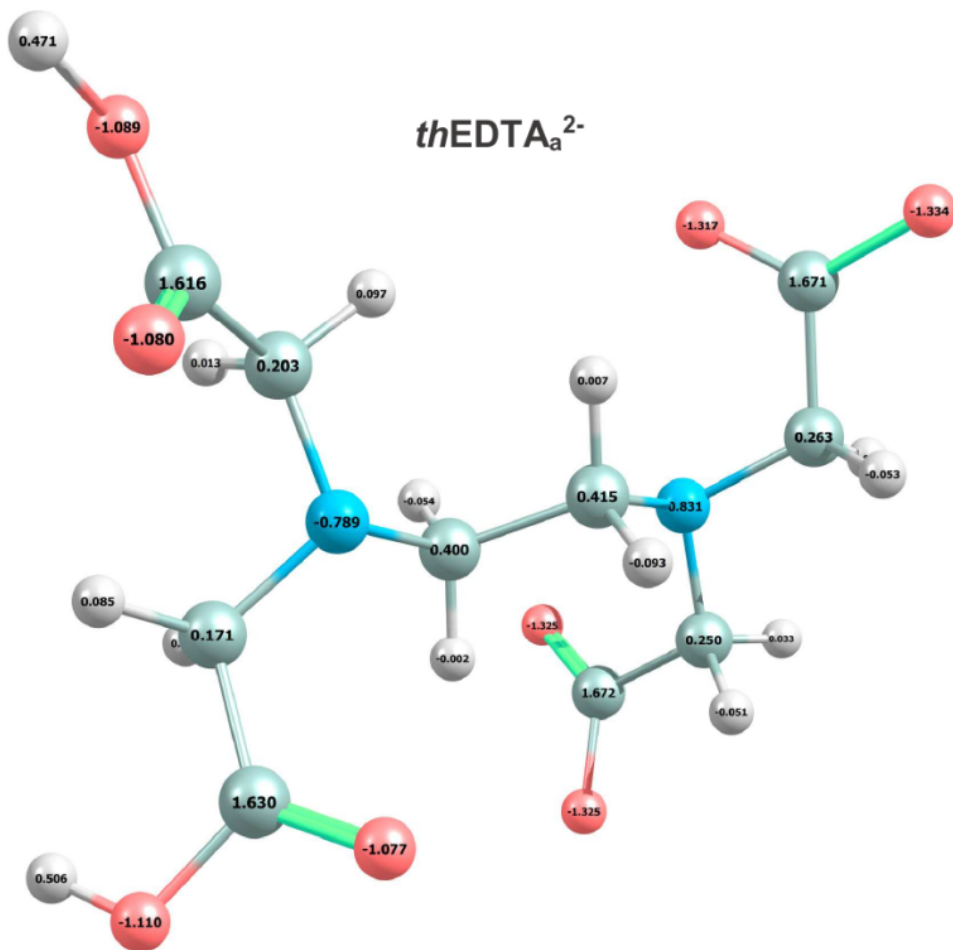
Type	Charge	Mass	ϵ (kcal mol ⁻¹)	σ (Å)
N1	-0.63	14.007	0.17	3.3
N2	-0.10	14.007	0.17	3.25
C1	0.09	12.011	0.066	3.5
C2	-0.22	12.011	0.066	3.5
C3	0.52	12.011	0.105	3.75
H1	0.06	1.008	0.03	2.5
H2	0.29	1.008	0.0	0.0
H3	0.45	1.008	0.0	0.0
O1	-0.44	15.999	0.21	2.96
O2	-0.53	15.999	0.17	3.0
O3	-0.80	15.999	0.21	2.96
Hg ²⁺	2.00	200.59	0.0409	2.36

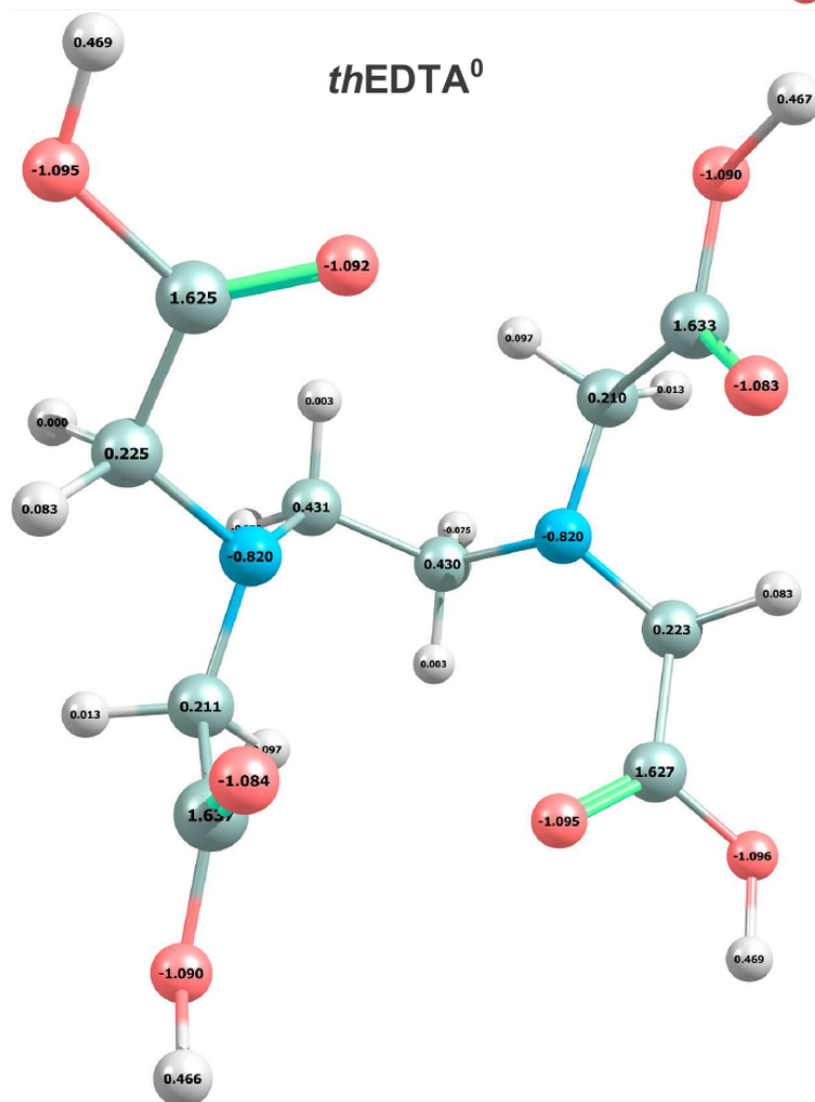
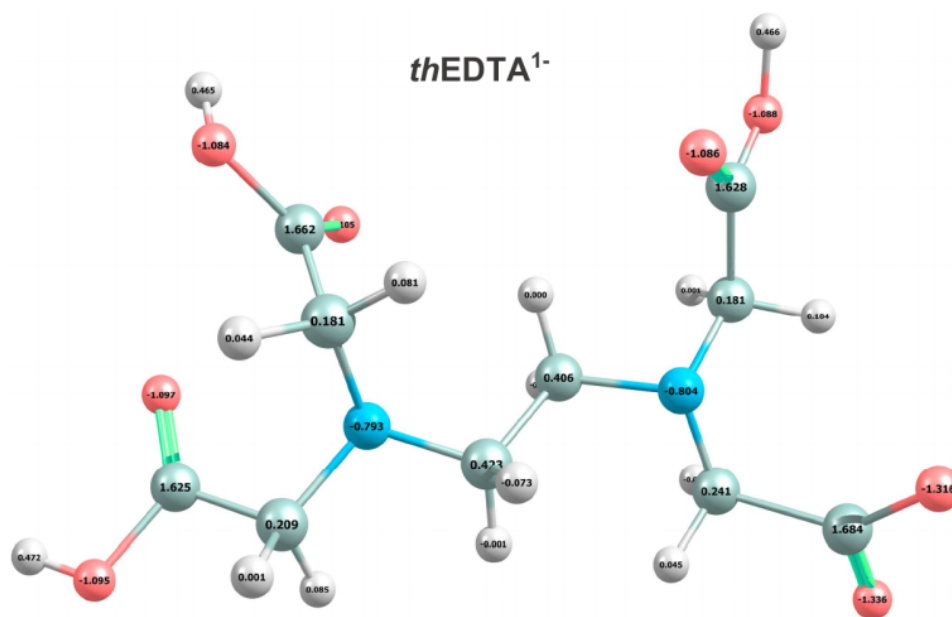
APPENDIX B

The APT partial charges of EDTA at different protonation states. The carbon, oxygen, nitrogen, hydrogen, and mercury atoms, are coloured in grey, red, blue, white, and yellow, respectively. Reproduced from Ref. [1] with permission from the PCCP Owner Societies.

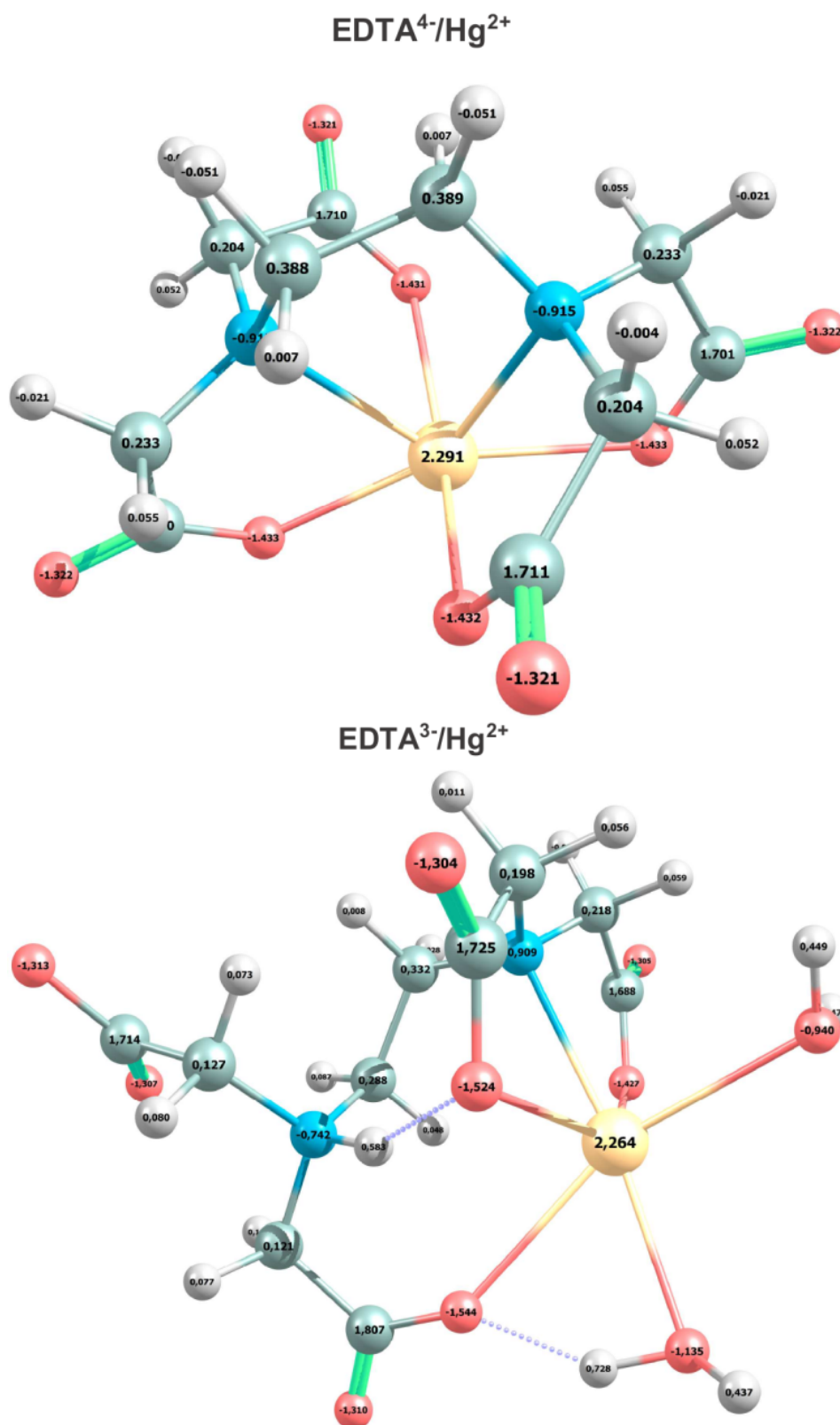








The APT partial charges of EDTA-Hg chelates at different protonation levels of EDTA. The carbon, oxygen, nitrogen, hydrogen, and mercury atoms, are coloured in grey, red, blue, white, and yellow, respectively. Reproduced from Ref. [1] with permission from the PCCP Owner Societies.



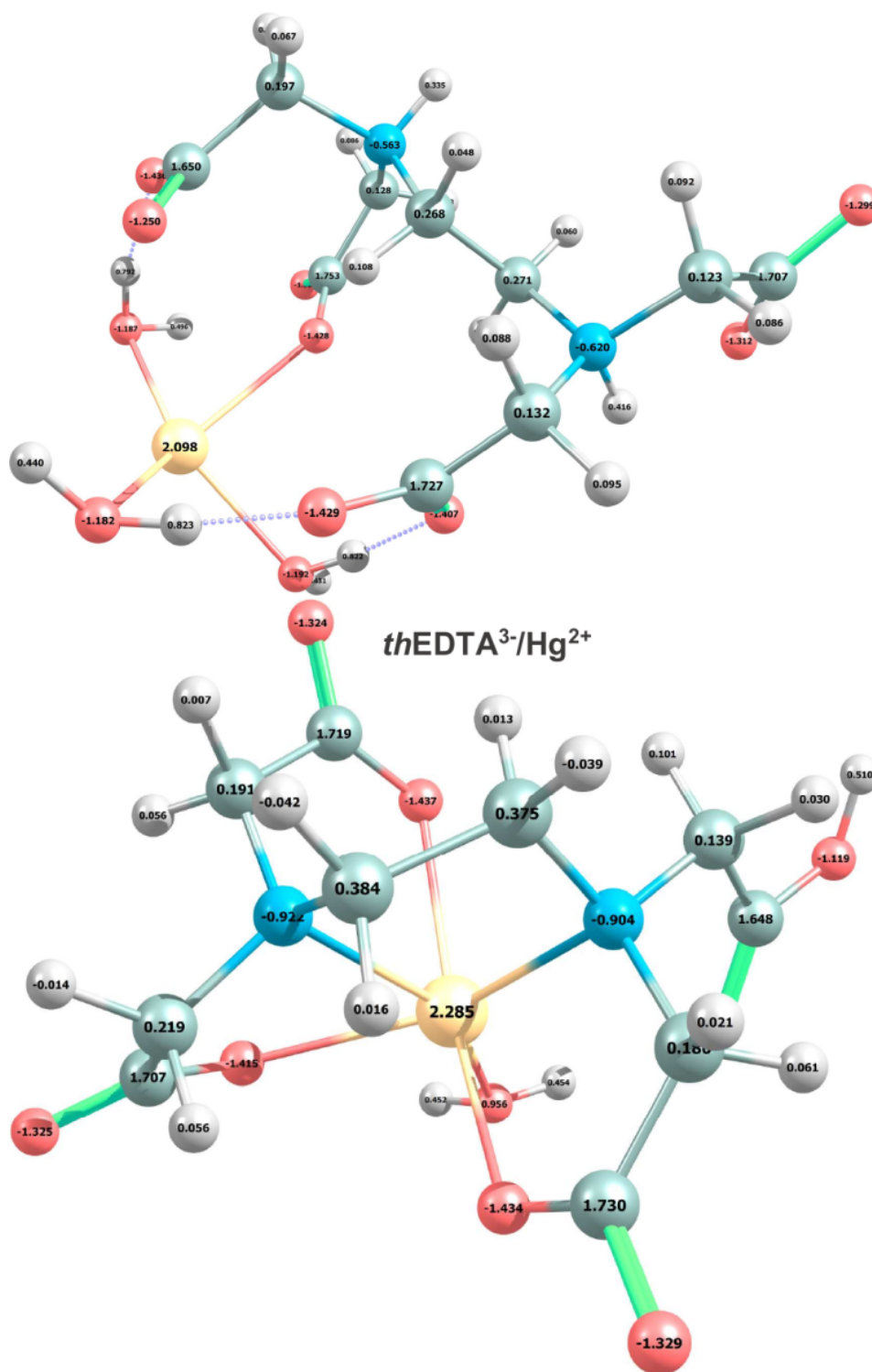


Table B.1. First hydration shell coordinates for Hg^{2+} ion.

1	Hg	0.015736	0.048400	-0.135129
2	O	-1.961405	0.045910	-1.623663
3	H	-2.662558	0.329512	-1.024617
4	H	-2.113559	-0.899202	-1.744871
5	O	1.789753	-0.380701	-1.721540
6	H	2.369011	-0.967586	-1.219336
7	H	2.262815	0.460019	-1.753428
8	O	1.593700	-0.332488	1.838121
9	H	2.343481	-0.708102	1.361731
10	H	1.140303	-1.097030	2.212828
11	O	-0.387962	-2.347033	0.259236
12	H	-1.091305	-2.559654	-0.366232
13	H	-0.826443	-2.312348	1.118405
14	O	-1.604763	0.477438	1.742720
15	H	-1.174709	1.235263	2.156925
16	H	-2.350825	0.856167	1.262404
17	O	0.491000	2.419533	0.024700
18	H	1.441314	2.463605	-0.138786
19	H	0.397128	2.585693	0.970922

Table B.2. First hydration shell coordinates for Cd^{2+} ion.

1	Cd	-0.044617	-0.016627	0.016712
2	O	-1.519237	1.473999	-1.161871
3	H	-1.737916	2.165767	-0.526168
4	H	-0.964773	1.910629	-1.819540
5	O	1.538623	-1.474917	1.090382
6	H	1.983888	-0.882708	1.708603
7	H	2.201596	-1.665779	0.415923
8	O	-1.023938	-1.851339	-1.110826
9	H	-1.648412	-1.468822	-1.738926
10	H	-1.577269	-2.293238	-0.454864
11	O	-1.711540	-0.220794	1.711907
12	H	-2.164515	-1.054528	1.538042
13	H	-2.365136	0.460515	1.513016
14	O	1.611088	0.225383	-1.695285
15	H	1.668718	1.168375	-1.889586
16	H	2.448615	0.008698	-1.268587
17	O	0.933759	1.771399	1.234401
18	H	0.548171	1.743678	2.118177
19	H	1.855646	1.514562	1.358947

Table B.3. First hydration shell coordinates for Pb^{2+} ion.

1	Pb	0.016351	-0.007314	-0.426688
2	O	2.361206	-0.972156	0.310368
3	H	2.585190	-1.707404	-0.272793
4	H	3.007134	-0.284980	0.106791
5	O	-0.150458	-2.503056	0.329591
6	H	-0.985124	-2.599827	0.804170
7	H	0.534046	-2.636833	0.996972
8	O	-2.520063	-0.538949	0.073826
9	H	-2.736098	-1.396441	-0.312205
10	H	-2.569586	-0.680608	1.027382
11	O	-1.332534	2.191711	0.105734
12	H	-2.081026	1.939783	0.660255
13	H	-0.737802	2.681641	0.687161
14	O	-0.116441	0.069425	2.009649
15	H	-0.522783	-0.749879	2.322547
16	H	-0.726159	0.773851	2.266256
17	O	1.581202	1.950764	0.390961
18	H	2.013956	1.635150	1.193952
19	H	2.294206	2.043415	-0.253070

Table B.4. Optimized coordinates of EDTA⁴⁻+Hg²⁺-H₂O complex.

1	O	-3.516899	2.621979	-0.044147
2	O	-2.565169	-3.443865	0.852247
3	O	3.457704	-2.694690	-0.021568
4	O	2.687067	3.466235	0.472874
5	O	-2.153901	1.238096	-1.131217
6	O	-0.892630	-2.413088	-0.198860
7	O	2.068245	-1.371097	-1.149582
8	O	0.928561	2.404278	-0.385515
9	N	-1.460428	-0.036460	1.245160
10	N	1.540020	0.120384	1.155841
11	C	-0.591461	-0.278019	2.396353
12	C	0.732540	0.461770	2.328550
13	C	-2.108948	1.263882	1.276788
14	C	-2.439544	-1.101417	1.092942
15	C	2.160595	-1.191493	1.251292
16	C	2.535009	1.148737	0.888322
17	C	-2.639642	1.734372	-0.078886
18	C	-1.902641	-2.426065	0.547414
19	C	2.601095	-1.787701	-0.087174
20	C	1.992078	2.444754	0.286346
21	H	-1.097001	0.007472	3.328313
22	H	-0.412382	-1.353715	2.452790
23	H	0.553329	1.538615	2.299915
24	H	1.290921	0.255732	3.250922
25	H	-1.386372	2.020541	1.593485
26	H	-2.935037	1.280598	1.998481
27	H	-3.198332	-0.776274	0.374816
28	H	-2.954618	-1.295076	2.041945
29	H	1.441789	-1.900531	1.669135
30	H	3.025637	-1.171078	1.925036
31	H	3.249304	0.758700	0.157033
32	H	3.098525	1.392030	1.797051
33	Hg	0.006857	0.043341	-0.690722
34	O	-0.336489	-0.384801	-3.097044
35	H	0.059894	-1.263387	-3.144846
36	H	-1.287755	-0.545679	-3.082524

Table B.5. Optimized coordinates of EDTA⁴⁻+Cd²⁺-H₂O complex.

1	O	3.587996	2.432266	0.122704
2	O	2.204397	-3.628974	-0.347973
3	O	-3.592309	-2.404739	0.146494
4	O	-2.235895	3.613179	-0.349340
5	O	2.117025	1.130959	1.173844
6	O	0.643464	-2.284244	0.495503
7	O	-2.151784	-1.061143	1.187944
8	O	-0.732617	2.258302	0.575737
9	N	1.482616	-0.197747	-1.153070
10	N	-1.469521	0.191561	-1.163799
11	C	0.639201	-0.435178	-2.323366
12	C	-0.618246	0.413047	-2.331517
13	C	2.232566	1.043280	-1.224692
14	C	2.353766	-1.331110	-0.890476
15	C	-2.209852	-1.056145	-1.214368
16	C	-2.350908	1.323790	-0.931429
17	C	2.687218	1.567990	0.137091
18	C	1.667732	-2.511395	-0.205630
19	C	-2.694537	-1.536487	0.153855
20	C	-1.708750	2.493805	-0.189723
21	H	1.195081	-0.250627	-3.252926
22	H	0.370006	-1.494010	-2.325328
23	H	-0.347979	1.471760	-2.343054
24	H	-1.168373	0.218373	-3.262631
25	H	1.597996	1.826209	-1.648622
26	H	3.109728	0.953877	-1.878375
27	H	3.145184	-1.015534	-0.203615
28	H	2.844465	-1.684417	-1.805800
29	H	-1.560888	-1.848556	-1.596515
30	H	-3.071797	-0.993647	-1.891243
31	H	-3.180395	1.002120	-0.294321
32	H	-2.790819	1.691571	-1.866301
33	Cd	0.008438	0.012386	0.761166
34	O	0.112155	-0.163097	3.158630
35	H	-0.803595	-0.048547	3.438688
36	H	0.556122	0.650548	3.425355

Table B.6. Optimized coordinates of EDTA⁴⁻+Pb²⁺ complex.

1	O	4.081283	-1.856600	0.097404
2	O	1.675907	3.907463	0.421179
3	O	-4.078696	1.860637	0.096876
4	O	-1.677767	-3.906994	0.422334
5	O	2.507795	-0.841192	-1.115327
6	O	0.187084	2.401543	-0.269958
7	O	-2.509189	0.839065	-1.115901
8	O	-0.187508	-2.402233	-0.268242
9	N	1.434988	0.380660	1.110959
10	N	-1.434984	-0.379968	1.111053
11	C	0.556081	0.510780	2.279784
12	C	-0.556086	-0.509751	2.279955
13	C	2.383733	-0.715501	1.272188
14	C	2.142286	1.634966	0.876604
15	C	-2.383381	0.716571	1.271765
16	C	-2.142712	-1.634174	0.877372
17	C	3.050411	-1.164400	-0.024254
18	C	1.260651	2.740843	0.309787
19	C	-3.049838	1.165407	-0.024825
20	C	-1.261599	-2.740672	0.311025
21	H	1.136639	0.413224	3.205810
22	H	0.137210	1.518492	2.275215
23	H	-0.137211	-1.517459	2.275675
24	H	-1.136649	-0.411855	3.205942
25	H	1.861169	-1.592250	1.662374
26	H	3.162241	-0.455381	1.999502
27	H	2.934609	1.464536	0.141906
28	H	2.622128	1.994669	1.794578
29	H	-1.860657	1.593227	1.661888
30	H	-3.162114	0.456926	1.999035
31	H	-2.935045	-1.463773	0.142696
32	H	-2.622579	-1.993252	1.795582
33	Pb	0.000143	-0.000608	-0.980946

APPENDIX C

LIST OF PUBLICATIONS

1. *H. Butovych, Y.V. Kalyuzhnyi, T. Patsahan, J. Ilnytskyi* Modeling of polymer-enzyme conjugates formation: Thermodynamic perturbation theory and computer simulations. // *J. Mol. Liq.* — 2023. — Vol. 385. — P. 122321. Scopus (Q1), DOI: 10.1016/j.molliq.2023.122321
2. *H. Butovych, F. Keshavarz, B. Barbiellini, E. Lähderanta, J. Ilnytskyi, T. Patsahan* Role of EDTA protonation in chelation-based removal of mercury ions from water. // *Phys. Chem. Chem. Phys.* — 2024. — Vol. 26. — P. 25402-25411. Scopus (Q2), DOI: 10.1039/D4CP02980A
3. *H. Butovych, J. Ilnytskyi, E. Lähderanta, T. Patsahan*, Chelation of the mercury ions by polyethyleneimine: Atomistic molecular dynamics study // arXiv preprint arXiv:2506.18835. 2025.
4. *H. Butovych, T. Patsahan, J. Ilnytskyi, E. Lähderanta, B. Barbiellini, F. Keshavarz*, “Chelation of heavy metals by ethylenediaminetetraacetic acid in aqueous solution”, EMLG-JMLG Annual Meeting 2024, Structure and Dynamics of Hydrogen-Bonded Systems, 8-13 September, 2024, Trieste, Italy, Book of Abstracts, p. 96. <https://indico.elettra.eu/event/39/>
5. *H. Butovych, F. Keshavarz, J. Ilnytskyi, B. Barbiellini, E. Lähderanta, T. Patsahan*, “Computer simulation of complexes formed by heavy metal ions and EDTA molecules in aqueous solution”, 1st annual workshop on the use of computer simulations in nanophysics “Nanophysics meets computer simulations”, November 8, 2024, Lviv, Ukraine, Book of Abstracts, p. 9. <https://icmp.lviv.ua/nanosim>
6. *Г. Бутович, Ф. Кешаварз, Б. Барб’єлліні, Е. Лахдеранта, Я. Ільницький, Т. Пацаган*, “ЕДТА при різних рівнях протонації для хелатування іонів ртуті у водному розчині”, 24-та Всеукраїнська школа-семінар молодих вчених зі статистичної фізики та теорії конденсованої речовини, 24-25 жовтня 2024 р., Львів, Збірка тез, с.32. <https://icmp.lviv.ua/konkurs/2024/index.html>

APPENDIX D

APPROBATION OF THE OBTAINED RESULTS

Key results of this research were presented at the conferences: EMLG-JMLG 2024 annual meeting 2024: Structure and dynamics of hydrogen bonded systems, Trieste, Italy (8-13 September, 2024) [4], the 1st annual workshop on the use of computer simulations in nanophysics “Nanophysics meets computer simulations”, Lviv (November 8, 2024) [5], 24-та Всеукраїнська школа-семінар молодих вчених зі статистичної фізики та теорії конденсованої речовини, Lviv (24-25 October, 2024) [6], as well as the ICMP seminars.