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REVEALING MOLECULAR INSIGHTS: A MOLECULAR DYNAMICS STUDY OF INTERACTIONS BETWEEN NANOPLASTICS AND ENDOCRINE DISRUPTORS

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Tese de Doutorado apresentada ao Programa de Pós-graduação em Engenharia Química, COPPE, da Universidade Federal do Rio de Janeiro, como parte dos requisitos necessários à obtenção do título de Doutor em Engenharia Química.

Orientadores: Nathalia Salles Vernin
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"Minha mãe achava estudo a coisa mais fina do mundo. Não é. A coisa mais fina do mundo é o sentimento."

Adélia Prado

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provided a home when I was only expecting a house. Meeting her was the best gift of this whole experience, and I'm genuinely thankful.

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REVELANDO PERCEPÇÕES MOLECULARES: UM ESTUDO DA DINÂMICA MOLECULAR DAS INTERAÇÕES ENTRE NANOPLÁSTICOS E DISRUPTORES ENDÓCRINOS

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Dezembro/2023

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Programa: Engenharia Química

O crescente consumo de plásticos e seu descarte inadequado são responsáveis por gerar uma cadeia de problemas. Os plásticos, quando descartados de forma incorreta, podem sofrer degradação devido à exposição ao clima, chegando a tamanhos na escala de nanometros, gerando nanoplásticos (NPs). Outra fonte de NPs inclui produtos que já foram pensados nesse formato nano. Esses NPs podem entrar em contato com os seres humanos de várias maneiras: pela ingestão de alimentos, exposição ao ar contaminados, etc. Pesquisas recentes indicam que esses NPs também podem atuar como vetores para outros contaminantes, uma vez que, devido às propriedades físico-químicas dos NPs, esses têm tendência a adsorver outros poluentes. Mais pesquisas ainda são necessárias para determinar os efeitos dessas interações e se são significativas sob condições ambientais. Bisfenol A (BPA) e Benzofenona (BZP) são possíveis contaminantes que poderiam ser co-transportados por meio de NPs. Mesmo em baixas concentrações, o BPA e a BZP podem atuar como desreguladores endócrinos e foram associados a diversas doenças. Neste estudo, utilizamos simulações de dinâmica molecular para obter o perfil de potencial de campo médio (PMF) entre uma nanopartícula de polietileno (PE) e uma molécula de BPA/BZP. Os resultados mostram que a BZP tem um potencial de atração muito maior para o PE do que o BPA. Pode-se inferir que a maior quantidade de ligações de hidrogênio que a BZP possui com a água contribui para a diferença entre a BZP e o BPA. Os resultados apontam o potencial que as nanopartículas de plástico possuem no co-transporte e bioacumulação de contaminantes em ecossistemas aquáticos.

Abstract of Thesis presented to COPPE/UFRJ as a partial fulfillment of the requirements for the degree of Doctor of Science (D.Sc.)

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The growing consumption of plastics and their improper disposal are responsible for generating a chain of problems. Plastics, when incorrectly discarded, can undergo degradation through weathering and reach sizes on the scale of nanometers, generating nanoplastics (NPs). Another source of NPs includes products that have already been designed in this nano format. NPs can come into contact with humans through different means, such as the ingestion of contaminated food or exposure to contaminated air. Recent research indicates that these NPs can also act as vectors for other contaminants, as the physicochemical properties of NPs can promote their interactions with other pollutants. Further research is still needed to determine the effects of these interactions and whether they are significant under environmental conditions. Bisphenol A (BPA) and Benzophenone (BZP) are possible contaminants that could be co-transported through NPs. Even in low concentrations, BPA and BZP can act as an endocrine disruptor and have been linked to several diseases. In this study, we use molecular dynamics simulations to obtain the potential of mean force (PMF) profile between a polyethylene (PE) nanoplastic and a BPA/BZP molecule. The results show that BZP has a much greater attractive potential to PE than BPA. We can infer that the higher quantity of hydrogen bonds that the BZP has with the water contributes to the difference between BZP and BPA. The results indicate the need to address the possibility of nanoplastics playing a role in the co-transport and bioaccumulation of contaminants in aquatic ecosystems.

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List of Abbreviations

4-NP	4-nonylphenol, p. 23
AFEST	*Archaeoglobus fulgidus*, p. 28
AMBER	Assisted Model Building and Energy Refinement, p. 14
ATB	Automated Topology Builder and Repository, p. 16
BDE47	2,2, 4,4-tetrabromodiphenyl ether, p. 23
BPA	bisphenol A, p. 4
BPF	4,4-dihydroxydiphenylmethane, p. 23
BZP	benzophenone, p. 4
BaP	benzo[a]pyrene, p. 27
CG	coarse-grained, p. 15
CHARMM	Chemistry at Harvard Macromolecular Mechanics, p. 15
COMPASS	Condensed-phase Optimized Molecular Potentials for Atomistic Simulation Studies, p. 15
CR	polychloroprene, p. 24
DFT	density functional theory, p. 27
DPPC	dipalmitoyl-phosphatidylcholine, p. 19
EC	emerging contaminant, p. 6
EDC	endocrine disruptor, p. 8
GAFF	General AMBER Force Field, p. 15
GROMOS	GROningen MOlecular Simulation, p. 15
HA	humic acid, p. 27
LAMMPS	Large-scale Atomic/Molecular Massively Parallel Simulator, p. 15
LS	lung surfactant, p. 29

MC	Monte Carlo, p. 3
MD	Molecular Dynamics, p. 3
MP	microplastic, p. 1
MS	molecular simulation, p. 5
NP	nanoplastic, p. 1
OPC	Organochlorine pesticides, p. 25
OPLS-AA	Optimized Potentials for Liquid Simulations, All-Atom, p. 14
PAE	phthalic acid esters, p. 28
PAH	polycyclic aromatic hydrocarbons, p. 2
PA	polyamide, p. 25
PBDE	polybrominated diphenyl ethers, p. 25
PCB	polychlorinated biphenyls, p. 25
PC	polycarbonate, p. 24
PET	polyethylene terephthalate, p. 22
PE	polyethylene, p. 4
PLA	polylactic acid, p. 28
PMMA	polymethyl methacrylate, p. 24
POPC	1-palmitoyl-2-oleoyl-phosphatidylcholine, p. 18
POP	persistent organic pollutants, p. 25
PP	polypropylene, p. 15
PS	polystyrene, p. 15
PVC	polyvinyl chloride, p. 25
SA	simulated annealing, p. 22
SPC/E	extended simple point charge model, p. 15
SPION	superparamagnetic iron oxide nanoparticles, p. 29
TraPPE	Transferable Potentials for Phase Equilibria, p. 15
WHAM	Weighted Histogram Analysis Method, p. 42

List of Symbols

Ω	degeneracy and the partitio function of microcanonical ensemble, p. 34
$\mathcal{H}$	Hamiltonian, p. 36
$\mathscr{U}$	potential energy, p. 32
μ	chemical potential, p. 35
$\mathbf{p}$	momentum, p. 36
$\mathbf{q}$	generalized coordinates, p. 36
ξ	reaction coordinate, p. 41
i	particle indexes, p. 32
k_B	Boltzmann constant, p. 35
m	particle mass, p. 32
r	particle coordinates, p. 32
A	free energy, p. 40
P	pressure, p. 35
S	entropy, p. 34
T	absolute temperature, p. 35
U	internal energy, p. 35
$\boldsymbol{F}$ (t)	force exerted on the particle, p. 32

Chapter 1

Introduction

Plastic materials are decisive in several aspects of human life because their physical and chemical properties result in high durability and strength, low production cost, and weight. Nonetheless, its high durability brings negative effects; even with its great potential to be recycled, tons of plastics become polluting agents in the environment in the course of time. In 2023, approximately 400 million tons of plastics were produced worldwide [2], but only 9% were recycled [3] and around 10% eventually ended up in the ocean [4, 5]. As a result of these plastics being exposed to chemical, physical and biological agents, they begin to degrade into secundary microplastics (MPs) (size $<$ 5 mm) and nanoplastics (NPs) (size $<$ 100 nm) [6–9]. Moreover, some plastics have already been designed to be MPs (primary MPs) or NPs, including fertilizers, plant protection products, cosmetics, household and industrial detergents, cleaning products, paints, and products used in the oil and gas industry [10] . Nowadays, MPs and NPs are omnipresent in the environment and can be found in the air (both indoors and outdoors), water, soil, and aquatic and terrestrial living beings. There is a variation in the source of these plastics but they mainly come from disposable plastics, fishing materials, dust, urban textiles, clothing, cosmetics, and tires [6].

The COVID-19 pandemic has been an aggravating factor in this regard, and, since its beginning, the use of disposable plastics in the health sector has greatly increased. It is estimated that, in 2020, 1.56 million masks might have ended up in the ocean [11, 12]. Among all the plastic disposals related to COVID-19, it was estimated that, from the beginning of the pandemic until August 2021, a total of approximately 25.9 tons had reached the oceans, including 12.3 tons of micro and NPs [12].

Several studies prove the bioaccumulation of these particles in living marine life, indicating a direct route for contact with humans through the food chain as shown in the literature review conducted by Toussaint *et al.* [6]. Other access routes would be ingestion or contact with contaminated water, exposure to aerosols containing NPs and contaminated air (associated mainly with the textile industry), as well as contact with skin care products and cleaning products that have NPs in their composition [13, 14]. It is already known that these NPs and MPs have a high surface/volume ratio and, consequently, a great tendency to adsorb other substances. It is important

to highlight that, depending on the polymer's glass transition temperature, the absorption of compounds can also occur. So, in addition to the presence of NPs and MPs, it is necessary to know how to deal with other types of pollutants adsorbed on its surface [15]. Many experimental studies which have been conducted in recent years proved the sorption of antibiotics [16–18], heavy metals [19, 20], pesticides [21], polycyclic aromatic hydrocarbons (PAH) [22, 23], and other organic pollutants [24] in NPs ans MPs; these studies also showed how MPs and NPs may act as transport vehicles for these contaminants. Figure 1.1 represents a possible transport scheme of pollutants adsorbed on NPs through the food chain.

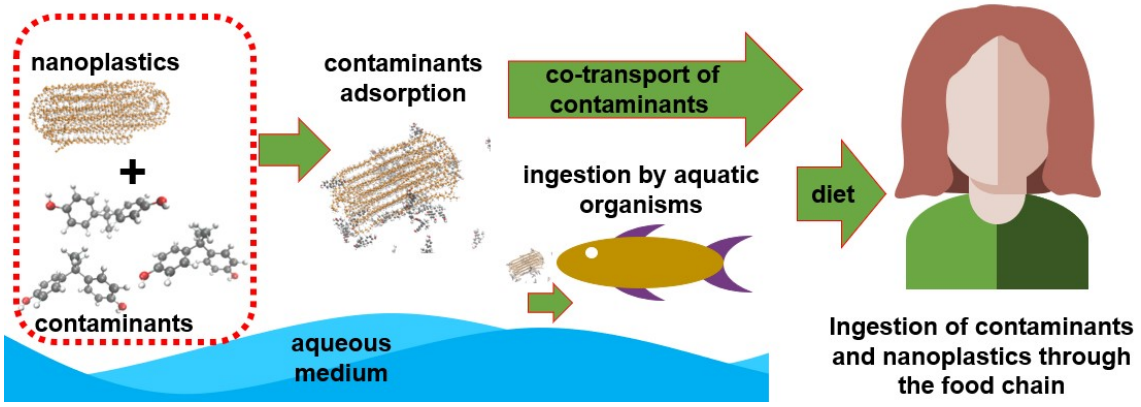


Figure 1.1: A possible transport scheme of pollutants adsorbed on NPs through the food chain.

MPs and NPs, with or without adsorbed pollutants, cause adverse effects on several species. Reichert *et al.* [25] and Okubo *et al.* [26, 27] demonstrated the negative effects in different corals when exposed to MPs. Some studies show that the combined exposure of MPs/NPs with adsorbed metals can heighten toxic effects in aquatic test organisms [28, 29]. Similar evidence was observed in other experimental studies carried out with organic pollutants such as phthalates, PAH, pharmaceuticals, and flame retardants accumulated on the MP surface [30–32]. It is important to highlight that most experiments are carried out under laboratory conditions and employ high concentrations of MPs/NPs and pollutants, which are unrealistic conditions when compared to aquatic environments. Few studies indicate that the presence of pollutants adhered to the surface of MPs can lead to synergistic, antagonistic, and additive effects under real conditions [33, 34]. Regarding human exposure to MPs and NPs, there is a great concern in determining, firstly, effectively how sizeable the contamination is, and, secondly, the main contact mechanisms [14, 35]. However, the possible effects on human health tend to be estimated, as studies that prove the problems with exposure to NPs have been materializing [36, 37].

The use of molecular simulation techniques, such as molecular dynamics and Monte Carlo simulations, is an alternative strategy to understand the possible health harms due to human exposure to NPs [38]. Molecular simulation is a powerful tool for studying phenomena at the nanometric scale and has been remarkably successful in predicting macroscopic thermodynamic and dynamic observables for various systems. It is continually growing as an option for describing system properties under conditions that experimental determinations are difficult to acquire [39]. It can also be applied together with experimental results to investigate the mechanisms behind a phenomenon of interest.

Molecular Dynamics (MD) simulation is a numerical technique that can be used to calculate the equilibrium and transport properties of many-body systems. The temporal evolution of a set of interacting classical particles is accompanied by the integration of Newton's equations of motion. The temporal averages of the trajectories and their fluctuations can be correlated with the macroscopic properties of the studied system [39].

Another approach is called Monte Carlo (MC), which is a method based on randomly finding the lowest energy states in the phase space, *i.e.*, in a stochastic way. Simplistically, this is accomplished by changing the system by one move for each Monte Carlo step, calculating the system's potential energy and the Boltzmann factor for this configuration, and accepting or not the move based on the Boltzmann factor of the potential energy difference between the old and proposed configurations [40]. A random number between 0 and 1 is generated and compared to the probability of accepting the trial move. It is accepted if the random number is less than the computed probability and rejected otherwise [41].

Both MD and MC can be applied to understand the adsorption of pollutants to NPs and study the interaction mechanism between NPs and biomolecules, such as phospholipids and proteins. Knowing the mechanism involved in these interactions is crucial to predicting dangerous combinations and outlining action strategies that reduce negative impacts on the ecosystems. Despite the potential of molecular simulation to describe the mechanism of interaction between molecules and predict system equilibrium and dynamic properties, it has only been used to analyze systems with NPs and MPs in recent years (further discussed in Chapter 3).

1.1 Motivation

There is recent interest in the scientific community in studies related to the possible impacts caused by MPs and NPs on the environment and human health. In 2020, Ragusa *et al.* [42] published the first evidence on MPs in the human placenta. In 2022, Jenner *et al.* [43] detected MPs in human lungs. Moreover, in 2023, Zhang

et al. [44] identified NPs in commercially bottled drinking water and Yang *et al.* [45] identified MPs in the human heart. This evidence extended beyond the scientific community and reached the press, circulating on social media [46, 47]. There are also several studies that investigate the adsorption of organic compounds (which are already in our aquatic systems) by MP [48–51]. The adsorption analysis shows a relation between the adsorption potential and the polymer type, although the quantitative effects of each polymer type were not easily determined through experimental studies [48]. The consequences of the contamination by MPs and NPs with or without other contaminants sorbed are still unknown and require further study. Within this context, molecular dynamics studies have also expanded, creating new opportunities for investigations on this subject [52]. Many challenges are mainly related to the methodologies used: understanding the appropriate equilibration and production times and properly building and equilibrating a simulation box with NP particle [52]. Furthermore, preliminary simulation studies can guide investments in experimental studies by providing information on the behavior of NPs and their interactions with other contaminants. Thus, it is interesting to validate molecular simulation methodologies that can contribute to experimental studies.

1.2 Objectives

The main goal of this study is to investigate, using MD, the possibility of PE NPs acting as a vehicle for two endocrine disruptors, bisphenol A (BPA) and benzophenone (BZP), in aqueous solution and understand whether there are differences in the results according to the contaminant studied. Polyethylene (PE) is chosen as our NP model because it is one of the most widely used in the plastics industry [17, 53].

Specifically, we aim to:

a) Investigate the structure of PE NP in water;

b) Develop a methodological protocol to analyze the adsorption behavior of the PE NP and the interaction energies between PE NP and BPA/BZP;

c) Calculate binding energies between PE NP and (BPA)/(BZP) using the potential mean force (PMF) methodology;

d) Calculate hydrogen bonds present in PE+H_2O+BPA and PE+H_2O+BZP simulations;

e) Compute the Henry constant between PE NP and BPA/BZP contaminants in aqueous media;

f) Investigate the angles formed between the NP and the contaminants (BPA/BZP) in the PE+H_2O+BPA and PE+H_2O+BZP simulations;

1.3 Organization of the Document

After this introduction, which presents the general aspects and motivation of the thesis, Chapter 3 presents the literature review on the current scenario of molecular simulation (MS) as a technology to study NPs and their interaction mechanisms.

The computational methods are presented in Chapter 4, where the main concepts of statistical thermodynamics and molecular simulation are summarized.

In Chapter 2, some key concepts in this field of emerging contaminants and endocrine disruptors are discussed as well as the current state of research.

In Chapter 5, the methodology and results for the application of molecular dynamics to the study of interactions of NPs, in this case PE, with two emerging contaminants, BPA and BZP, are presented. The expression "emerging contaminants" is an allusion to potential toxic products that, in general, are not fully removed or eliminated by traditional processes in water treatment for human consumption. They are contaminants that are not yet regulated by any ordinance but that should enter the regulation in the near future if a real risk to human health and the ecosystem is proven at concentrations normally found in the environment. Hence the importance of studying them.

The thesis conclusion is in Chapter 6, followed by References. The thesis has two appendices:

a) Force field and charge parameters are presented in Appendix A

b) The Appendix B is our published paper: Pollution caused by NPs: adverse effects and mechanisms of interaction via molecular simulation [52]

Chapter 2

Emerging Contaminants

In this chapter, we will analyze some key concepts on the topic of emerging contaminants in this field and provide an overview of the current state of research.

2.1 Introduction

In 2016, Noguera-Oviedo and Aga [54] published an article discussing the main lessons learned in the last 20 years of research on emerging contaminants. They emphasized that the growth of research in this field is attributed to the improved methods for analyzing, detecting, and identifying these contaminants, even at low concentrations. Due to the presence of these emerging contaminants in both aquatic and terrestrial environments and their diverse chemical compositions, the definition of emerging contaminants tends to be quite general. In general terms, emerging contaminants (ECs) can be described as pollutants that are not yet regulated by responsible agencies.

According to the United States Environmental Protection Agency [55], these materials have the potential or are already considered a real threat to human health or the environment, or they lack sufficient published information (health standards). These materials can originate from chemical or natural processes and may include pharmaceuticals, everyday products, illicit drugs, nanomaterials, and more. Although they can be related to natural processes, the growth of pollution from these contaminants is linked to human activities. Another significant source of ECs related to human activity is agriculture and livestock, particularly due to antibiotics and hormonal treatments [54].

The major concern with these contaminants is that, due to the lack of regulation, water treatment plants are not required to consider their presence in their efficiency analysis. Consequently, the extent of the problem is challenging to estimate, and long-term effects may include public health issues and serious environmental risks [56]. Research on emerging contaminants can be divided into the following areas: the study of the occurrence and fate of ECs in both natural and human-controlled environments, research on available water treatments and their efficiency in removing ECs, the study of the processes ECs undergo and their outcomes, and finally, the study of the identification of these contaminants and their toxicity [54].

Due to the variety of emerging contaminants, one of the greatest challenges lies in characterizing them and proposing an appropriate action plan for each situation. Ideally, individual studies of each potential contaminant and monitoring of each one would be a standard procedure. Unfortunately, this process becomes impractical due to the sheer number of substances that can be considered as ECs [56]. Taheran *et al.* [56] emphasized the need for closer collaboration between the scientific community and those in positions of power to develop strategies and prevent adverse effects from ECs. When used in conjunction with experimental tools, simulations can help guide research directions, saving time and resources [40]. In studies that aim to identify these contaminants and their toxicity, molecular simulation can provide information about interactions at the atomic scale, making it easier to identify potential issues. Additionally, molecular simulation aids in understanding the tools that can help remove these components from the environment. Therefore, this work uses this tool to study some emerging contaminants.

2.2 Microplastics ans Nanoplastics

Even though we extensively discuss numerous aspects of nanoplastics (NPs) throughout this thesis, in this section, we will focus on the fact that they are also considered emerging contaminants. A literature review by Liu *et al.* [57] addresses the presence of microplastics (MPs) and NPs in food for human consumption. The authors highlight that environmental pollution and changes in human lifestyles have led to the emergence of contaminants in food, a problem that is gradually gaining recognition from the public and regulatory authorities.

Exposure to MPs and NPs in food stems from two main sources: food that has been contaminated along the food chain and food contaminated by plastic packaging [57]. Several studies have reported the presence of MPs and NPs in drinking water in various parts of the world [58–62]. In the case of fruits and vegetables, contamination occurs when planting soil interacts with water contaminated by these MPs and NPs [63]. Additionally, the use of agricultural film, sludge, organic fertilizer, and contamination from landfill leachate seepage contribute to the issue [64, 65].

Reports of the toxic effects of MPs/NPs have only emerged in recent years [66–68]. Some studies indicate that this contamination can affect the human immune system of the intestines, causing inflammation, and triggering inflammatory responses in surrounding tissues in the body [57, 69]. NPs may pose even greater risks to human health than MPs, as their smaller size enables them to reach and penetrate all organs, including the placenta and brain [70].

2.3 Endocrine Disruptors

This work also focuses on EDCs, which are endocrine disruptors . These substances are characterized by their ability to alter hormonal functions in the body and they can be related to a variety of health issues. One of the major challenges is that these effects can occur even at extremely low concentrations. Additionally, these substances are not typically associated with acute toxicity; in other words, the harmful effects often manifest only in the long term, making problem identification difficult [55]. The term "Endocrine Disruptor" began to be used after a meeting held at the Wingspread Conference Center in Racine, Wisconsin. This meeting involved various scientists from different fields, including wildlife biologists, endocrinologists, reproductive physiologists, and toxicologists, who reached a consensus that certain chemicals in the environment had the potential to disrupt the endocrine systems of both humans and wildlife. Although the term is relatively new, it was based on results from studies conducted in the previous 30 years [71].

Here, a brief literature review on the use of molecular dynamics to study EDCs is presented to gain a general understanding of the current research landscape and to position our study within this context.

Boateng *et al.* [72] employed molecular simulation to investigate the adsorptive capacity of carbon nanotubes for the removal of EDCs. The authors evaluated the binding energies between Bisphenol A and 17-α-ethinyl estradiol (EE2) with graphene, single-walled carbon nanotubes, and multi-walled carbon nanotubes. The simulation results align with experimental findings, demonstrating preferential adsorption of EE2 over BPA on carbon nanotubes. They also showed that the most favorable adsorption of EE2 occurred on multi-walled carbon nanotubes, with binding energies increasing with the number of walls.

Bope *et al.* [73] investigated the capacity of polymer membranes to operate in water treatment for the removal of EE2. The molecular dynamics methodology predicted the interaction energies between EE2 and polyether sulfone (PES) and polyvinylidene fluoride (PVDF) membranes. The results revealed significantly different interactions depending on the membrane, with strong interaction for PES membranes and low interaction with PVDF membranes. Once again, the simulation results were consistent with experimental findings.

Borthakur *et al.* [74] conducted simulation experiments with aqueous solutions of 17-α-ethinyl estradiol and β-estradiol on graphene oxide surfaces in different ionization states (neutral and ionic) to understand the influence of pH on the adsorption process. The objective was to determine if graphene oxide could be used for the removal of these contaminants during water treatment and under which conditions this process was most favorable. The adsorption was facilitated in an acidic environ-

ment. The simulations provided a deeper understanding of interaction mechanisms and once again corroborated with experimental results. The information obtained from simulations goes beyond experimental data and includes details about preferred angles and the stability of the adsorption process, as well as information about which atoms of the contaminants interact more with graphene and their interaction energies.

Ng *et al.* [75] proposed the use of microporous polyethersulfone (PES) hollow fiber membranes for water treatment to remove EE2. They conducted both experimental studies and molecular dynamics simulations. Experimental results demonstrated a high adsorption capacity of the membrane (nearly 100%), and simulation results delved deeper, explaining this phenomenon by suggesting a strong affinity between PES and EE2, primarily due to hydrogen bonding and $\pi - \pi$ interactions. EE2 removal by PES membranes occurs even at lower concentrations, and in the case of high concentrations, EE2 removal can be achieved through multiple cycles in a sustainable manner.

The studies mentioned so far focus on finding solutions for the removal of EDCs from water. However, accurate analytical methods are needed to identify these contaminants in the first place. Zhang *et al.* [76] sought simpler and less costly alternatives to this problem. They proposed the use of chem/biosensors for the detection of these pollutants, particularly employing aptamers (short-chain oligonucleotides). The authors conducted MD simulations on the ligand–aptamer complex systems with the goal of understanding intermolecular mechanisms and affinity in the aptasensor fabrication process. The results revealed differences in affinities, indicating that MD can provide crucial information for the fabrication of aptasensors.

The above-mentioned works demonstrated how molecular dynamics can help address this type of problem. In most cases, they employed MD to gain a deeper understanding of the experimental data presented in their work. However, it is essential to note that the reverse path is also possible, where MD can be used in advance to provide clearer guidance for experimental researchers.

2.3.1 Bisphenol A and Benzophenone

Among the numerous emerging contaminants known as EDCs, we have chosen two to be concerned with in this thesis: Bisphenol A (BPA) and Benzophenone (BZP). A brief description of each, their significance within the industry, and potential risks to human health are presented below.

Bisphenol A is a chemical compound that was first synthesized in 1891. Around the 1930s, researchers in the field of medicine sought to explore BPA's ability to act as a synthetic estrogen. However, other substances took precedence over BPA

in these studies, and it was never used as a drug. Years later, its purpose was found as a plastic additive. Thus, the first epoxy resin using BPA was synthesized, and its commercial use began in the 1950s. Due to its properties, the use of epoxy resins spread rapidly, and nowadays, they are integral to the production of a variety of products. In general, these products have a direct or indirect relationship with various industries [77, 78].

In 1957, another discovery was made: the polymerization of BPA forms polycarbonate. Polycarbonate is exceptionally tough and durable, with the capacity to replace steel in some situations. Furthermore, its transparency allows it to be used as a substitute for glass [79]. As a result, several industries began using BPA in this manner, and it became present in electronics, automobiles, safety equipment, and more. Over the years, BPA gained an increasingly significant share of the market, and exposure to this compound became a constant reality [78]. Although initially its ability to act as a synthetic estrogen was what interested scientists, the safety of BPA came to be defined solely by its toxicity in plastic usage, rather than its capacity to act as a hormone. The safety of BPA and most chemicals was presumed based on the logic that effects increase and decrease with concentration; therefore, if the concentration is very low, the effects are negligible [80]. Due to early studies in the 1950s that showed low toxicity and the fact that it is a quickly metabolized substance [81], BPA was considered safe for use in food packaging. In the early 1990s, a significant discussion was taking place in the scientific community regarding the hypothesis that certain chemicals could interfere with the production, processing, and transmission of hormones in the body, disrupting the normal functions of the endocrine system, and these would come to be called “endocrine disruption" [71]. Although the name was new at the time, it was built upon years of prior experiments and research. Following this discussion, tests began to be conducted with BPA at levels well below the toxic concentration and far below the previously established safety threshold, but at a concentration level where it could be physiologically active as a synthetic estrogen. The results demonstrated that BPA acted as a weak estrogen [71]. These findings sparked new debates about the safety of BPA, and regulatory agencies demanded further studies on the subject. At the conclusion of the research, the findings included changes in fetal prostate and mammary gland development, disruption of chromosomal alignment in developing eggs in females of different animals, altered immune function, metabolic abnormalities, and changes in the brain and behavior [82]. Studies continue to occur and are periodically reviewed by the Public Health Service Food and Drug Administration (FDA). The current dosage in the United States for the “No Observed Adverse Effect Level" (NOAEL) is 5 mg/kg bw/day. Which means 5 mg per kg body weight per day. The acceptable daily intake (ADI) is defined as the daily intake of a substance which, over the entire

lifetime of a human, appears to be without adverse effects or harm to the health of that human [83]. The ADI is calculated by applying a safety or uncertainty factor, which is commonly 100, to the NOAEL obtained from the most sensitive test species [83]. What this really means here is that if a 50kg person intakes less than 2,5mg (50 * 0.05) of BPA per day, there should be no risk. This dosage was last reviewed in 2014 and became effective in 2008 [84]. On April 19, 2023, the European Food Safety Authority (EFSA) issued an updated scientific opinion that deemed BPA a consumer health risk. Based on all the new scientific evidence assessed, EFSA's experts established a tolerable daily intake (TDI) - which is basically the same as the American ADI- of 0.2 nanograms (0.2 billionths of a gram) mg/kg bw/day, replacing the previous temporary level of 4 micrograms (4 millionths of a gram) mg/kg bw/day set in its previous assessment in 2015 [85].

Benzophenone is also associated with a series of health problems. It is an established carcinogen, proven to cause damage to the genetic information within a cell, leading to mutations [86]. It also has a direct interaction with hormone receptors, classifying it as an endocrine disruptor [87]. BZP can occur naturally in wine grapes (*Vitis vinifera L.*), black tea and mountain papaya [88, 89]. However, despite its natural occurrence, most people come in contact with BZP through personal care products, including sunscreens, anti-aging creams, and moisturizers [90]. Additionally, due to its widespread use as a photo-initiator in UV-cured inks on the external face of paperboard packaging, there is a significant risk of exposure to BZP through food packaging [88, 89]. Downs *et al.* [91] demonstrated that benzophenone accumulates over time from the degradation of octocrylene in commercial sunscreen products. Dermatologists recommend the daily use of sunscreen with reapplication depending on the level of exposure and activities undertaken [92, 93]. Consumers would be using octocrylene-based sunscreens every day, with multiple reapplications throughout the day, and throughout the entire year. If people follow these basic recommendations, they may be exposed to more than 100 mg/kg/day of BZP [94]. As with other chemicals in mass-produced personal care products, BZP can be a potential emerging contaminant that we should pay attention to. The contamination of environmental systems can come from at least three routes: swimmer discharges (sunscreen and fragrances), sewage discharges (sunscreen, cosmetics, and fragrances), garbage and debris leachate (personal care products, paper, and plastic packaging) [91].

2.4 Final Considerations

The broader context of emerging contaminants is characterized by their unregulated status, posing threats to human health and the environment. From the information

presented it is possible to infer the need for collaboration between the scientific community and policymakers aligns with the call for strategic interventions to prevent adverse effects from emerging contaminants. The multifaceted research areas, including the occurrence and fate of contaminants, water treatment efficiency, processes and outcomes, and contaminant identification and toxicity, collectively contribute to a comprehensive understanding of the challenges posed by emerging contaminants.

Chapter 3

Adverse Effects and Mechanisms of Interaction of Nanoplastics via Molecular Simulation: a literature review

This review aims to examine how research has been conducted in the field of nanoplastics (NPs) and microplastics (MPs) studied *via* molecular simulations, identify how NPs are being characterized within the molecular dynamics (MD) methodology, and to identify and analyze gaps in knowledge. These discussions become important to point out the paths to be followed and the difficulties that need to be addressed more urgently, as well as to guide us who are starting to simulate NPs and MPs while providing an overview of those already working in the area. This review focuses on four points: (i) summarize the force fields conventionally used to describe NPs by molecular simulations; (ii) discuss the effects of NPs in the structural and dynamical properties of biological membranes; (iii) evaluate how NPs affect the folding of proteins; (iv) and discuss mechanisms by which NPs and MPs sorb contaminants from the environment.

This chapter resulted in a review article entitled Pollution caused by nanoplastics: adverse effects and mechanisms of interaction via molecular simulation, published on PeerJ Life and Environment Special Issue: Nano- and Microplastics in the Environment [52].

3.1 Survey Methodology

The combinations of keywords used in this research were: "nanoplastics molecular dynamics", "microplastics molecular dynamics", "nanoplastics Monte Carlo", and "microplastics Monte Carlo". To guarantee a thorough assessment of the literature, the keywords were searched in five different repositories: acs.org, Science.gov, sciencedirect.com, scopus.com, webofknowledge.com, and scienceresearch.com. In total, 706 results were found. Most of the articles were in more than one repository, thus guaranteeing an unbiased search. We also used Google Scholar mainly to check if any additional articles could be found. After analyzing the documents and excluding duplicates, 28 articles were considered with data that could be used in this

review - basically, those that used MD and/or Monte Carlo (MC) to study NPs. It is interesting to highlight that, due to computational limitations, NPs are generally simulated rather than MPs. However, the observations can be extrapolated to systems containing MPs in many cases. Meanwhile, there are situations in which the focus of interest is the NP itself.

3.2 General remarks of the simulations and force field analysis

The number of scientific articles using the MD approach has been growing since 2011, with five published papers from 2011 to 2017 and more than 30 published papers from 2018 to 2023. The number of papers that report the use of MC is smaller (less than 5) than the ones that use MD. Before discussing each point proposed in the study, it is necessary to comment on how research is being conducted in this area and on important differences between the studies. Initially, it is possible to identify two groups of studies: the first focused exclusively on MD, and the second focused on MD as a complement to an experimental study or with other models. Within the first group, one can see the time scale used is mainly in the nanosecond range, reaching the microsecond range when using coarse-grained force fields [95, 96]. Within the second group, there are studies that simulate systems at the nanosecond scale, but also other studies that simulate at the picosecond scale, the latter mostly following an experimental study [15–17, 19, 97, 98]. Considering that all studies work with the same type of system, *i.e.*, a simulation box containing NP, a disparity between simulation times is noted, making a discussion about it worth having. This concern applies not only to studies of NPs through MD, but also to any study that uses this methodology. Braun et al. (2019), in their practice guide for MD methodology, emphasize that for condensed systems, the properties may exhibit a dependence on the fluctuations and correlations of movement between molecules, depending on the type of information desired. It is also mentioned that systems involving polymers and proteins have relevant timescales ranging from nanoseconds to microseconds, depending on the specific information sought.

The potential energy computed by MD and MC is associated with parameters related to pairwise interactions, bonds, angles dihedrals, among others. This information is computed into force fields that can be defined as the functional forms used to describe the system's intramolecular and intermolecular potential energies. Many are the force fields available in the literature and their modifications, such as OPLS-AA (Optimized Potentials for Liquid Simulations, All-Atom) [99, 100]; AMBER (Assisted Model Building and Energy Refinement) [101]; CHARMM (Chem-

istry at Harvard Macromolecular Mechanics) [102]; GAFF (General AMBER Force Field) [103];TraPPE (Transferable Potentials for Phase Equilibria) [104]; COMPASS (Condensed-phase Optimized Molecular Potentials for Atomistic Simulation Studies) [105]; DREIDING [106]; and CFF93 [107]. They were developed based on quantum mechanical calculations or experimental data. When working with molecular simulation, one of the main concerns must be the force field selection. In this session, we discuss the main force fields used in the parameterization of NPs, the software used, and the chosen water models.

We noticed that seven studies used some version of the Material Studio program [108] with the COMPASS force field [105], which is already available in the simulator program. These studies have both MD and experimental sections and deal with the sorption of contaminants on NP. Most experiments use between 100 and 500 ps of simulation time before analyzing the results. As already mentioned, relevant time scales for these systems should be higher in magnitude. An interesting fact is that none of them considered the presence of water, even if implicit, neglecting the fact that the phenomenon of interest occurs in an aqueous medium.

The most used MD simulator to study NP was Gromacs [109], with 16 studies using its versions. The force fields were more varied in this case, but eight of those studies chose to use the GROMOS (GROningen MOlecular Simulation) [110] in one of its versions. Five investigations used the LAMMPS simulator (Large-scale Atomic/Molecular Massively Parallel Simulator) [111], while one used the Gabedit program (along with the AMBER force field) [112]) and another used the Amber molecular dynamic package [113]. Of the works that used LAMMPS, two used OPLS-AA with SPC/E (extended simple point charge model) water model [114].

Regarding the force field only, two publications combined GAFF [103] and TIP3P water model [115]. The studies that used GAFF obtained the partial charges from different methods of quantum calculations. One publication combined TIP3P to water and a version of GROMOS to the NP. Two publications combined TIP3P to water and a version of CHARMM to the NP. Three publications used OPLS-UA [100]. Unfortunately, many papers fail to report essential details on models and software used. The references in this paragraph are related to force fields. To check which force fields each author used in their work, see table 3.1.

A force field development was carried out for polystyrene (PS) and polypropylene (PP). It was specially designed for the interaction between these plastics and lipids. In other words, this force field (MARTINI coarse-grained (CG)) [116] is compatible with the popular MARTINI force field for lipids [117], and it was used in five different publications for experiments with simulation time from at least 100 ns to 20000 ns. Tables 3.1 and 3.2 summarize the number of published studies on NP simulations via MD and the force fields used to model NP and water in each of them. Moreover,

Hollóczki and Gehrke [118] used the Automated Topology Builder and Repository (ATB) version 3.0, a website that provides classical molecular force fields for novel compounds [119]. The investigation of Hollóczki and Gehrke [118] together with the studies from Table 3.1 are the referred articles in this literature review. Figure 3.1 presents the number of articles that use each force field to model NP/MP and water model.

Force Field	Number of articles	References
AMBER	1	[120]
Compass	6	[15–17, 19, 121, 122]
CHARMM	2	[123, 124]
GAFF	3	[125–127]
GROMOS	8	[21, 97, 98, 128–132]
MARTINI	6	[95, 116, 133–136]
OPLS AA	3	[38, 135, 137]
OPLS UA	3	[95, 116, 138]

Table 3.1: Scientific publications applied to simulate NP via MD or MP and the force fields used to model the NP in each of them, identified by authors.

Water Model	Number of articles	References
MARTINI	1	[134]
SPC	1	[129]
SPC/E	2	[38, 137]
TIP3P	5	[98, 123–125, 127]
TIP4P	1	[139]

Table 3.2: Scientific publications applied to simulate NP via MD or MP and the force fields used to model the water in each of them, identified by authors.

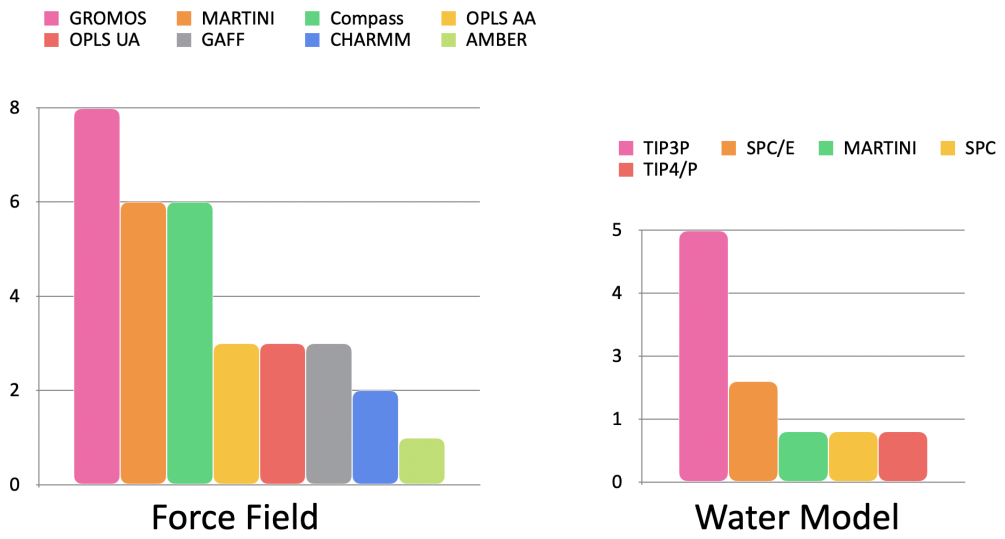


Figure 3.1: Distribution of scientific publications according to the force fields used to model NPs and MP and water.

3.3 The effects of nanoplastics in the structural and dynamic properties of biological membranes

Biological membranes are complex structures composed basically of proteins and lipids stabilized by dynamic cooperative non-covalent interactions [140]. They are a permeable protective barrier of the cells involved in relevant functions, namely sensing, transport, adhesion, and recognition processes. They consist of a bilayer of lipid molecules and have functions such as the control of substances (*e.g.*, ions, nutrients, waste) into and/or out of cells, keeping toxic substances outside the cells as well as separating vital, and often incompatible processes inside the cells [141]. In the membrane, it is possible to have different compositions of lipids with different cell receptors, their composition and formation being a universe of possibilities. Biological membranes are very complex and, in addition to the lipid layers that allow or prevent the diffusion of smaller molecules, some proteins form channels that can allow free diffusion into and out of the cell and channels that only allow specific passage of some compound [142]. The imbalance of these processes may be linked to diseases such as cancer, neurodegeneration, and muscular dystrophies [143]. The manipulation of membrane dynamics has also been associated with anesthetic effects [144]. Thus, understanding, at the molecular level, how the interaction mechanisms of membranes with the environment work can influence our comprehension in more than one field of knowledge. In this session, we discuss recent advances in studies to understand how lipid membranes behave in the presence of NP contaminants through molecular simulation techniques.

The simulations made by Hollóczki and Gehrke [118] show that a membrane

composed of 2 × 300 molecules of 1-palmitoyl-2-oleoyl-phosphatidylcholine (POPC) (*i.e.*, phospholipid commonly found in cell membranes) readjusts itself in the presence of nanoparticles of polyethylene with about 5 nm in diameter. The membrane rearranges to cover more of the NP, and the NP surface can rearrange itself to almost double in size in the presence of the membrane. The conformational changes gradually cause the membrane thickness to increase and the average area of each lipid to decrease during the 200 ns course of simulation time. In addition to structural changes, the results show that there are changes in dynamics as well since the presence of NP facilitates lipid movement within the membrane. Thus, it is suggested that the presence of NP significantly affects biological membranes [118].

Bochicchio *et al.* [95] conducted a study using coarse-grained simulations of polyethylene (PE), PS, and PP with diameters around 7 nm interacting with lipid membranes of POPC. All NPs quickly entered the membrane and changed their behavior from solid to liquid at room temperature. Depending on the type of NPs and on their degrees of polymerization, one can observe different situations: PE chains tend to aggregate when in a high polymerization degree, unlike the other two NPs whose chains tend to separate from each other, as shown in Figure 3.2. An interesting topic of this work is the study of heterogeneous membrane systems (those composed of ternary lipid mixture exhibiting liquid-ordered/liquid-disordered phase separation). These membranes are formed by more than one type of lipid, so it is possible to observe the dynamics of a more complex system. Once again, the result was related to the type of polymer: the separation of the lipid phase is disadvantaged by PP, while PS stabilizes the lipid phase, and PE modifies the boundary topology and causes cholesterol depletion from the liquid-ordered phase. The authors emphasize the need for further studies to better understand the toxicity of NPs that humans have come increasingly in contact with recently. This study follows up a previous one [96], which is simpler in the variety of compounds and deals with different sizes of sets of PS chains and a POPC membrane. Initially, they carried out an extensive study of the coarse-grained force field and, only after good agreement with the OPLS-UA force field, they performed the simulations with PS chains formed by 10, 20, and 100 monomers. In equilibrium, the set of shorter chains (with less than 100 monomers) were dispersed in the membrane, not aggregating, whereas the one with 100 monomers preferred to be concentrated in the center of the membrane. The results show that PS alters the properties of the membrane, significantly increasing its compressibility modulus and decreasing its bending modulus, which indicates a structural change in the membrane in addition to affecting its lateral organization.

In the wake of these last two investigations, a recent publication presented experimental and MD results, introducing small chains (with 25 styrene monomers) in

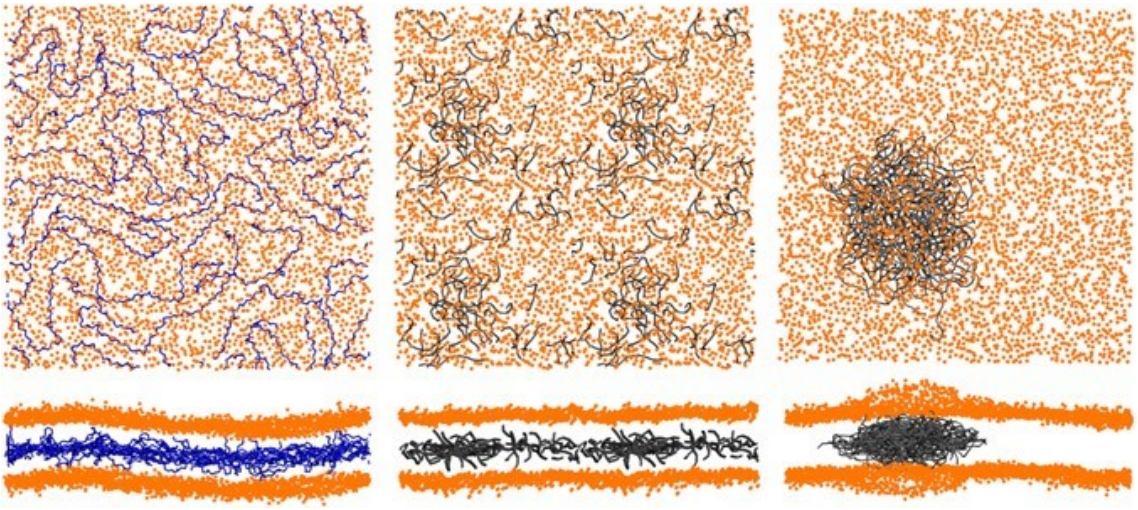


Figure 3.2: Typical distributions of the polymers inside pure POPC membranes (lipid:polymer mass ratio of 6.6%). Two views of the membrane (only head beads, in orange) are shown for each configuration: from the top and from the side. Left: long PP chains (in blue). Middle: short PE chains (in gray). Right: long PE chains (in gray). Reproduced from Bochicchio *et al.* - License CC BY 4.0 (http://creativecommons.org/licenses/by/4.0/).

dipalmitoyl-phosphatidylcholine (DPPC) lipid bilayers [138]. The authors carried out the equilibration for 50 ns of 3 systems composed of the DPPC membrane and different mass ratios of PS. Because they use the OPLS-UA force field (*i.e.*, coarse grained-force field), it was possible to simulate up to 2 microseconds in the production stage. The experimental and MD results complement each other and lead the authors to believe there is a critical concentration in partial segregation of PS chains within the membrane. Similarly to Hollóczki and Gehrke [118], Bochicchio *et al.* [138] demonstrated changes in membrane thickness. The authors reported a nonlinear increase in diffusion coefficients with the PS mass ratio [138]. For the bending modulus values, the behavior was inverse in the presence of PS. There was a substantial decrease in bending modulus compared to the total absence of PS. The results confirm that the effects of NPs on human health cannot be underestimated, and that concentration is a factor to be analyzed.

The last study to be covered by this section brings an exciting view of how treating water with chlorine can affect the MPs to the point of increasing the toxicity of these MPs [98]. The authors conducted experiments, and, using MD in accordance with the methodology of [138], they compared the system of pristine PS MPs and chlorinated pristine PS MPs (with chlorine- and oxygen-containing functional groups) in contact with bilayer membranes of phosphatidylcholine. Simulations showed that MPs interact differently with membranes. The authors speculate that the increased cell membrane permeability caused by chlorinated PS might be due to

C-Cl bonds. Consequently, the presence of chlorinated pristine PS MPs can cause even more damage at the cellular level [98].

Dai *et al.* [136] performed extensively both experimental and molecular simulation investigations seeking to better understand the interaction between different types of bacteria with neutral, positively charged (PS-NH_3^+) and negatively charged (PS-COO^-) PS. Considering the indispensable work of bacteria in the environment, the authors point out that one of the problems for the plastic biodegradation process is not yet a practical remediation nor a proven recycling strategy is the lack of understanding of the mechanisms of molecular interaction between NPs and bacteria. Moreover, this knowledge is important for comprehending the cycle of NPs in the ecosystem and their potential risks. In addition to experimental work, three independent 100 ns simulations were performed for each type of NP. The positively charged PS showed a stronger interaction with the membrane and the negatively charged one showed a weaker interaction with the membrane. These results indicated that electrostatic attraction promoted the ready penetration of PS-NH2 with the overall anionic phospholipid cell membrane. There is a dependency on the surface charge properties of NPs and bacterial cell envelope structures so the bacteria can absorb the contaminants.

One of the major concerns regarding NPs is their ability to interact with the largest organ in the human body: the skin. With this in mind, through experiments and molecular simulations, Cheng *et al.* [123] investigated the interactions between PS NPs and a model of the Stratum corneum membrane, which is the first barrier of the skin. The authors investigated whether the functionalization of the surface of the NPs and its size will influence the interactions. The information was generated from the results of density profiles, the order parameter responsible for quantifying the disorganization caused in the membrane due to the NPs, and the radial distribution function for the analysis of different functional groups of the PS with the membrane. The simulation results confirm the experimental results and show that PS altered with functional groups present a greater interaction with the lipid membrane. This study shows the potential risk to human health from exposure to NPs through the skin.

Yuan *et al.* [132] used the classification of emerging contaminants to define NPs and simulated the first interactions that NPs would have when coming into contact with humans through breathing. When inhaling contaminated air, the first surface that NPs come into contact with is covered by a layer of hydration. Thus, they carried out simulations containing water. First, they analyzed the radial distribution function. The dynamics with the water changed the arrangement of NPs, and the NPs became more compact in the initial layer of the pulmonary membrane. After this first analysis, the NPs were placed in contact with the lung membrane model

composed of DPPC bilayers. Depending on the type of NP, the results show two possible behaviors:

i) the NPs are brought into the cell (PP and PVC);

ii) an adhesion to the surface of the lipid layer is observed (PS, PET, PLA).

When the former case occurred, after crossing the first lipid membrane, the NPs began to break into smaller chains that ended up dispersing within the membrane. In the latter case, when the NPs adhered to the membrane, they remained in this state throughout the simulation and might suffer only slight deformations. The membrane also presented some changes because of the interaction with the NPs. A pore was formed if NPs passed through the membrane; however, with time, the membrane rearranged itself and the pore was closed. NPs also contributed to decreasing the thickness of the lipid layer which led to increased premature destruction of red blood cells by disruption of the plasma membrane. This result differs from the work of Hollóczki and Gehrke [118] whose analysis showed an increase in the thickness of the lipid layer of the membrane formed by POPC when in contact with NPs.

All studies showed changes in lipid membranes when in contact with NPs or MPs. Considering the biological functions of membranes, it is plausible to conclude that these NPs/MPs somehow affect the cellular environment. All investigations suggested that further investments in this field are needed to properly assess and control the potential negative effects of NPs/MPs on the environment and human health.

3.4 Nanoplastics affecting the folding of proteins

Proteins play a substantial role in human health. They are critical for tissue growth and maintenance [145], and for various biochemical reactions when proteins take the form of enzymes, with functions such as digestion, blood clotting, energy production, and muscle contraction [146]. Equally important, proteins can also act as hormones that are chemical messengers responsible by the communication between cells, tissues, and organs [147]. Additionally, they regulate the pH of body fluids [148], such as blood and stomach acid, act acting in the body's defense [149]. In recent years, studies that associate the presence of NPs in the human body with changes in protein structures began to emerge. The MD methodology is currently used to understand the dimension of these changes and their possible effects on human health. It is proven that NPs tend to interact with proteins to the point of modifying their secondary structures, with the result being protein denaturation [137].

Hollóczki and Gehrke [137] studied four types of NPs interacting with a series of proteins, namely PE, PP, nylon-6,6, and polyethylene terephthalate (PET). They showed, for instance, that the amino acids polarity is a relevant factor in their adsorption on NPs. Non-polar amino acids such as phenylalanine and tryptophan tend to interact highly with NPs and basically all amino acids in solution adsorb to NPs. They can form a micelle around NP, showing that the hydrophobicity of NPs can be masked by proteins, affecting their solubility and ability to aggregate [137]. It is interesting to highlight that the interaction between NPs and tryptophan zipper induced no significant changes in its overall structure, regardless of the adsorption of the peptide on the surface of the NP. However, the lack of structural rearrangement at the end of the simulation does not mean that the NP does not affect the protein structure because the time scale for this to happen could be greater than that available for the simulation. Moreover, a α-helix composed of 12 alanine adsorbed on hydrophobic surfaces of NP, and mainly for PE and PP, the nanoparticle rearranged to incorporate the peptide, affecting the conformation of the protein, as shown in Figure 3.3. Another relevant point is that the results differ greatly depending on the NP, *i.e.*, the problems caused by exposure to NPs are different depending on their type. This was the first article that had Hollóczi, notably the author of most publications regarding the study of NPs with molecular simulations, published in said field.

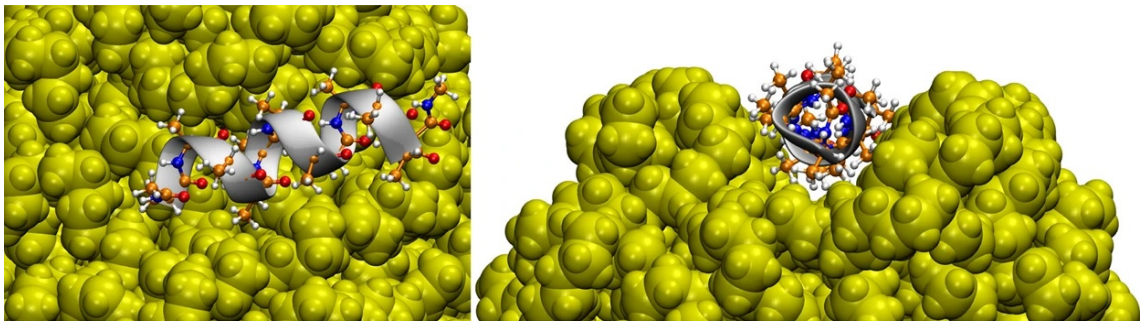


Figure 3.3: Three-dimensional structure of the helical peptide (composed of 12 alanine units) on the surface of a PE nanoparticle (yellow) from two views. Reproduced from Holloczki *et al.*- License CC BY 4.0 (http://creativecommons.org/licenses/by/4.0/).

Later, Hollóczki [38] single-handedly published an article on the same topic. In addition to MD, the study used the semiempirical extended tight-binding GFN2-xTB calculations. In order to overcome the limitations of MD regarding the time scale and the energetic barriers of the restructuring of compounds as proteins, which were pointed out even in the previous work [137], Hollóczki [38] discussed the use of simulated annealing (SA) strategy. In this methodology, one runs a simulation with

the system at high temperatures, allowing several conformations to be accessed, and then, the system is gradually cooled to, ideally, the minimum free energy level. Hollóczki [38] used this methodology to find the best conformations for MD and nylon-6,6, reaching the conclusion that the temperature range for carrying out the SA must be approximately between the condensation and freezing temperatures of the compound. This methodology proved to be quite efficient in revealing important structural information. Peptides' structures were, thus, optimized through quantum chemistry and then submitted to SA. The next step was to run SA for the peptide-NP pair. About 150 simulations were performed for each one of them. Further optimizations were performed using MD and quantum chemistry at the end of the simulations, and then the adsorption, interaction, and reorganization energies were defined and calculated. In this study, the two plastics influenced the stability of the secondary structure of the simulated peptide, corroborating previous results. The need for further studies to understand the consequences of these changes is also highlighted.

In a more recent study, Li *et al.* [21] performed an experimental investigation coupled with a simulation study of insulin fibrillation in the presence of NPs and various contaminants. They chose insulin to represent the protein because it is a model already widely used in the literature in the study of protein fibrillation and conformational changes. The research aimed to examine whether common organic contaminants (pyrene, bisphenol A (BPA), 2,2, 4,4-tetrabromodiphenyl ether (BDE47), 4,4-dihydroxydiphenylmethane (BPF), and 4-nonylphenol (4-NP)) enhance the abilities of NPs to accelerate insulin fibrillation as well as carry out a molecular study of the mechanism of action. By MD, the insulin, pyrene insulin and PS insulin systems were studied. Although one of the goals was to examine the three molecules' dynamics together, the simulations were done in pairs: pyrene + insulin, insulin + PS, and PS + pyrene. The presence of PS had a more significant effect on the conformational transition of insulin than that which was observed with the presence of pyrene, which is consistent with the trends in the experimental results. It was possible to conclude that van der Waals forces predominate by decomposing the binding free energy of the interaction of insulin with PS or pyrene. These results complemented previous studies of the mechanism by which NPs promote insulin fibrillation.

Another study involving NPs and proteins deals with the effect of using a PEG spacer on small peptides in a process commonly called PEGylation [129]. This process is used to stabilize, immobilize or modify biomolecule properties. The authors sought to understand, at the molecular level, the structural effects caused on the peptides due to the PEGylation. The peptides were simulated under three conditions: free in solution, attached to a PEG spacer, and attached to a PEG spacer

constrained to a 2-dimensional lattice to mimic the display of a peptide on the surface of a microsphere. The results suggested that the non-charged peptides in a PEGylation situation do not undergo a noticeable conformational change. However, peptides with high charges, both negative and positive, suffered conformational changes. These results show the need for specific studies and significant investment in this research field. Since we could not find additional reports focusing on this specific subject, comparing results was impossible. This fact alone justifies the relevance of more investment to develop the field further. The mentioned study is presented here distinctly from the other works in this section because its author discusses a process in which peptides are artificially changed through a PEG spacer while acting as a single unit instead of examining the interaction between the protein and the MPs separately.

Enyoh *et al.* [150] used different simulation methodologies (molecular docking, MD simulation, and the factorial design method) to indicate dangerous combinations of MPs for aquatic organisms. The organism chosen was the zebrafish, which is an organism commonly used as a model in research due to its physiological and genetic characteristics close to mammals, especially considering the fact that 84% of the genes associated with human genetic diseases are also present in this organism [150–152] . Therefore, the protein chosen for the study was the cytochrome P450 present in zebrafish liver. This protein is commonly used to obtain biological responses to pollution effects. Ten types of MPs were part of the study, namely: PS, PVC, PU, polymethyl methacrylate (PMMA), PA, PET, PE, PP, polychloroprene (CR) and polycarbonate (PC). The PC had the highest binding affinity to the cytochrome P450 and the highest zebrafish toxicity. The study also concludes that hydrogen bonds and hydrophobic effects are the main factors that influence the affinity between MPs and the protein. The authors showed that with different MPs combinations, the results of aquatic toxicity vary. The combinations of PU, PS, and PP with PA and a single CR chain are the ones that most affect the zebrafish protein. Even though the results are quite interesting, it should be noted that the MD simulation ran for only 10 ps.

In general, the study of interactions between NPs and proteins shows that a single type of NP cannot be used to understand the possible effects of contacting all types of NPs with proteins. More than one of these studies discussed that polarity is fundamental in how NPs and proteins interact. It is also possible to conclude that this type of study is necessary to indicate new research routes in the subject. Different pairs, peptides, and NPs should be analyzed to understand the problem to a greater. The present number of investigations in this area is still insufficient to provide a general dimension of what must be approached.

3.5 Sorption of contaminants on nanoplastics

Research has been conducted to investigate the effects of microplastic as a vector for POP (persistent organic pollutants) contamination in the aquatic environment [33, 34, 153]. MPs and NPs present physicochemical properties, such as surface area, shape, chemical composition, functional groups, and surface charge, that promote their interactions with different organic and inorganic pollutants in the environment [33, 154–156]. Although some studies emphasize that the ingestion of microplastic contaminated with POP does not increase the risk for marine animals when compared to the flux of POPs bioaccumulated from natural prey [153], others have focused on the potential harm of this combination, highlighting a possible existence of synergistic effects between MPs and POPs [33, 34]. However, it remains unclear whether this relationship actually exists under environmentally relevant conditions. More studies are needed to determine the actual capacity of NPs/MPs to transport associated pollutants that result in trophic transfer and bioaccumulation in the food chain. Experimental studies should be more widely developed based on actual conditions, mainly the concentration and nature of both MPs/NPs and contaminants commonly found in aquatic environments, combined with ecotoxicity assays with organisms of different trophic levels. While the experimental development continues to grow, simulation studies help indicate the paths to follow. In this field, in terms of MPs, the most studied polymer types consist of PE, PP, PS, polyamide (PA), and polyvinyl chloride (PVC) [157, 158].

Organochlorine pesticides (OCP), polychlorinated biphenyls (PCB), polybrominated diphenyl ethers (PBDE), and PAH are some chemicals that can accumulate on MPs [34, 157, 158]. In addition, MPs and NPs can release chemical monomers and additives with proven toxicity that are incorporated into materials during their manufacture, such as plasticizers, flame retardants, antimicrobial agents, and pigments [158]. The sorption depends on the system properties (*e.g.*, temperature and pH) and the physical-chemical characteristics of the polymer and the contaminant.

The main sorption mechanisms between chemical compounds and MPs/NPs are hydrophobic interaction, electrostatic interaction, pore filling, van der Waals forces, hydrogen bonding, and $\pi - \pi$ interaction [159]. Experimental investigations can provide evidence of the most likely type and mechanism of interaction; however, computer simulations are a valuable tool to predict and understand these processes. Moreover, the simulations can guide the experimental assays to look for compounds with harmful chemical characteristics or interact more with a specific polymer.

Guo *et al.* [16] analyzed the sorption of sulfamethazine on PE, PS, PP, PA, PET, and PVC. To build the simulation box, only one polymer chain and one molecule of sulfamethazine were added to the vacuum for each system. The number

of monomers varies from 100 to 300 depending on the polymer type. Besides, the simulation was performed in NVT ensemble at 298 K for 500 ps. Guo *et al.* [16] observed a dominance of electrostatic interactions in the sorption of PA, PS, PVC, and PP. In contrast, van der Waals interactions were dominant in the sorption on PE and PET. The computed adsorption energies decreased in the order PA > PET > PE > PVC > PS > PP.

Another antibiotic that was investigated was the tetracycline hydrochloride and its derivatives (chlortetracycline hydrochloride and oxytetracycline hydrochloride) [17]. The simulation box consists of a PE chain with 300 monomers degree of polymerization, and one antibiotic molecule. The simulation was performed in NVT ensemble at 298 K. The interaction energy reveals that the adsorption capacity of tetracycline hydrochloride on PE is the weakest, whereas the chlortetracycline hydrochloride presents the highest adsorption capacity. Chen *et al.* [17] also analyzed the radial distribution functions, which indicated a preference for the non-bond interaction between the carbon atoms of PE and the oxygens in the tetracycline molecules.

The adsorption of three pesticides (difenoconazole, buprofezin, and imidacloprid) on the PE molecular chain with polymerization degree equal to 160 was also evaluated, and interaction energies were found to be statistically the same among them [15]. Besides the MD simulation, they used the Grand Canonical MC method without giving more details about the properties analyzed with this method [15].

Regarding PAH, Yang *et al.* [121] analyzed the sorption of pyrene, 1-methylpyrene, 1-hydroxypyrene, 1-aminopyrene, and 1-pyrenecarboxylic acid on PS with polymerization degree equal to 100, and found that said interaction had higher levels of energy when compared to sulfamethazine [16]; however, the values for pyrene and its derivative were very close one another. Following the experimental evidence and the computational results, the pore-filling and the hydrophobic and $\pi - \pi$ interactions played an essential role in these adsorptions [121].

The sorption of $SrCl_2$ on PA, PS, and PP was also investigated, and the electrostatic interactions were the dominant mechanism by decomposing the energy contribution of MPs-$SrCl_2$ systems [19]. The simulation box was built following the methodology described by Guo *et al.* [16]. For $SrCl_2$, the adsorption energies followed the order PP > PS > PA, smaller than NPs and organic pollutants.

It is important to highlight that the simulations reported by Guo *et al.* [16, 19], Li *et al.* [15], Chen *et al.* [17], and Yang *et al.* [121] were conducted in the absence of water, even if implicit. The simulation boxes were composed of one polymer chain, one molecule of the pollutant and a vacuum layer. Although this kind of simulation requires less computational time, the validation is questionable when compared to experimental results in an aqueous medium, since the simulations were conducted

in a vacuum.

Cortés-Arriagada [120] studied the co-transport of BPA with PET using density functional theory (DFT) with B3LYP functional at def2-SVP basis sets. The solvent was considered implicitly, *i.e.*, a continuum medium characterized by the dielectric constant. To obtain an initial configuration of the PET NP, MD was performed by folding a single polymer chain described by AMBER force field using NVT ensemble, followed by energy minimization steps. The final nanoPET model was optimized *a posteriori* at the DFT level. Due to the nucleophilic nature of the outer surface of nanoPET and the hydrophobic characteristic of BPA, mass transfer and intraparticle diffusion of the pollutant into the NP were observed. An interplay between dispersion and electrostatics intermolecular interactions occurred, with the former dominating the inner surface adsorption, whereas the latter dominated the outer surface adsorption.

Feng *et al.* [128] aimed to understand the process of aggregation of humic acid molecules (HA) with the contaminant benzo[a]pyrene (BaP) and heavy metal ions (Cu^{2+}) in an aqueous solution, as well as the influence of PS, PP, PVC, PET, and PE in these systems. Simulations with NP, HA, BaP, and Cu^{2+} show a competition between HA and BaP to adsorb on NP. When HA wins the competition and adsorbs on NP, it exposes carboxyl groups that offer interesting binding sites for Cu^{2+} adsorption. The results indicate that PS has the highest capacity of adsorbing BaPs. The motivation to study this system comes from the fact that environmental factors (such as dissolved organic matter - in the article represented by HA) can influence the interactions of NPs and contaminants. Hence the necessity to consider these factors in more detailed studies.

PFAS are substances widely used in industry because they are responsible for several interesting features that make everyday life easier, such as making stain-resistant fabrics and creating non-stick surfaces. Because of the fluorine carbon bond in these components, they have difficulty reintegrating into the environment. Thus, PFAS are pollutants present in different environments and they end up following paths similar to those of micro and NPs. Enyoh *et al.* [122] use both MD and MC simulations to understand the adsorption of PFAS and MPs, more specifically, PE MPs. They showed that MPs can be PFAS carriers. However, some considerations about the methodology are necessary. The first is that Grand Canonical MC is used with a single chain of PE as the sorbent and the PFAS as the sorbate in a box without water until equilibrium is reached. Furthermore, MD studies were also conducted in the absence of water. Moreover, the results obtained from MD were all equilibrium properties, which does not justify the use of both methodologies since both can be used to acquire these properties. However, MC is not ideally used for anisotropic systems because of its high computational cost [160].

There are infinite combinations of pollutants and NPs that humans can contact, and each one can result in different consequences. However, the more studies in this field, the more it is possible to extrapolate behavioral trends. Thus, the investment of resources in this area becomes essential.

3.6 Other Applications of Molecular Simulation in the Study of Nanoplastics

Although most MP/NP studies via molecular simulation can be fitted into the sections above, some address more specific issues. Zhang *et al.* [97] conducted combined research with MD, ratio normalization, and molecular docking methods to understand biodegradation of phthalic acid esters (PAE) derivatives in marine and fresh-water environments. PAE are commonly present in plastics and confers their characteristics of malleability and plasticity; thus, they were chosen to serve as a model for the study of the biodegradability of plastics in marine environments. As we have already discussed, the marine environment ends up being the final destination for tons of plastics every year, which is why the study is so important. As a result, five PAE derivatives were designed with excellent biodegradability as a goal, as well as functionality in mind.

Following the same train of thought, MD and molecular docking appeared in Lameh *et al.* [131] study about mutating existing enzymes to facilitate the biodegradation of PET and PP. The authors modified certain amino acids of the enzyme *Archaeoglobus fulgidus* (AFEST) and compared the changes in binding energy with plastics. They claimed that modifying an existing enzyme is the best approach, from the biotechnology viewpoint, to solve our plastic waste problem.

Moreover, Wang *et al.* [125] shared some exciting findings using molecular mechanics Poisson-Boltzmann surface area method and MD simulations. Through prior knowledge that the hydrolase enzyme RPA1511 (obtainable from *Rhodopseudomonas palustris* bacterium) can efficiently depolymerize polylactic acid (PLA) plastic, they sought to understand which amino acids are responsible for this action. The binding affinity data showed that the RPA1511 could also degrade other polyester plastics. These results open doors for the study of more biodegradable plastics.

Another interesting research was that of Ramalho *et al.* [127]. They carried out extensive molecular dynamic simulations of PE, nylon 6 and PET in an aqueous environment to achieve aggregation, mainly from their conformational behavior, of polymer chains after exposure to water. Over 200 ns of simulation, the three plastics had different responses, and, in the end, their chains equilibrated in the following

ways: compacted and ordered, almost like a crystal, for PE, globular chains for nylon6, and, for PET, tangled chains with the aromatic rings preferably oriented in parallel. Understanding how these plastics organize themselves when they are in such small particle form demonstrates how other contaminants can adsorb onto their surfaces and cause even more damage.

Since the presence of NPs in water is a reality, and, with research showing their potentially harmful effects, a line of study arises naturally: the search for methodologies for the removal of NPs from aqueous environments. Sarcletti *et al.* [126] came up with the idea of removing NPs from water by applying an external magnetic field. They developed superparamagnetic iron oxide nanoparticles (SPION) that attract NP; they worked with both structural analysis, and MD which supported the experimental results.

As far as we could track, the work by Dettmann *et al.* [133] is the only one that applied MD to NPs found in soils. The authors used carbon nanotubes (with and without functional grouping) as a model for hydrophobic cavities and surfaces to represent an existing structure in organic soil particles. They carried out up to 500 ns of coarse-grained MD simulations of hydrophilic and hydrophobic NP, carbon nanotubes, and water. NPs behave as expected concerning the hydrophobic carbon nanotubes according to their hydrophobicity. Regardless, the study stand out for being the first step in understanding processes in environments such as soils.

The work by Li *et al.* [134] about the impact of NP inhalation on the lungs is extremely interesting and pertinent. They investigated five types of NPs varying their sizes, surface charges, and molecular weights, and exposed them to interaction with lung surfactant (LS) film, both in the alveolar fluid and at the air-water interface. The authors pointed out that although the study does not yet represent an authentic environment in its complexity, the type of NP, its size, surface charge, and molecular weight were factors that modified the results in the interaction with LS. A study along the same lines (except that, rather than the concern about inhaling MPs and NPs, it deals with impacts on the human intestinal tract) is that of Tan *et al.* [130], which shows results on how five different types of MPs reduce lipid digestion when ingested with food. This work presents MD and experimental results, with the former corroborating the latter's findings and going further in understanding interactions between NPs and lipids. The authors conclude that the interaction between lipid droplets and MP is expected to play an influential role in reducing lipid digestion.

Zheng *et al.* [161] addresses an interesting problem in the fashion industry: the abundant use of water and the cost of waste treatment in the process of dyeing polyester fabrics. Because of its nanostructure, the PET fiber presents difficulties in allowing dye penetration. The purpose of the work was to use MD simulation to

understand the swelling mechanism of PET, besides the study of PET dyeing with non-aqueous solvents. The changes in the microstructure of PET were analyzed in three systems: pure PET, PET/water, and PET/decamethylcyclopentasiloxane, both water and decamethylcyclopentasiloxane were used as solvents. The authors found that just by adding water to the PET, the glass transition temperature of the polymer decreases, the diffusion coefficient increases, and the free volume increases. When adding the decamethylcyclopentasiloxane, the glass transition temperature is further reduced, and it is easier to dye PET. Although the interesting conclusions, the equilibration time is something to be aware because systems were considered equilibrated only after 600 ps of annealing plus 200 ps of simulation.

Zhang *et al.* [162] investigated by MD simulation the interplay between two very current topics: MPs and the virus responsible for COVID-19. Considering that environmental pollution and habitat conditions can facilitate transmission and increase the risk of exposure to SARS-CoV-2, the authors decided to investigate what behavioral changes the virus could acquire due to the interaction with MPs. Due to the undeniable presence of MPS in the environment, it is plausible to conclude that the virus may be coming into contact with these contaminants. Interactions between 5 types of MPs and a SARS-CoV-2 RNA fragment were investigated. They also compared the interaction results with the results obtained with RNAs from the SARS-CoV-1 virus and the Hepatitis B virus. Hence, they used structures of the following RNA fragments and nucleocapsid proteins, identified by the PDB ID: 6XRZ (SARS-CoV-2), 1XJR (SARS-CoV-1), 6VAR (HBV), 6M3M (SARS-CoV-2), 1SSK (SARS-CoV-1), and 6HU7 (HBV). The simulations ranged between 223 and 310 K and were performed both in vacuum and in water. As a result, they showed that the affinity between the MPs and the virus responsible for COVID-19 is higher regardless of all the other variables. Bearing in mind the fact that other studies have already shown the presence of MPS in human lungs [43], it is worth seeking to understand the consequences of these interactions. However, the production time was very short: only 100 ps. Due to the urgency of the topic, further investigations would be interesting. The work also reviews the means of contact of MPs and the coronavirus with humans.

Bearing in mind the possible ways to monitor the presence of MPs in the environment, Awada *et al.* [124] used MD to complement experimental data on the development of a probe for selective detection of MPs. This probe was constructed from conjugated polymer NPs. The simulation brought light on the interactions and affinities between probe components and MPS. The research reported by Awada *et al.* [124] is very promising as it develops new ways of detecting a problem that is still being identified, which is the presence of MPs in the environment.

3.7 Final Considerations

The present literature review focuses on molecular simulation methodologies to study MPs and NPs, the force fields used, and the main findings.

Most scientific publications are very recent, which strongly indicates that the subject is growing in importance. It is important to highlight that MPs/NPs are released in very high quantities by human activities and they end up, mostly, in aquatic and marine environments. However, the interactions with and potential impacts on living organisms are largely unknown. All studies regarding the consequences of human contact with MPs/NPs have been simplified while showing that MPs/NPs interact with their surroundings, fundamentally modifying their characteristics.

The MD was the most used computational technique out of the theoretical methodologies applied, and, based on the results, it fulfilled its objective of showing the interactions at the molecular level. An obstacle, however, is the level of simplification that is necessary during simulation since natural systems, given their concrete complexities, are still outside the reality of investigations on a molecular level. Despite this, simulations can help a great deal to understand experimental data. The use of both experimental and computational approaches is in many scientific reports, and, in their conclusions, the authors have pointed out that they complement each other.

As this is a fairly new field of study, there is no protocol to date on how to simulate NPs. Therefore, the steps in the methodological approaches vary considerably from study to study. Among these differences, one can highlight the simulation time, the force fields applied, the presence or absence of water models, and how the polymer chains are built to be considered NP particles. It is necessary to discuss the validity of certain practices within the molecular simulation to create a more mature protocol based on the information accumulated.

In addition to MD, another promising option is the MC methodology. Although many publications presented equilibrium properties that can be accessed through both MC and MD, only one investigation reported the use of MC methodology. Due to how the system reaches equilibrium, MC could be an alternative to achieve simulations with shorter computational times. Between the results obtained, it is interesting to highlight that the interactions between all NPs and the environment cannot be understood through a single NP model. Depending on the NP type, the interactions, whether with proteins, lipids, or contaminants, are expected to differ significantly. Thus, each NP may cause a different impact when in contact with humans and other living organisms, which makes further studies even more pressing.

Chapter 4

Computational Methods: Molecular Dynamics

The theoretical understanding of physical phenomena plays a significant role in the development of knowledge. As technological advancements continue to unfold, new resources become available to advance science. Consequently, theoretical and computational methods have become indispensable in the field of chemical engineering [39, 163]. Within this domain, decisions must be made regarding the most appropriate methodology for each type of study. Much of this decision-making process is based on the scale at which the desired phenomena occur. For instance, when using the quantum mechanics theory, it is possible to monitor the behavior of electrons and atomic nuclei. In contrast, classical models employ statistical mechanics theory to study particle interactions [164]. The more detailed the model, the more complex the calculations become, leading to longer times required to obtain results and increased resource expenditure [163]. In simpler terms, the use of a model and theory of choice ultimately comes down to a cost-benefit analysis and one's specific interests.

The computational methodology of molecular dynamics (MD) simulation uses the fundamental concepts of Newtonian dynamics to obtain thermodynamic and transport properties, as well as to enable a deep understanding of the dynamics of a multi-particle universe [165]. In a MD simulation, we have the temporal evolution of particle motion governed by the classical equations of motion [40]. For a system with N particles, we have:

$$\begin{aligned} m_i \frac{\mathrm{d}^2 \boldsymbol{r_i}(t)}{\mathrm{d}t^2} &= \boldsymbol{F_i}(t) \\ \boldsymbol{F_i}(t) &= -\frac{\partial \mathscr{U}(\boldsymbol{r^N})}{\partial \boldsymbol{r_i}} \end{aligned} \tag{4.1}$$

where i is the particle index, m is the particle mass, $\mathscr{U}(\boldsymbol{r^N})$ is the interaction potential or potential energy, $\mathbf{r}$ are the particle coordinates, and $\boldsymbol{F_i}(t)$ is the force exerted on the particle i by the $(N$ - $1)$ other particles.

Potential energy governs the pairwise interaction between particles, and in most

systems, it can be described as:

$$\mathscr{U}(\boldsymbol{r}^{\boldsymbol{N}}) = \sum_{i} \sum_{j>i} \mathscr{U}_{ij}(\boldsymbol{r_{ij}}) \tag{4.2}$$

with $\boldsymbol{r_{ij}} = |\boldsymbol{r_i} - \boldsymbol{r_j}|$.

This is a system of differential equations that can be numerically solved by their discretization on a time step δt, which must be small enough that forces can be considered constant in this time interval. The first step of MD is to assign the initial position and velocity for each particle of the system. Then, the forces acting upon the particles are calculated, and finally, the new positions and velocities are deduced. Repeating this procedure many times makes it possible to obtain trajectories in the phase space [40]. At the end of equilibration, all the memory from the initial configuration should be lost. The next step is production, which provides information about time dependence and the magnitude of fluctuations in positions and velocities. It is important to highlight that the time average of a property can be related to the observable macroscopic property [41]. The information obtained during production time makes it possible to calculate the equilibrium and nonequilibrium desirable properties [40, 41].

In practical terms, due to computational limitations, the size of the particle set is on a nanoscale, as is the simulation time, which usually varies from femtosecond to nanosecond. A crucial aspect of these simulations is to know the timescale in which the desired observed motion resides. Macromolecules, in general, exert a huge range of characteristic motions on a large difference of timescales. These motions can vary from very fast and localized motions, like the stretching vibration of a hydrogen bond, to very slow and large-scale motions, like the folding of a whole protein. An overview of the different motions and their timescale can be found in Table 4.1 [1].

In a conventional MD, the total energy and the total linear momentum are constant, and therefore, it is most naturally formulated in the microcanonical ensemble (specified number of particles, total volume, and energy). Nevertheless, it is not an impediment to carrying out MD in other ensembles due to the existence of techniques that enable the correlation of the fluctuations in different ensembles. Thermostats can be included in the simulation to control the temperature; barostats can control the system's pressure; and particle baths can control the chemical potential [41].

4.1 Gibbs' Hypotheses

Since MD is based on statistical thermodynamics, it is subject to Gibbs' assumptions and to the statistical mechanics postulates [166].

The ergodicity principle applied to thermodynamic systems stipulates that the

Type of motion	Example	Timescale
Local		
Atomic fluctuations	Stretching vibrations	Femtoseconds (fs) to picoseconds (ps)
Side chain motion		
Medium-scale		
Loop motion	Active site conformation adaption, binding specificity	Nanoseconds (ns) to microseconds (μs)
Terminal-arm motion		
Rigid-body motion		
Large-scale		
Domain motion	Hinge bending motion, allosteric transitions	Mircroseconds (μs) to milliseconds (ms)
Subunit motion		
Global		
Helix-coil transition	Hormone activation, protein functionality	Milliseconds (ms) to hours (h)
Folding/unfolding		
Subunit association		

Table 4.1: An overview of the different types of motions and their timescale with some examples of phenomena [1].

time average is equivalent to an average calculated on a set of representative microstates of the system. This statistical average is also called the ensemble average. Considering M any macroscopic mechanical property, the ensemble average of M can be written as [166]

$$\langle M \rangle = \lim_{\Delta t \to \infty} \frac{1}{\Delta t} \int_{t_o}^{t} M(t) \mathrm{d}t \tag{4.3}$$

for a time Δt sufficiently long.Figure 4.1 illustrates the Ergodic Hypothesis.

Another important hypothesis is the principle of equal *a priori* probabilities, which determines that states with the same energy, volume, and number of specified particles (NVE, microcanonical ensemble) have the same probability of existence. A simple way to illustrate this is by considering 10 possible states, where the probability of each one's existence is 1/10. There is no state with a higher probability than the others [166]. Thus, mathematically:

$$P_j = \frac{1}{\Omega(N, V, E)} \tag{4.4}$$

where $\Omega(N, V, E)$ represents the system's degeneracy, *i.e.*, the number of possible microstates for the same NVE (number of particles, volume, and energy) condition. In this ensemble, degeneracy also serves as the system's partition function.

The entropy S is defined by

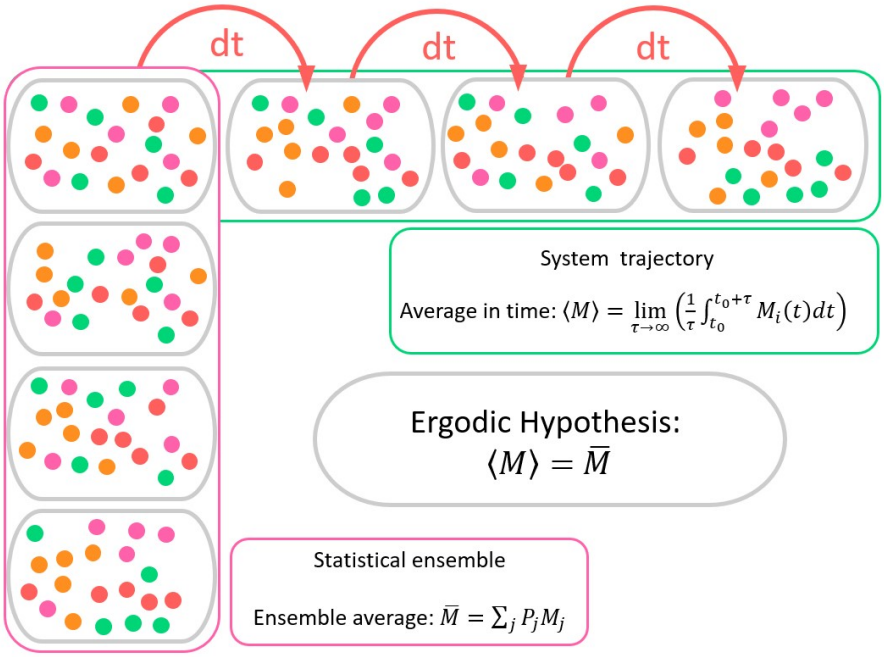


Figure 4.1: Ergodic Hypothesis Illustration

$$S(U, V, N) = k_B \ln \Omega(N, V, E). \tag{4.5}$$

where U is the internal energy, and T is the absolute temperature and k_B is the Boltzmann constant . From the first law of thermodynamics

$$\mathrm{d}U = T\mathrm{d}S - P\mathrm{d}V + \mu\mathrm{d}N, \tag{4.6}$$

therefore

$$\mathrm{d}S = \frac{1}{T}\mathrm{d}U + \frac{P}{T}\mathrm{d}V - \frac{\mu}{T}\mathrm{d}N. \tag{4.7}$$

where P is the pressure and μ is the chemical potential.

If the partition function Ω is known, the entropy is known, and other properties can be obtained as follows:

$$\frac{1}{T} = \left(\frac{\partial S}{\partial U}\right)_{V,N} = k_B \left(\frac{\partial \ln \Omega}{\partial E}\right)_{V,N} \tag{4.8}$$

With the internal energy U being the ensemble average of the values of E_j, in the microcanonical ensemble, energy is a specified variable, representing a single fixed value; thus, the average will be the same as E, hence $U = E$.

Therefore,

$$\frac{P}{T} = \left(\frac{\partial S}{\partial V}\right)_{U,N} = k_B \left(\frac{\partial \ln \Omega}{\partial V}\right)_{E,N} \tag{4.9}$$

and

$$\frac{\mu}{T} = -k_B \left(\frac{\partial \ln \Omega}{\partial N} \right)_{E,V,N_{i \neq k}} . \tag{4.10}$$

In classical Hamiltonian mechanics, we have that:

$$\frac{\mathrm{d}\mathcal{H}}{\mathrm{d}t} = 0 \Rightarrow \mathcal{H}(x) = \ const. \tag{4.11}$$

And, if we have a system evolving according to Hamilton's equations:

$$\dot{p}_i = -\frac{\partial \mathcal{H}}{\partial \mathbf{q}} \tag{4.12}$$

$$\dot{q}_i = \frac{\partial \mathcal{H}}{\partial \mathbf{p}} \tag{4.13}$$

where $\mathbf{p}$ is the momentum and $\mathbf{q}$ is the generalized coordinates of particle i and the dot $(\cdot)$ represents the time derivative. As the Hamiltonian $\mathcal{H}(x)$ is conserved through the equations of motion, the trajectory calculated through Equation 4.13 will naturally generate microscopic configurations belonging to a microcanonical ensemble with energy E.

The coordinate and momentum possibilities for each atom in a $6N$ dimensional space are defined as the phase space. The trajectory through the phase space is then the time evolution of a system in a MD simulation. The number of accessible microstates, as written before, defined by the partition function or the density of states, is given by the following equation for the microcanonical ensemble:

$$\Omega(N, V, E) = \frac{\epsilon_0}{h^{3N} N!} \int \delta[\mathcal{H}(\mathbf{p}^N, \mathbf{r}^N) - E] \mathrm{d}\mathbf{p}^N \mathrm{d}\mathbf{r}^N \tag{4.14}$$

Note that the constant ϵ_0 is irrelevant and will not affect any thermodynamic or equilibrium properties. However, this normalization constant has an energy unit and renders $\Omega(N, V, E)$ dimensionless, h is the Planck constant, and δ is the Dirac delta function. The macroscopic properties from MD are then obtained from the relation of the microcanonical partition function to the entropy S.

For the sake of convenience, it is not always desirable to fix the number of atoms, volume, and energy. Therefore, it is possible to work with different independent variables using various statistical ensembles. Each statistical ensemble corresponds to a different thermodynamic potential, resulting from a Legendre transformation of entropy or internal energy.

It is important to note that statistical ensembles are equivalent in a macroscopic system. In small systems where fluctuations are very large, they may not necessarily be equivalent, but they become so in the thermodynamic limit.

4.2 Statistical Ensembles

Table 4.2 summarizes the essential information about the main statistical ensembles.

Partition Function	Ensemble Name	Fixed Variable
Ω	Microcanonical	N, V, E
Q	Canonical	N, V, T
Δ	Isothermal-isobaric	N, P, T
Ξ	Grand canonical	μ , V, T

Table 4.2: Statistical Ensembles, partition function, and properties

The choice of the statistical ensemble to work with is made based on the most convenient properties to be fixed. The simulations conducted in this study were mostly performed in the isothermal-isobaric ensemble; for this reason, we delve a bit deeper into its theory. The theory regarding the other ensembles can be better explored in [39].

4.2.1 Isothermal-isobaric Ensemble

Let's consider a finite yet arbitrarily large system L enclosed by an isolated boundary. This means that L does not vary in total energy, total volume, or total number of particles, signifying that it has specified NVE (number of particles, volume, and energy). System L is further subdivided into numerous subsystems with specified numbers of particles, pressure, and temperature. Within these subsystems, the walls allow for heat exchange, and they are also capable of movement to adjust the volume and maintain pressure, but they do not permit mass exchange. For a given subsystem, we consider a set of $L-1$ other subsystems that function as thermal and pressure reservoirs. More details on this ensemble can be seen in [167].

We can then define different systems $\{n_{il}\}$ with some restrictions:

$$E_t = \sum_i \sum_l n_{il} E_{il} \tag{4.15}$$

$$V_t = \sum_i \sum_l n_{il} V_l \tag{4.16}$$

$$L_t = \sum_i \sum_l n_{il} \tag{4.17}$$

where n_{il} is the number of small systems with specified N, P, T, with volume V_l and quantum state i, and E_t, V_t, and L_t are the total energy, total volume and total number of systems of the supersystem, respectively.

The probability of each state in this ensemble will be:

$$P_{il} = \frac{\langle n_{il} \rangle}{L_t} = \frac{1}{L_t} \frac{\sum_{\{n_{il}\}} \Omega(\{n_{il}\}) n_{il}}{\sum_{\{n_{il}\}} \Omega(\{n_{il}\})} \tag{4.18}$$

The number of microstates possible given a distribution $\{n_{il}\}$, that is, its degeneracy, is:

$$\Omega(\{n_{il}\}) = \frac{(\sum_i \sum_l n_{il})!}{\Pi_i \Pi_l n_{il}!} \tag{4.19}$$

Taking into account that all states have the same probability, given a possible set $\{n_{il}\}$, we have various ways to organize the system. It is also worth noting that a maximum term dominates multinomial distributions. Therefore, we have:

$$P_{il} = \frac{\langle n_{il} \rangle}{L_t} = \frac{1}{L_t} \frac{\sum_{\{n_{il}\}} \Omega(\{n_{il}\}) n_{il}}{\sum_{\{n_{il}\}} \Omega(\{n_{il}\})} = \frac{n^*_{il}}{L_t} \tag{4.20}$$

with n^*_{il} being the most likely n_{il} given a distribution.

After some mathematical manipulations [167], it is possible to demonstrate that the probability can also be expressed in terms of thermodynamic properties as follows:

$$P_{il} = \frac{e^{\frac{-E_i}{k_B T}} e^{\frac{-PV_l}{k_B T}}}{\sum_j \sum_k e^{\frac{-E_j}{k_B T}} e^{\frac{-PV_k}{k_B T}}} \tag{4.21}$$

It is possible to relate it to thermodynamics as follows:

$$\Delta(N, P, T) = \sum_j \sum_l e^{\frac{-E_j}{k_B T}} e^{\frac{-PV_l}{k_B T}} \tag{4.22}$$

where $\Delta(N, P, T)$ is the partition function of the isothermal-isobaric ensemble.

We also have that entropy is:

$$S = -k_B \sum_i \sum_l P_{il} \ln P_{il} \tag{4.23}$$

Replacing equation 4.21 into equation 4.23, we have

$$S = -k_B \sum_i \sum_l P_{il} \left[-\frac{E_i}{k_B T} - \frac{PV_l}{k_B T} - \ln \Delta \right]. \tag{4.24}$$

Multiplying by $k_B T$:

$$TS = \sum_i \sum_l P_{il} E_{il} + P \sum_i \sum_l P_{il} V_l + k_B T \ln \Delta \left(\sum_i \sum_l P_{il} \right) \tag{4.25}$$

Only, we have by definition:

$$\sum_i \sum_l P_{il} = 1 \tag{4.26}$$

$$\sum_i \sum_l P_{il} E_{il} = \langle E \rangle \tag{4.27}$$

and

$$\sum_i \sum_l P_{il} V_l = \langle V \rangle \tag{4.28}$$

Therefore,

$$TS - \langle E \rangle - P \langle V \rangle = k_B T \ln \Delta = -G \tag{4.29}$$

$$G(T, P, N) = -k_B T \ln \Delta(T, P, N) \tag{4.30}$$

It is possible to relate it to thermodynamic properties, knowing that:

$$dG = -SdT + VdP + \mu dN \tag{4.31}$$

then we can obtain the entropy as follows:

$$-S = \left(\frac{\partial G}{\partial T} \right)_{P,N} \tag{4.32}$$

$$S = k_B T \left(\frac{\partial ln \Delta}{\partial T} \right)_{P,N} + k_B \ \ln \Delta \tag{4.33}$$

similarly, the volume:

$$V = \left(\frac{\partial G}{\partial P} \right)_{T,N} \tag{4.34}$$

$$V = -k_B \left(\frac{\partial \ln \Delta}{\partial P} \right)_{T,N} \tag{4.35}$$

and the chemical potential:

$$\mu = \left(\frac{\partial G}{\partial N}\right)_{T,P} \tag{4.36}$$

$$\mu = -k_B T \left(\frac{\partial \ln \Delta}{\partial N}\right)_{T,P} \tag{4.37}$$

There is also the possibility of writing the probability as follows:

$$P_{El} = \frac{\Omega(E, V_l, N) e^{\frac{-E}{k_B T}} e^{\frac{-PV_l}{k_B T}}}{\Delta(N, P, T)} \tag{4.38}$$

In other words, the probability of the existence of a quantum state j with energy E.

Thus, we can relate the partition function of the isothermal-isobaric ensemble to the partition function of the microcanonical ensemble as follows:

$$\Delta(N, P, T) = \sum_E \sum_l \Omega(E, V_l, N) e^{\frac{-E}{k_B T}} e^{\frac{-PV_l}{k_B T}} \tag{4.39}$$

4.3 Potential of Mean Force

The calculation of the Potential of Mean Force (PMF) allows us to assess the free energy along a reaction coordinate. In the case of the simulations conducted in this thesis, the chosen reaction coordinate is the distance between the centers of mass of the nanoplastic and the contaminant. Through the profile of the PMF, it is possible to evaluate the change in free energy between these two selected groups of atoms.

The free energy (A) difference between two generic states denoted as $\mathscr{A}$ and $\mathscr{A}_0$ can be related to the logarithm of the ratio between their canonical partition functions $Q_{\mathscr{A}}$ and $Q_{\mathscr{A}_0}$ by:

$$A_{\mathscr{A}} - A_{\mathscr{A}_0} = \Delta F_{\mathscr{A}_0 \mathscr{A}} = -\frac{1}{\beta} \ln \left(\frac{Q_{\mathscr{A}}}{Q_{\mathscr{A}_0}}\right) = -\frac{1}{\beta} \ln \left(\frac{Z_{\mathscr{A}}}{Z_{\mathscr{A}_0}}\right) \tag{4.40}$$

where:

$$\beta = \frac{1}{k_B T} \tag{4.41}$$

The ratio $\frac{Q_{\mathscr{A}}}{Q_{\mathscr{A}_0}}$ is equivalent to the ratio between their configuration partition functions $\frac{Z_{\mathscr{A}}}{Z_{\mathscr{A}_0}}$ since the momentum integrations cancel out the ratio. The practical implementation of Eq. 4.40 is not trivial because MD conventionally do not provide direct access to the partition function, but, on the other hand, it could be computed if expressed in terms of phase space average.

To this end, a set of configurations $\{\mathbf{r}_1, \ldots, \mathbf{r}_N\}$ is sampled from the canonical distribution of state $\mathscr{A}_0$ and used, without alteration, to sample the canonical distribution of state $\mathscr{A}$. However, to obtain the free energy difference between the two states, the unbiased of the sampling is required by removing the distribution of state $\mathscr{A}_0$ through the factor $\exp\left(-\beta\mathscr{U}_{\mathscr{A}_0}\right)$ and reweighting with $\exp\left(-\beta\mathscr{U}_{\mathscr{A}}\right)$. Unfortunately, the results from the free energy perturbation formula are only meaningful if the configuration spaces of states $\mathscr{A}_0$ and $\mathscr{A}$ overlap sufficiently, i.e. the state $\mathscr{A}$ must be a small perturbation to the state $\mathscr{A}_0$. If this is not the case, one alternative is the introduction of intermediate states with potential energy functions $\mathscr{U}_k\left(\mathbf{r}^N\right)$, where k is the index of the state. Since the free energy is a state of function, the system can be transformed from state $\mathscr{A}_0$ to $\mathscr{A}$ along a path through each of the intermediate states - which might be physical or unphysical - without affecting the free energy difference. With this artifice, $\Delta A_{\mathscr{A}_0\mathscr{A}}$ corresponds to the sum of contributions obtained using the free energy perturbation formula for each pair of consecutive states along the path:

$$\Delta A_{\mathscr{A}_0\mathscr{A}} = -\frac{1}{\beta}\sum_{k=1}^{w-1}\ln\left\langle\exp\left(-\beta\Delta\mathscr{U}_{k,k+1}\right)\right\rangle_k \tag{4.42}$$

where $\langle\cdots\rangle_k$ denotes an average over the distribution of the state k, also known as simulation window, and w is the total number of states or windows including the initial and final ones represented by $\mathscr{A}_0$ and $\mathscr{A}$, respectively. The accuracy of the free energy perturbation formula is correlated to the values of $\beta\Delta\mathscr{U}_{k,k+1}$. For some systems, Boltzmann sampling leads us to substantial statistical error. One example of such a situation is the presence of a large energy barrier along a reaction coordinate, preventing the achievement of meaningful statistics within the available computer time.

Umbrella sampling, which is based on non-Boltzmann sampling in order to achieve a more accurate estimate of the PMF, avoids this statistical problem by modifying the potential function through an artificial biasing potential; therefore, the unfavorable states are sampled sufficiently along some predefined reaction coordinate away from an equilibrium value ξ_0 without wasting time with irrelevant although accessible configurations.

As mentioned before, in order to obtain the PMF covering the whole range of interest, it is usually divided into successive intermediate states, and one simulation is performed for each window k biasing the variation of $\xi'\left(\mathbf{r}^N\right)$ within a small interval around some $\xi_0^{(k)}$. Hence, the biasing potential provides a better sampling of the window k yielding w biased probability functions, Which leads us to a biased configurational partition function associated with umbrella window k

The biased distribution function may be calculated by building up a biased

histogram for each window with data from all simulations. Then, the unbiased probability functions may be recovered from the biased simulations for the various windows. To carry out this last step, several methods were developed and one of them is WHAM (Weighted Histogram Analysis Method) proposed by Kumar *et al.* [168]. WHAM aims to estimate the unbiased distribution function as a weighted sum over the data obtained in all w simulations

Chapter 5

Interaction between endocrine disruptors and polyethylene nanoplastic by molecular dynamics simulations

5.1 Introduction

More research is needed to determine the actual ability of these NPs to act as vehicles for other contaminants and to understand the consequences in the food chain. While the experimental development regarding the interaction between NPs and contaminants grows, simulation studies can help to indicate the paths to be followed. Our previous paper, a literature review involving studies of NPs by molecular simulation, found that further research is necessary to accurately map the possible combinations of NPs and EC that present environmental and public health risks [52]. We highlighted the need to develop methodology protocols when dealing with these systems with molecular simulation based on the principles of good practice given by Braun *et al.* [163]. In this context, particular attention must be given to the size and complexity of the system, thus requiring a correspondence with the time scale used.

With that in mind, here we investigate the interaction between two endocrine disruptors that are also emerging contaminants and a NP in an aqueous solution. Endocrine disruptors are characterized by altering the body's hormonal functions and may be related to various health problems. One of the biggest problems is that this effect can happen even at very low concentrations. In addition, they are substances not characterized by acute toxicity; the harmful effect usually only manifests in the long term, making it challenging to identify the problem [55]. The plastic model used here is polyethylene (PE) since it is one of the most widely used plastics in the industry because of its attractive properties, high financial viability and large production capacity [17, 53]. The two endocrine disruptors chosen are bisphenol A (BPA) and benzophenone (BZP). BPA is an organic component widely used as a building block of several plastics and plastic additives. It can be found in polycarbonate plastics, tissue paper, and epoxy resins. Its structure contains two hydrophilic hydroxyl groups and a hydrophobic aromatic group, causing it to display

amphiphilic properties [169]. It has been linked to cardiovascular diseases, breast cancer, diabetes, and benign liver tumors, among others [120]. BZP is also associated with a series of health problems and considered to be a carcinogen proven to cause damage to the genetic information within a cell causing mutations [86]. Most people come in contact with BZP through personal care products, including sunscreens, anti-aging creams, and moisturizers [90]. With these emerging contaminants in water supplies, there needs to be more research on the interaction between these contaminants and NPs in aqueous environment.

In this work, we aim to understand from a theoretical-computational point of view the interactions between BPA/BZP and PE NP. We primarily seek to understand if the PE NP can possibly act as a vehicle for these two contaminants, and seek to shed light on which of them would adsorb more and binds more strongly. Another goal of this paper is to build a reliable protocol suitable for studying systems containing NPs using molecular dynamics simulations.

5.2 Computational details

We have employed standard molecular dynamics, as well as center of mass pulling and umbrella sampling. Umbrella Sampling generates a series of configurations along a reaction coordinate, ξ, between two interacting species. One of these species serves as a reference, while the other group is placed at increasing center of mass distance from the reference with its position maintained by a biasing potential. These center of mass distances represent so-called "sampling windows," wherein independent simulations are conducted to generate an ensemble of structures in each sampling window along the reaction coordinate. The figure 5.1 illustrates this methodology[170]. Within each of these windows, values of the potential of mean force (PMF) can be calculated. To assemble a PMF curve as a function of the entire reaction coordinate (ξ), energy values in adjacent windows are reassembled to produce a continuous function. The results presented here are in the form of a PMF profile. This approach allows us to examine how the system's free energy changes as a function of the distance between the BPA/BZP and the PE.

Due to the low solubility limit of BPA and BZP in water, it is challenging to create a large enough system to represent their actual concentrations in water. The maximum concentration of BPA in water is 300 mg L^{-1} at 25 °C [120], which means for a system containing 80,000 water molecules and a PE particle, there would be only approximately 2 BPAs. Creating a smaller system to maintain reasonable concentrations to simulate the adsorption process is also problematic as the amount of contaminant becomes very low, resulting in poor statistical accuracy. For the BZP, it is an even more complicated situation since its maximum concentration in water is

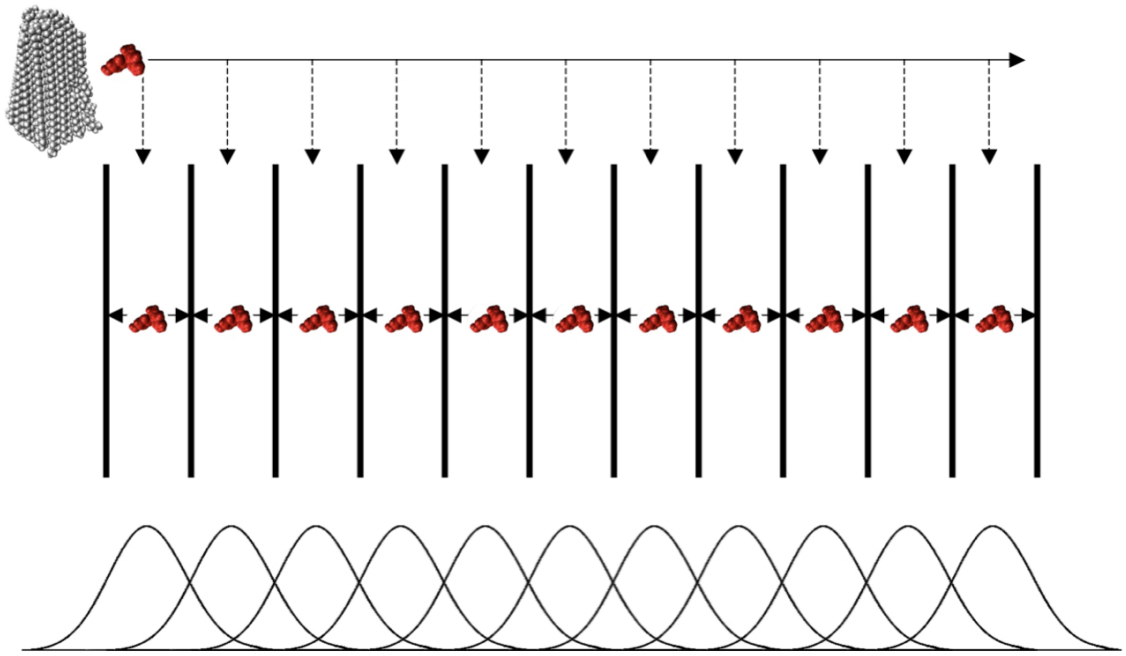


Figure 5.1: The top image illustrates the pulling simulation conducted in order to generate a series of configurations along the reaction coordinate. These configurations are extracted after the simulation is complete (dashed arrows in between the top and middle images). The middle image corresponds to the independent simulations conducted within each sampling window, with the distance between the center of mass of the nanoplastic and the BPA restrained in that window by an umbrella biasing potential. The bottom images show hypothetical histograms of configurations, with neighboring windows overlapping such that a continuous energy function can later be derived from these simulations.

137 mg L^{-1} at 25 °C [171]. To overcome this problem, we use the umbrella sampling approach to computes the free energy profile along a chosen reaction coordinate, determined by the Boltzmann-weighted average over all degrees of freedom except for the chosen reaction coordinate.

All simulations presented in this study were performed with the GROMACS package [172]. For BPA, BZP, and PE, the OPLS-AA force field was used [173], and for the water, the SPC/E force field was applied [114]. The BPA and BZP parameters were acquired from the LigParGen website [173–175] and the PE parameters were acquired from the PolyParGen website [176]. The chemical structure of the two contaminants are shown in Figure 5.2, along with their identification indexes used to identify the force field parameters and charges, which are shown in the supplementary material. Figure 5.2 (c) shows a partial polyethylene molecule and their atomic indexes; the atoms that are not shown follow the same identification logic. The supplementary material shows the force field parameters and atomic charges of polyethylene.

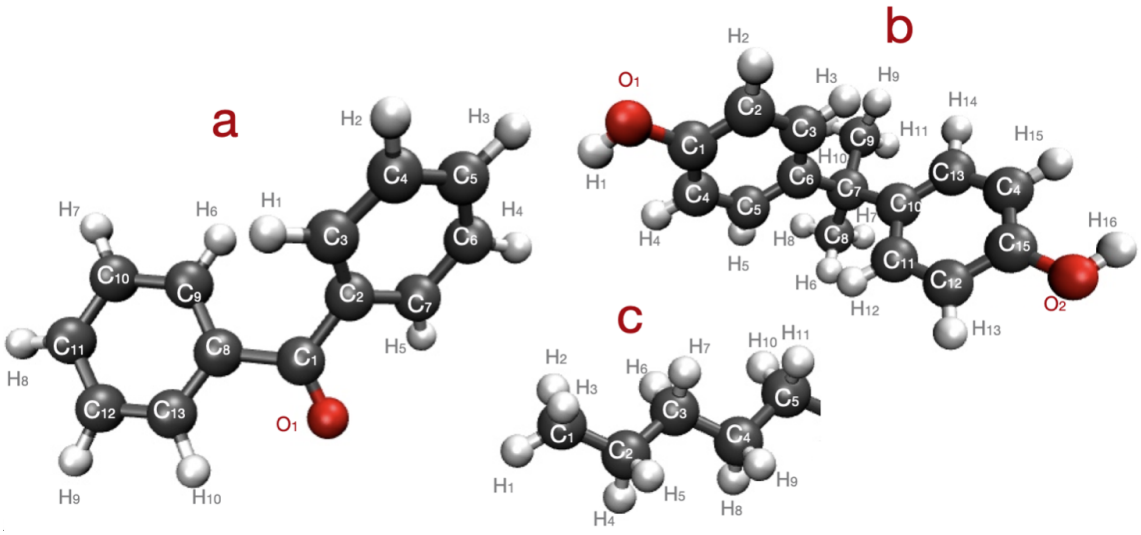


Figure 5.2: Chemical structure depiction for (a) BZP, (b) BPA, (c) and PE, along with their atom labels used to identify the force field parameters and atomic charges, which are shown in the supplementary material.

A PE NP was constructed from 16 PE chains with 36 monomers each. The chains were initially straight and were packed with some proximity, as shown in Figure 5.3 (a). The molecular configurations used to initialize the simulations were generated using the Playmol package [177]. The system underwent an initial minimization process, followed by 5 ns of simulation in the isothermal-isobaric ensemble (NpT) at 500 K and 1 atm (Figure 5.3 (b)).

To couple temperature and pressure, the V-rescale thermostat [178] and the Parrinello-Rahman barostat [179] were used, respectively. The smooth Particle-Mesh Ewald electrostatics [180] with an interpolation order parameter of 4 was used to take into account the long-range Coulombic interactions. The cutoff radius was chosen to be 10 Å for both Coulombic and van der Waals interactions, the time step was 1 fs, the thermostat coupling time constant was 100 fs, and the time constant for pressure coupling was 1200 fs.

It is necessary to be aware of some limitations of the molecular dynamics methodology. One of them is that the system can equilibrate at a local minimum because barriers greater than a few kJ mol^{-1} at room temperature are difficult to overcome. We followed the Hollóczki and Gehrke [118] instructions to get around this situation. They performed extensive simulations to find the best temperature ranges for the simulated annealing (SA) with different nanoplastics and identified the temperature between 200 and 400 K as ideal for PE to achieve a configuration with a minimum energy. After the equilibration process, we transferred the PE particle to a larger box with length of 155 Å. The system was simulated for 24 ns in the canonical ensemble. During the first 2 ns, the temperature dropped linearly from 500 to 400

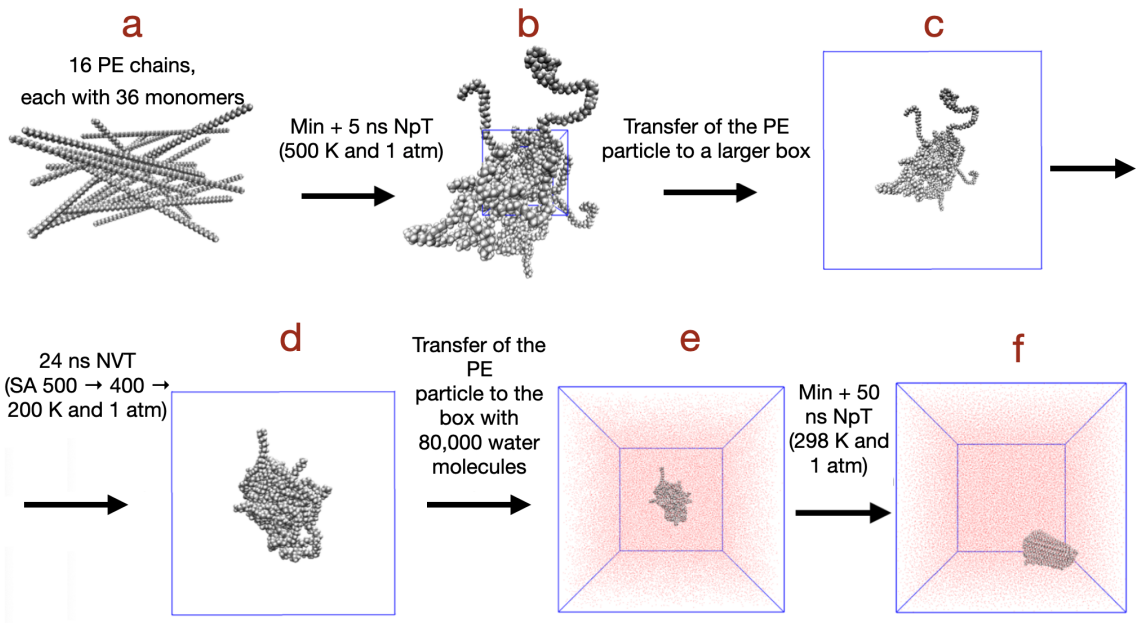


Figure 5.3: Steps for the building of the PE NP. Simulation box with (a) 16 straight chains of PE with 36 monomers each just after assembly. (b) After the minimization and 5 ns of NpT simulation at 500 K and 1 atm. (c) Simulation box with a length of 155 Å with the particle from the last step. (d) After 24 ns of simulation in the canonical ensemble executing the process of simulated annealing (SA) with temperatures dropping from 500 to 400, then to 200 K. (e) After the transference of the PE particle from the last step and the insertion of 80,000 water molecules. (f) After the minimization and 50 ns of NpT simulations in water at 298 K and 1 atm.

K; the SA simulation was performed during the following 20 ns, the temperature reached 200 K at the end, and stayed at this temperature for another 2 ns. The final configuration is shown in Figure 5.3 (d).

Three aspects were taken into consideration to determine the number of water molecules that we should use: the limited concentration of contaminants in the aqueous environment regarding their solubility, the computational time, and the available computational resources. Based on those aspects, we decided to use 80,000 water molecules.

The final PE configuration was placed in the center of a box with 80,000 water molecules (Figure 5.3 (e)). The system was simulated for another 50 ns in the NpT ensemble at 298 K and 1 atm so that the PE/water system could relax. At the end of the simulation, the PE had a cylindrical crystal shape with rugged surfaces on both ends (Figure 5.3 (f)).

To choose the proper reaction coordinate for the PMF calculation, we ran two test simulations for 10 ns each in the NpT ensemble with the same parameters as before. In one simulation, the BPA molecule was placed at the side of the PE cylinder and in the other simulation the BPA was placed at the end of the PE

cylinder, as shown in Figure 5.4 (a) and (b). At the end of the simulations, it was found that the BPA that started at the cylinder end position migrated to the side whereas the one that started at the side remained at the same location, suggesting the position at the cylinder side has lower energy. Therefore, the configuration with the BPA on the side of PE cylinder was considered in the PMF simulation. The box for the PMF was assembled with the normal of the end of the PE NP aligned with the Y Cartesian coordinate (Figure 5.4 (c)), and the BPA was placed at the side of the PE NP aligned with the Z Cartesian coordinate. The reaction coordinate was chosen to be the distance between the center of mass of the contaminant and the center of mass of the PE. We restrained all the atoms in one of the chains of PE located near the center of the PE NP by applying a restraint of 1000 kJ mol^{-1} nm^{-2} in all directions (Figure 5.4 (d)). This allowed us to have a flexible PE NP that remained in the same position and did not rotate.

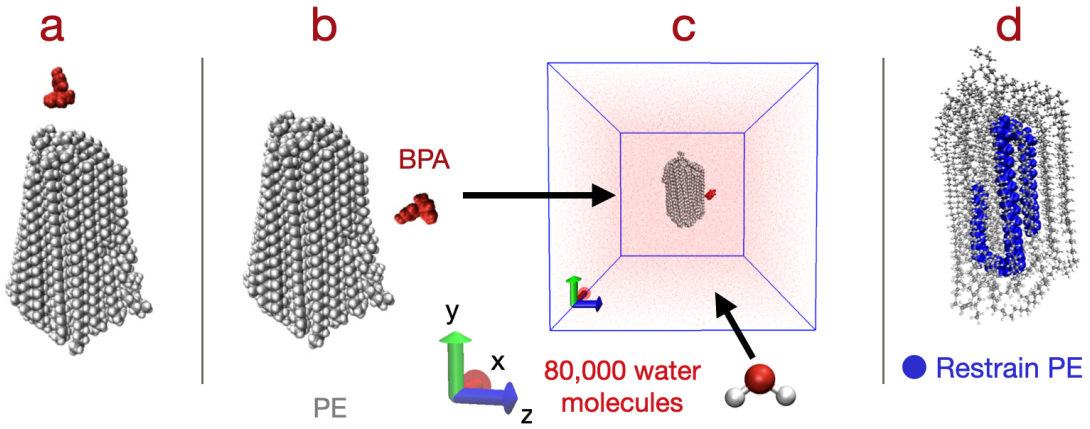


Figure 5.4: Representation of the initial BPA placement positions relative to the PE NP for the PMF simulation. (a) BPA is placed at the cylindrical-shaped PE's end. After a 10 ns simulation in the NpT ensemble, the BPA migrated to the side of the cylinder (water not shown for clarity). (b) BPA is placed at the cylindrical-shaped PE's side. After a 10 ns simulation in the NpT ensemble, BPA stayed close to where it was started (water not shown for clarity). (c) Assembly of the umbrella sampling simulation box with the PE's ends aligned with the Y Cartesian coordinate (in green) and the BPA aligned with the Z Cartesian coordinate (in blue). (d) IN blue is the PE chain chosen to be restrained by a force restraint of 1000 kJ mol^{-1} nm^{-2} in x, y, and z directions.

To ensure that the BPA molecule does not move a significant distance in the X or Y directions during the PMF simulation, we applied a force restraint in both the X and Y directions to the carbon C_7 between both carbon rings as shown in Figure 5.2 (b). We then performed a 5 ns equilibration simulation in the NpT ensemble before conducting two simulations with the BPA molecule being pulled along the Z direction. Both simulations started with the center of mass distance

between PE NP and BPA being 2.379 nm. In one simulation, we forced the BPA molecule to move closer to the PE; in the other, we forced it to move away from the PE. Both simulations were performed in the NpT ensemble with a harmonic pulling force of 1000 kJ mol^{-1} nm^{-2} along the Z axis and a pulling rate of 0.5 nm/ns. The simulation with the BPA molecule moving away from the PE ran for 7 ns, while the simulation with the BPA molecule moving toward the PE ran for 3 ns. Configurations were saved during these simulations and were used as the initial configuration for each window of the umbrella sampling simulation. After these simulations, we simulated each window with a spacing of approximately 0.5 nm for 2 ns of equilibrium and 5 ns of production. For BZP, we modified the initial configuration files for each BPA window, replacing the BPA by the BZP. For the equilibrium and production simulations involving the umbrella sampling, the time step was 2 fs and the LINCS constraint algorithm was used for the hydrogen bonds [181]; this allowed us to spend less computational time without losing precision in the results. The simulation boxes went through 2 ns of equilibrium and 5 ns of production. Similarly to BPA, restraints in both the X and Y directions were applied to BZP. Further details about the restriction of the contaminants during the umbrella sampling are shown in the supporting information. Analysis of results was performed with the weighted histogram analysis method (WHAM) [168]. After analyzing the umbrella sampling histograms, some other windows were added at low probability sampling points, ensuring sufficient energy overlap to integrate and calculate the free energy change, which yields the PMF. Further simulation details are provided in the supporting information.

5.3 Results

The first step in this study was to create a realistic model of a PE NP. This involves the transformation of PE from an amorphous to a crystalline state during simulation in water. Figure 5.3 (a) represents the starting point for creating the PE NP, Figure 5.3 (e) shows the PE inserted in the box with water, and Figure 5.3 (f) shows PE crystallized in the form of a cylinder. This result is consistent with previous simulation [127] and experimental [182] results for high-density PE formed by chains without branches, similar to the ones used in this study. In the formation of the cylindrical crystal of PE, it was noticed that the chains follow the packing pattern stacked on the sides and, in the ends of the cylinder, they tend to fold in on themselves so that the ends of the chains are not exposed at the ends of the cylinder. Chains that were initially 10.05 nm long when fully extended generated an NP approximately 4.7 nm long and 2.6 nm in diameter.

The two profiles in Figure 5.5 were obtained with a force restraint of 1000 kJ

mol^{-1} nm^{-2} applied in both the X and Y directions to the carbon between the two carbon rings from both BZP and BPA (C_1 in Figure 5.2 (a) and C_7 in Figure 5.2 (b)). It restricts the translational movement of the contaminates in the X and Y direction, allowing only rotational movement. The goal is to ensure that the region in which the BPA interacts with the PE is the same during all simulation windows. We also note that the side surface of the PE cylinder is not homogeneous and, as the intention of the current work is to compare the PMF of two different contaminants, the restraint in the X and Y directions ensures that the region of interaction between the two contaminants and the PE is the same during the PMF simulations such that the calculated PMFs are directly comparable.

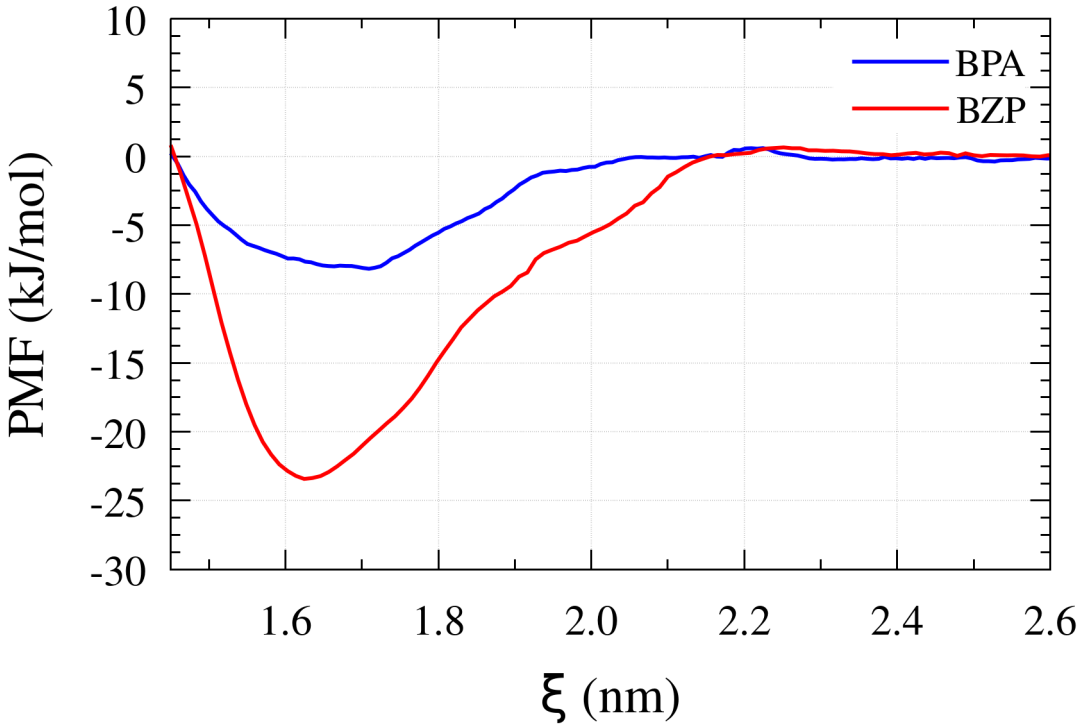


Figure 5.5: PMF curve as a function of the center of mass distance between a BPA/BZP molecule and a PE NP in water with a force restraint of 1000 kJ mol^{-1} nm^{-2} applied at the central carbon of BPA/BZP.

It was observed that the influence of polyethylene on BZP at a distance of 2.18 nm is similar to that of the bulk (end of the profile). For BPA, the interaction energy begins to change when the centers of mass of PE and BPA are at a distance of 2.01 nm from each other. That is, the distance at which the interaction between BZP and PE is attractive is greater than the distance between BPA and PE. This indicates that beyond these distances, both contaminants do not feel the attraction from the PE; when the distance is shorter than these critical values,the PMF between BZP and PE is always lower (more negative) than that between BPA and PE. Despite

the fact that the PMF profiles have different magnitudes, the minima are located basically at the same place at a distance of 1.65 nm. At this point, the ΔG of BPA is -8.0 $\pm$ 0.1 kJ mol^{-1}, whereas the ΔG of BZP is -23.5 $\pm$ 1.4 kJ mol^{-1}, statistical errors were estimated with bootstrap analysis.

Because of the flexibility of the polyethylene nanoplastic, the PE geometry changes during simulations; the shape of the ends varies between a circle and an ellipse, and the average radius of the ellipse varies between 2.4 and 3.0 nm. The shape varies even more when the contaminants are forced to approach the PE surface. The non-polar characteristic of PE chains causes them to readjust their configuration in a way that the contaminants do not penetrate into the NP since both BPA and BZP are characterized by a greater polarity than the PE. The two contaminants exhibit an attractive interaction with the surface of PE compared to the bulk, but BZP is much more attractive than BPA. The order of magnitude of the BPA PMF minimum is the same as the thermal energy (RT) at room temperature, which is -2.48 kJ mol^{-1}. We know that adsorption will likely occur when the PMF is lower than the magnitude of -RT [183]. Although the two molecules are similar sizes in terms of the number of atoms, both present two carbon rings, and both have oxygen in their formula, the PMF profiles are quite different. This is because BPA has two hydroxyl functional groups, unlike BZP, which has only a carbonyl functional group. The presence of this extra oxygen in BPA causes this molecule to form more hydrogen bonds with water, so this molecule has more affinity with the aqueous environment than BZP.

To illustrate this phenomenon, we calculated the number of hydrogen bonds formed between BZP/BPA and water in the simulation windows where the PMFs show a minimum at around 1.65 nm (Figure 5.6). Hydrogen bonds were computed over the 5 ns simulations. A hydrogen bond involves three atoms: a donor, a receptor (both highly electronegative), and a hydrogen atom shared between them. In this work, the hydrogen bond is determined solely based on the geometry and distance of interactions. Two cutoffs are considered: the angle between the receptor, donor, and hydrogen (here we use a value of 30°) and the distance between the donor and receptor (0.35 nm). The number of hydrogen bonds between BPA and water mostly ranges between 4 and 6, while the number of hydrogen bonds between BZP and water mostly ranges between 1 and 2. These results corroborate the hypothesis that the presence of the extra oxygen in the BPA molecule causes it to interact more strongly with water than BZP, which could cause the significant difference shown in the PMF profiles.

We can use the PMF to obtain the Henry constant, which is usually expressed as the adsorption equilibrium constant in chromatography, defined as the ratio of the adsorbed concentration over the bulk concentration in diluted conditions (i.e.,

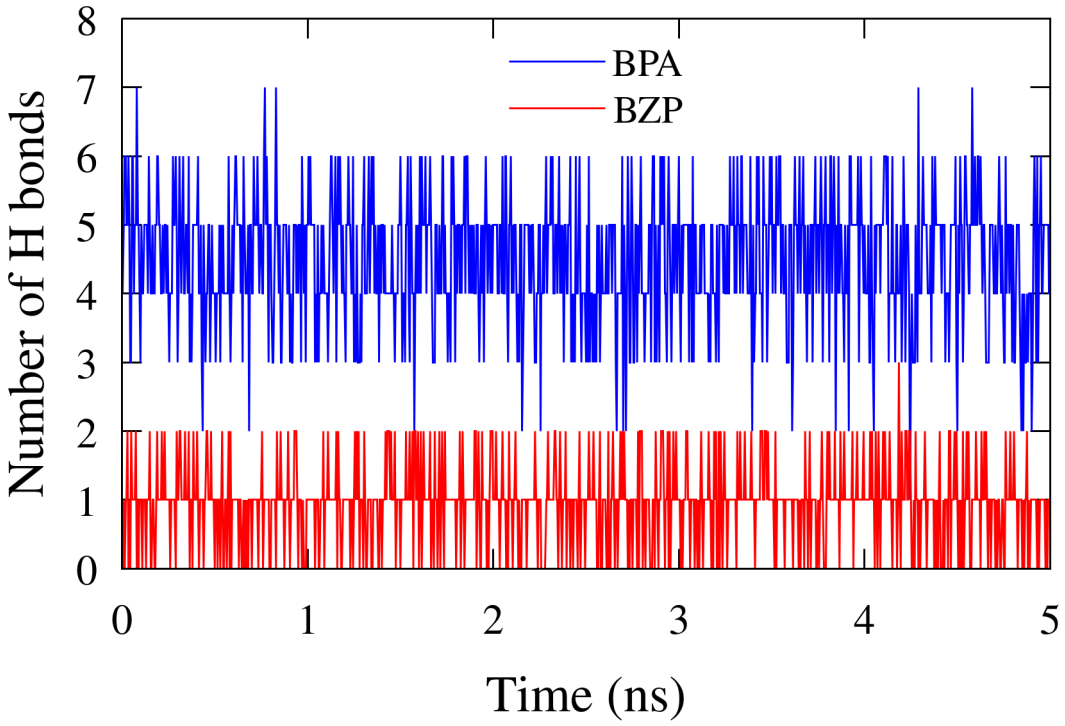


Figure 5.6: Number of Hydrogen bonds between BZP/BPA and water as a function of time (ns).

no interactions between the NP and the contaminants). The Henry constant (K) can be written as a function of the PMF (the free energy, G) in the following way:

$$K = \int_0^\infty \left[\exp\left(-\frac{\Delta G}{kT} \right) - 1 \right] \mathrm{d}h \tag{5.1}$$

where h is the distance between the NP and the contaminant, k is the Boltzmann constant, and T is the absolute temperature. The development of this equation is shown in the works of Staahlberg *et al.* [184], Guélat *et al.* [185], de Souza Gama *et al.* [186]. According to the way it is written, the equation gives us the Henry constant with a distance unit. To make it dimensionless, we divide it by the average of the sum of the distance between BZP/BPA + PE complex. The Henry constant for BZP is 570.4, and for BPA, it is 2.3.

Our results agree with preliminary experimental results of batch equilibrium studies on the sorptive capacity of polyethylene microplastics to sorb different pollutants including BPA and BZP in an aqueous solution. BPA did not show great sorption while BZP was the pollutant among the ones tested (Bisphenol A, S and Z, benzophenone, diethylphthalate, diazepam, bromazepam, carbamazepine, sulfamethoxazole, andibuprofen) that exhibited the greatest sorption, with 39% of removal from the initial aqueous solution [187]. Our results are also consistent with

the work of Jiang *et al.* [188], who carried out an experimental study of the adsorption and desorption behavior of BPA on five different microplastics (polyamide, thermoplastic polyurethane, polyvinyl chloride, polyethylene, and polystyrene). The results showed that the adsorption capacity depends on the microplastics and that polyethylene was the one that presented the second lowest adsorption, with a maximum of 3.7861 mg g $^{-1}$. This corroborates our results that BPA adsorbs but does not show such high adsorption.

We also analyzed the angles formed between specific vectors to help us better understand the preferred molecular interaction. The first vector is traced between the center of mass of the contaminants and PE, while the second vector is traced between two atoms of the contaminants. For BZP, this vector connects carbons C_2 and C_8, and for BPA, it connects carbons C_6 and C_{10}. The results obtained are presented in Table 5.1. Figure 5.7 shows a representation of the vectors chosen to form the angles in Table 5.1, the elements in the figure are not to scale.

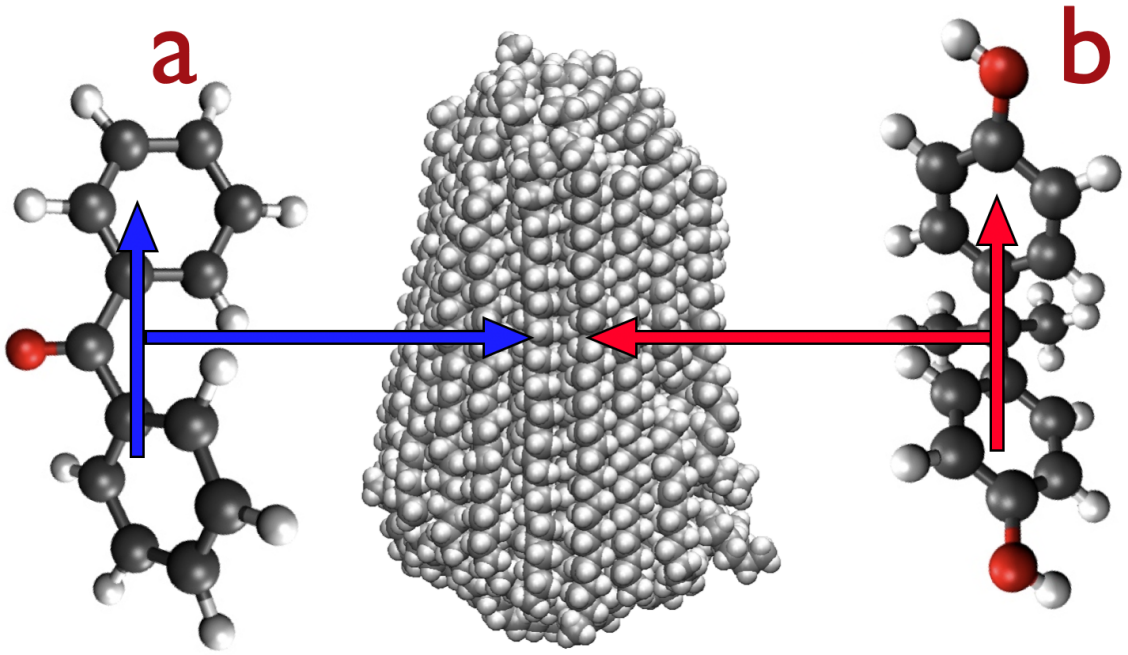


Figure 5.7: Representation of the vectors chosen to form the angles in Table 5.1. (a) Vectors between carbons C_2 and C_8 and between the center of mass of the BZP and the PE. (b) Vectors between carbons C_6 and C_10 and between the center of mass of the BPA and the PE.

The first column in the table presents the distance in nm between the center of mass of PE and the contaminants. The subsequent two columns display the average angles formed between the described vectors. It is important to notice that a precise analysis based on these data is not feasible because the interaction of the contaminants with PE occurs at the surface rather than at the center of mass of PE. Moreover, the vector traced in the contaminants varies with the positions of the

Distance from PE center of mass (nm)	BZP angle (°)	BPA angle (°)
1.6	96.2±0.1	114.0±0.2
1.85	83.5±0.2	100.0±0.2
2.05	88.1±0.3	109.3±0.2
2.34	93.5±0.2	92.1±0.2
2.5	96.9±0.2	78.6±0.2

Table 5.1: Average angles between BZP/BPA and PE. In the second column, the average angles were obtained between the vector from the center of mass of BZP to the center of mass of PE, and the vector from carbon C_2 to carbon C_8 of BZP as a function of the distance between the center of mass of BZP and center of mass of PE (first column). In the third column, the average angles were obtained between the vectors from the center of mass between BPA to the center of mass of PE, and the vector from carbon C_6 to carbon C_{10} of BPA.

selected atoms during the simulation. However, despite these circumstances, we can deduce from these results that both contaminants aim to enhance their aromatic rings' interaction with the PE. Interestingly, despite the presence of oxygen atoms capable of forming hydrogen bonds with water, both BZP and BPA prefer adsorbing onto the surface of PE. This suggests that the interactions between the aromatic rings and PE are stronger than those involving the oxygen atoms. Consequently, the contaminants position themselves to facilitate this interaction on the surface of PE.

5.3.1 Effects of the Applied Restrictions

Considering that the restriction applied to the central carbon limited translational movements, we explored the possibility of constructing PMF profiles without this restriction, thereby increasing the area of PE that interacts with the contaminants. We initially tested this without restriction using BPA, and the profile results are shown in Figure 5.8. Due to the significant difference between the results with and without restriction, we analyzed the trajectories of each simulation window. It became evident that BPA remained on the side (as in Figure 3 (b)) in the windows closest to PE. However, as the windows moved further apart, and consequently, the attractive interaction potential decreased, BPA began to interact not only with the side of PE but also with its ends (as in Figure 3 (a)). Consequently, the windows began to sample interactions between entirely different regions, rendering the rightmost part of the profile in Figure 5.8 invalid.

Nevertheless, in an effort to keep the system as flexible as possible, we decided not to discard the energies from the windows closest to PE, as the contaminants were in the region of interest. For the more distant windows, we applied a force restraint of 200 kJ mol^{-1} nm^{-2} in both the X and Y directions to the carbon between the

two carbon rings from both BZP and BPA (C_1 in Figure 1 (a) and C_7 in Figure 1 (b)). The free energy profiles are displayed in Figure 5.9. Although they closely resemble the profiles in Figure 5, the new profiles exhibit higher ΔG values, -6.3 kJ mol^{-1} for BPA and -21.4 kJ mol^{-1} for BZP. The expectation was that the changes in free energy (ΔG) would be larger as the system became more flexible because, technically, the contaminants would have the opportunity to explore more areas of PE, making it easier to find a lower-energy configuration. However, this was not the result obtained, leading to further investigation.

We compared the BZP profile with three different force restraints: 200 kJ mol^{-1} nm^{-2}, 1000 kJ mol^{-1} nm^{-2} in both the X and Y directions to the carbon between the two carbon rings from both BZP and BPA (C_1 in Figure 1 (a) and C_7 in Figure 1 (b)), and no force restraint near PE but with a force restraint from distance of 1.9 nm from PE onwards (Figure 5.10). The results demonstrate that there is no proportional relationship between the force constant and the minimum of the profile since we observed no trend in the behavior of the PMFs when varying the force constants. Recognizing this fact and understanding that less-restricted contaminants lead to a greater number of uncontrollable degrees of freedom in the simulation, we chose to restrict them with a force constant of 1000 kJ mol^{-1} nm^{-2}, which produced the results presented in the main manuscript.

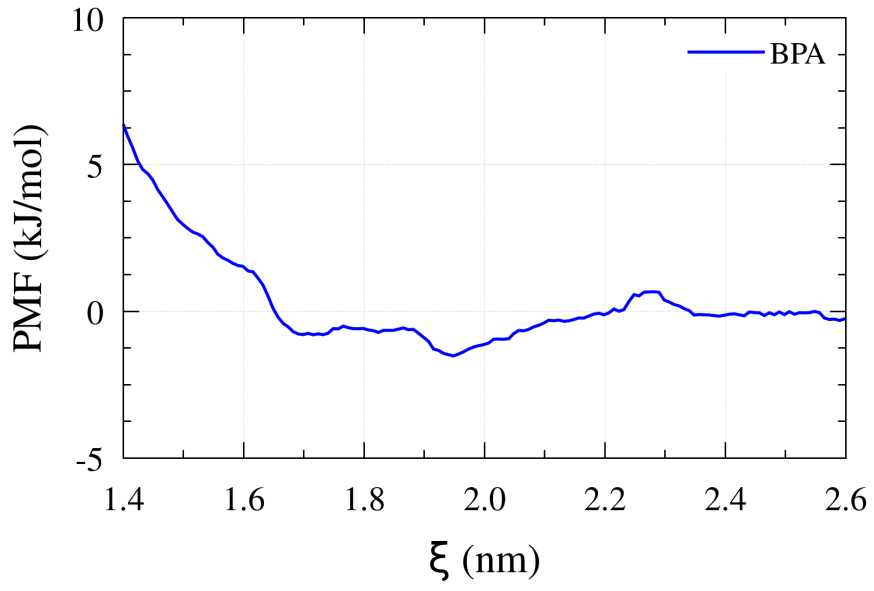


Figure 5.8: PMF curve as a function of the COM distance between a BPA molecule and a PE NP in water without a force restrain applied at the central carbon of both BPA.

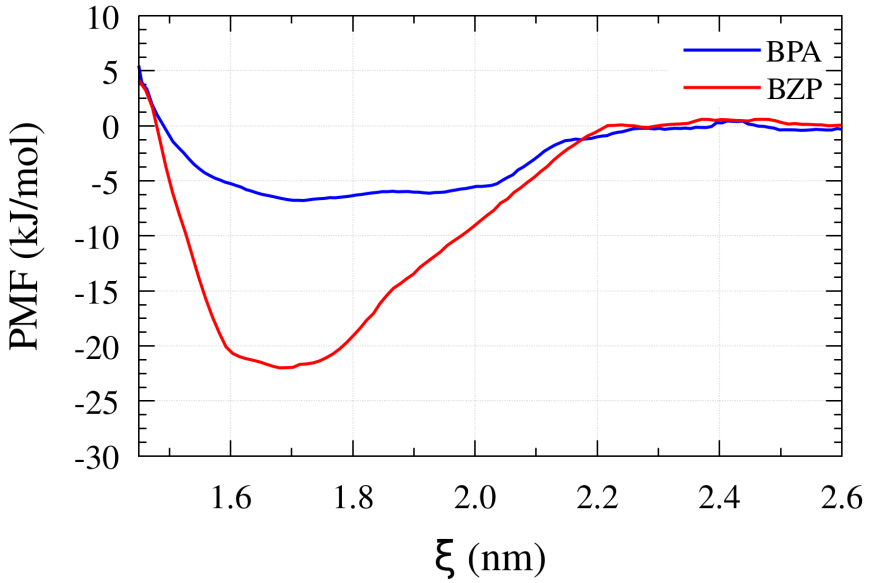


Figure 5.9: PMF curve as a function of the COM distance between a BPA/BZP molecule and a PE NP in water with a force restrain of 200 kJ mol^{-1} nm^{-2} applied at the central carbon of both BPA and BZP for the long distance region and without a force restrain applied at the central carbon of both BPA and BP for the short distance region (until 1.9 nm).

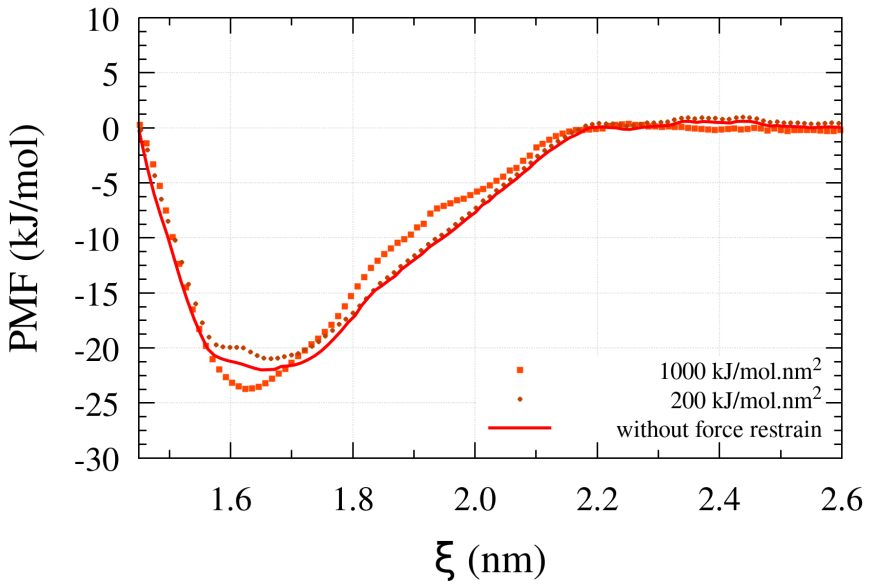


Figure 5.10: PMF curve as a function of the COM distance between a BZP molecule and a PE NP in water with different force restrains applied at the central carbon of BZP.

5.4 Umbrella Sampling Details

The umbrella histogram consists of discrete bins that provide a relative probability of observing the states of interest. From these probabilities, we can calculate the

free energy profile. In order to construct a reliable profile, it is necessary for the histogram curves to overlap. Once we have established that the profile is well-sampled, we can analyze the information and obtain the PMF.

It is important to emphasize that the force constant applied in the position restraint does not influence the final PMF result, but it does have a significant impact on the shape and size of each histogram. A higher force constant will result in a narrower and taller histogram. Different force constants can be applied to the same histogram depending on the system's requirements. Typically, in regions of higher energy (in this case, the region where the contaminants are closer to the PE), a higher force constant is needed to ensure that the chosen distance is respected, and thus, more simulation windows are required to ensure the overlap of the histograms. The number of windows or the shape and height of the curves will not necessarily influence the PMF profile; what matters is their overlap.

In this work, we chose to perform simulations with each window using a harmonic potential force of 1000 kJ mol^{-1} nm^{-2} to restrict the center of mass distance between the contaminants and the PE while keeping the windows as widely spaced as possible to save computational resources. The initial distance chosen was 0.5 nm. Upon analyzing the histograms, we observed some gaps between them. To address this, we conducted independent simulations in these spaces, increasing the force constant to 2000, 5000, or 8000 kJ mol^{-1} nm^{-2} as needed. The corresponding histograms of the simulations can be seen in Figures 5.11 and 5.12. More histograms are noticeable in the Figure corresponding to the BZP.

Figure 5.11: Converged umbrella histograms for the BZP system for the PMF profile shown in Figure 5 .

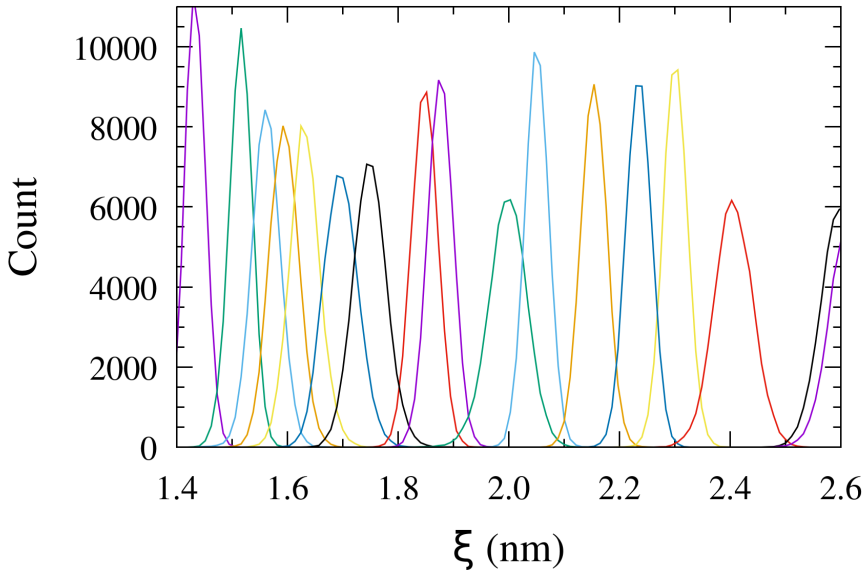
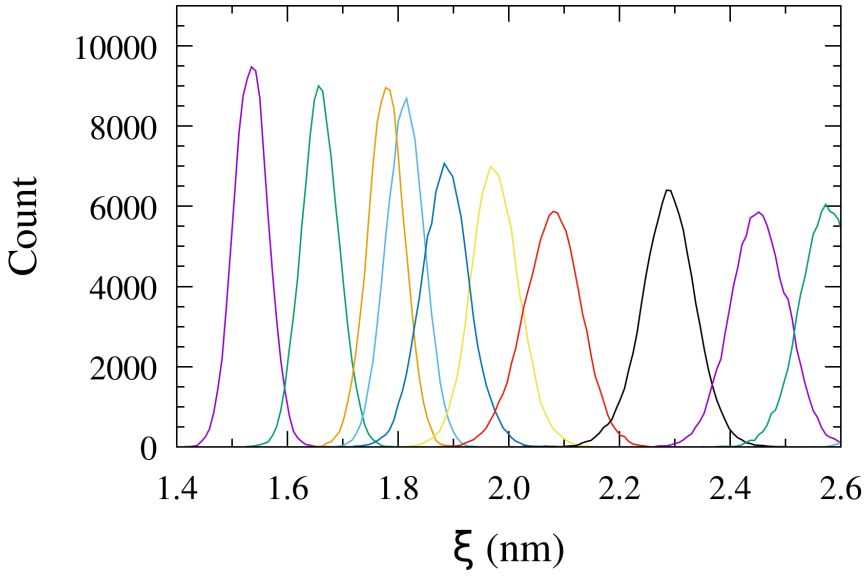


Figure 5.12: Converged umbrella histograms for the BPA system for the PMF profile shown in Figure 5 .



5.5 Final Considerations

This chapter focuses on showing the development of a methodology for simulating PE NP interactions with emerging contaminants, BPA and BZP, in water. Employing PMF analysis, the study reveals that BZP exhibits significantly stronger attractive potential (-23.5 kJ mol^{-1}) towards PE NP compared to BPA (-8.0 kJ mol^{-1}). Derived Henry constants (570.38 for BZP and 2.31 for BPA) emphasize BZP's higher environmental persistence. The investigation extends to hydrogen bonds and preferred angles, providing insights into the molecular dynamics of NP-contaminant interactions, crucial for understanding the environmental fate and risks associated with nanoplastic and contaminant coexistence.

Chapter 6

Conclusion

In this thesis, we present a robust methodology for simulating polyethylene (PE) nanoplastic (NP) in water and obtaining the interaction energy between two emerging contaminants (bisphenol A (BPA) and benzophenone (BZP)) and PE. This work is a first step towards conducting interaction analysis between NPs and other substances using the potential of mean force (PMF) methodology.

In addition, we derived Henry constants from the studied interactions, conducted calculations on the hydrogen bonds observed in the simulations, and explored the formation of preferred angles between BPA/BZP and PE.

PMF analysis shows that BZP has a much greater attractive potential than BPA towards the PE NP. The interaction energy reaches -8.02 kJ mol^{-1} for the BPA whereas it is -23.75 kJ mol^{-1} for the BZP. We can infer that the higher quantity of hydrogen bonds that the BZP has with the water contributes to the difference between both contaminants. The result for the Henry constant for BZP is 570.38, and for BPA, it is 2.31. Regarding the angles between both contaminants and the PE's surface, we can deduce that both contaminants aim to enhance their aromatic rings' interaction with the PE.

During the period when this work was conducted, we also made contributions to the literature through presentations at conferences:

1. "Separation of Methane and Carbon Dioxide mixtures by Adsorption on Calcite Nanopores via Molecular Dynamics" presented at the 13th Brazillian Meeting on Adsorption, 2021 [189];

2. "Adsorption of Bisphenol A in Polyethylene Nanoplastics via Molecular Simulation" presented at the XII Iberoamerican Conference on Phase Equilibria And Fluid Properties for Process Design, 2022 [190];

3. "Adsorption of Bisphenol A in Polyethylene Nanoplastics via Molecular Simulation" presented at the 14th Brazillian Meeting on Adsorption, 2022 [191];

4. "Potential of Mean Force between Polyethylene Nanoplastic and Bisphenol A" presented at the XXXII Midwest Thermodynamics and Statistical Mechanics Conference, 2023 [192];

5. "Interaction Between Endocrine Disruptors and Polyethylene Nanoplastic by Molecular Dynamics Simulations" presented at the XXIV Brazilian Congress of Chemical Engineering, 2023 [193].

The results of our study may help guide experimental investigations by identifying which parts of the molecules are more likely to interact with one another. To the best of our knowledge, this work is the first to employ the PMF methodology for analyzing interactions between NPs and other contaminants. This approach enabled us to generate results at concentrations that closely resemble real-world scenarios rather than substantially higher levels. Further research is needed to understand the implications of the co-transport of contaminants by NPs, but with the results presented here, we can conclude that the co-transport is a possibility and that the system will play a high role in this effect.

This work stands out for the development of the methodological protocol; therefore, the interesting aspect would be the application of this protocol in various other contexts. These may include but are not limited to simulating other nanoplastics and comparing their structures, studying aggregation patterns between nanoplastics, conducting comparative studies by modifying the force fields of both nanoplastics and contaminants along with the water model, exploring comparisons with other endocrine disruptors, examining other emerging contaminant classes like pharmaceuticals and per- and polyfluorinated substances (PFAS), and incorporating ions in water and functional groups to represent modifications occurring throughout the life of the nanoplastic to introduce greater complexity to the model. Additionally, exploring the possibility of applying metal–organic frameworks (MOFs) or other types of adsorbents for separating these nanoplastics from water could be a valuable avenue for future research.

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Appendix A

Force Field and Charge Parameters

PE			BPA		
Atom	σ (nm)	ϵ (kJ/mol)	Atom	σ (nm)	ϵ (kJ/mol)
C	0.35000	0.27614	C	0.35000	0.29288
H	0.25000	0.12552	C_7	0.35000	0.27614
			H_1, H_{16}	0.00000	0.00000
BZP			H_{2-5}, H_{12-15}	0.24200	0.12552
Atom	σ (nm)	ϵ (kJ/mol)	H_{6-11}	0.25000	0.12552
C	0.35000	0.29288	O	0.31200	0.71128
H	0.24200	0.12552			
O	0.29600	0.87864			

Table A.1: OPLA-AA parameters for PE, BPA, and BZP.

BPA				BZP			
Atom	Charge	Atom	Charge	Atom	Charge	Atom	Charge
C_1	0.3512	H_1	0.4270	C_1	0.4090	H_1	0.1713
C_2	-0.2404	H_2	0.1415	C_2	-0.1596	H_2	0.1557
C_3	-0.0831	H_3	0.1455	C_3	-0.0876	H_3	0.1517
C_4	-0.1772	H_4	0.1572	C_4	-0.1615	H_4	0.1531
C_5	-0.0944	H_5	0.1432	C_5	-0.1206	H_5	0.1595
C_6	-0.1101	H_6	0.0868	C_6	-0.1635	H_6	0.1718
C_7	0.0569	H_7	0.0868	C_7	-0.1050	H_7	0.1558
C_8	-0.2304	H_8	0.0868	C_8	-0.1599	H_8	0.1515
C_9	-0.2308	H_9	0.0872	C_9	-0.0867	H_9	0.1532
C_{10}	-0.1100	H_{10}	0.0872	C_{10}	-0.1621	H_{10}	0.1593
C_{11}	-0.0821	H_{11}	0.0872	C_{11}	-0.1201		
C_{12}	-0.2403	H_{12}	0.1444	C_{12}	-0.1638		
C_{13}	-0.0959	H_{13}	0.1414	C_{13}	-0.1047		
C_{14}	-0.1776	H_{14}	0.1438	O_1	-0.3968		
C_{15}	0.3521	H_{15}	0.1575				
O_1	-0.7196	H_{16}	0.4272				
O_2	-0.7189						

Table A.2: Partial charges (in e) on the molecules of BPA and BPZ calculated by the 1.14*CM1A charge model*.

*Dodda *et al.* [175]

PE

Atom	Charge	Atom	Charge	Atom	Charge	Atom	Charge
		H_1	0.0805	C_{19}	-0.1662	H_{38}	0.0832
C_1	-0.2370	H_2	0.0804			H_{39}	0.0831
		H_3	0.0804	C_{20}	-0.1661	H_{40}	0.0830
C_2	-0.1742	H_4	0.0856			H_{41}	0.0830
		H_5	0.0856	C_{21}	-0.1660	H_{42}	0.0831
C_3	-0.1726	H_6	0.0862			H_{43}	0.0831
		H_7	0.0861	C_{22}	-0.1661	H_{44}	0.0830
C_4	-0.1708	H_8	0.0880			H_{45}	0.0831
		H_9	0.0880	C_{23}	-0.1659	H_{46}	0.0830
C_5	-0.1752	H_{10}	0.0852			H_{47}	0.0830
		H_{11}	0.0852	C_{24}	-0.1659	H_{48}	0.0829
C_6	-0.1699	H_{12}	0.0859			H_{49}	0.0829
		H_{13}	0.0859	C_{25}	-0.1656	H_{50}	0.0829
C_7	-0.1711	H_{14}	0.0847			H_{51}	0.0829
		H_{15}	0.0847	C_{26}	-0.1658	H_{52}	0.0828
C_8	-0.1690	H_{16}	0.0848			H_{53}	0.0828
		H_{17}	0.0848	C_{27}	-0.1655	H_{54}	0.0828
C_9	-0.1688	H_{18}	0.0841			H_{55}	0.0828
		H_{19}	0.0841	C_{28}	-0.1656	H_{56}	0.0828
C_{10}	-0.1679	H_{20}	0.0840			H_{57}	0.0828
		H_{21}	0.0840	C_{29}	-0.1655	H_{58}	0.0827
C_{11}	-0.1678	H_{22}	0.0837			H_{59}	0.0828
		H_{23}	0.0837	C_{30}	-0.1654	H_{60}	0.0827
C_{12}	-0.1671	H_{24}	0.0837			H_{61}	0.0827
		H_{25}	0.0836	C_{31}	-0.1653	H_{62}	0.0827
C_{13}	-0.1670	H_{26}	0.0835			H_{63}	0.0827
		H_{27}	0.0836	C_{32}	-0.1654	H_{64}	0.0826
C_{14}	-0.1669	H_{28}	0.0834			H_{65}	0.0826
		H_{29}	0.0834	C_{33}	-0.1651	H_{66}	0.0826
C_{15}	-0.1665	H_{30}	0.0833			H_{67}	0.0826
		H_{31}	0.0833	C_{34}	-0.1652	H_{68}	0.0826
C_{16}	-0.1665	H_{32}	0.0832			H_{69}	0.0826
		H_{33}	0.0832	C_{35}	-0.1653	H_{70}	0.0826
C_{17}	-0.1664	H_{34}	0.0833			H_{71}	0.0827
		H_{35}	0.0832	C_{36}	-0.1652	H_{72}	0.0827
C_{18}	-0.1663	H_{36}	0.0832			H_{73}	0.0826
		H_{37}	0.0832				

Atom	Charge	Atom	Charge	Atom	Charge	Atom	Charge
C_{37}	-0.1650	H_{74}	0.0826	C_{55}	-0.1689	H_{110}	0.0838
		H_{75}	0.0827			H_{111}	0.0838
C_{38}	-0.1653	H_{76}	0.0825	C_{56}	-0.1696	H_{112}	0.0838
		H_{77}	0.0825			H_{113}	0.0838
C_{39}	-0.1650	H_{78}	0.0827	C_{57}	-0.1667	H_{114}	0.0848
		H_{79}	0.0826			H_{115}	0.0848
C_{40}	-0.1651	H_{80}	0.0825	C_{58}	-0.1718	H_{116}	0.0841
		H_{81}	0.0826			H_{117}	0.0841
C_{41}	-0.1651	H_{82}	0.0824	C_{59}	-0.1676	H_{118}	0.0858
		H_{83}	0.0824			H_{119}	0.0857
C_{42}	-0.1657	H_{84}	0.0823	C_{60}	-0.1741	H_{120}	0.0846
		H_{85}	0.0823			H_{121}	0.0846
C_{43}	-0.1650	H_{86}	0.0827	C_{61}	-0.1698	H_{122}	0.0866
		H_{87}	0.0827			H_{123}	0.0866
C_{44}	-0.1655	H_{88}	0.0824	C_{62}	-0.1760	H_{124}	0.0851
		H_{89}	0.0825			H_{125}	0.0851
C_{45}	-0.1654	H_{90}	0.0824	C_{63}	-0.1684	H_{126}	0.0884
		H_{91}	0.0825			H_{127}	0.0884
C_{46}	-0.1653	H_{92}	0.0826	C_{64}	-0.1729	H_{128}	0.0841
		H_{93}	0.0826			H_{129}	0.0840
C_{47}	-0.1658	H_{94}	0.0826	C_{65}	-0.1737	H_{130}	0.0848
		H_{95}	0.0826			H_{131}	0.0848
C_{48}	-0.1654	H_{96}	0.0827	C_{66}	-0.1808	H_{132}	0.0939
		H_{97}	0.0827			H_{133}	0.0939
C_{49}	-0.1660	H_{98}	0.0828	C_{67}	-0.1688	H_{134}	0.0853
		H_{99}	0.0828			H_{135}	0.0853
C_{50}	-0.1657	H_{100}	0.0830	C_{68}	-0.1689	H_{136}	0.0847
		H_{101}	0.0830			H_{137}	0.0846
C_{51}	-0.1663	H_{102}	0.0832	C_{69}	-0.1709	H_{138}	0.0821
		H_{103}	0.0832			H_{139}	0.0821
C_{52}	-0.1668	H_{104}	0.0833	C_{70}	-0.1687	H_{140}	0.0848
		H_{105}	0.0833			H_{141}	0.0847
C_{53}	-0.1676	H_{106}	0.0833	C_{71}	-0.1739	H_{142}	0.0884
		H_{107}	0.0833			H_{143}	0.0884
C_{54}	-0.1677	H_{108}	0.0848	C_{72}	-0.2398	H_{144}	0.0804
		H_{109}	0.0848			H_{145}	0.0804
						H_{146}	0.0804

Table A.3: Partial charges (in e) on the molecules of PE calculated by the electrostatic potential fitting method.

Appendix B

Pollution caused by nanoplastics: adverse effects and mechanisms of interaction *via* molecular simulation

Pollution caused by nanoplastics: adverse effects and mechanisms of interaction *via* molecular simulation

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ABSTRACT

The continuous increase in the production of synthetic plastics for decades and the inadequate disposal of plastic waste have resulted in a considerable increase of these materials in aquatic environments, which has developed into a major environmental concern. In addition to conventional parameters, the relevance of the environmental monitoring of microplastics (MPs) and nanoplastics (NPs) has been highlighted by the scientific community due to the potential adverse effects these materials pose to the ecosystem as well as to human health. The literature has registered an increasing interest in understanding the mechanisms, at the molecular level, of the interaction between NPs and other compounds using molecular simulation techniques. The present review aims to: (i) summarize the force fields conventionally used to describe NPs by molecular simulations; (ii) discuss the effects of NPs in the structural and dynamical properties of biological membranes; (iii) evaluate how NPs affect the folding of proteins; (iv) discuss the mechanisms by which NPs adsorb contaminants from the environment. NPs can affect the secondary structure of proteins and change the lateral organization and diffusion of lipid membranes. As a result, they may alter the lipid digestion in the gastrointestinal system representing a risk to the assimilation of the nutrients by humans. The adsorption of contaminants on MPs and NPs can potentiate their harmful effects on human health, due to a possible synergism. Therefore, understanding the mechanisms involved in these interactions is crucial to predict dangerous combinations and outline action strategies that reduce negative impacts on ecosystems and human health. Depending on the chemical properties of contaminants and NPs, electrostatic and/or van der Waals interactions can be more relevant in explaining the adsorption process. Finally, we conclude by highlighting gaps in the literature and the critical aspects for future investigations.

Subjects Aquatic and Marine Chemistry, Environmental Contamination and Remediation, Environmental Impacts

Keywords Microplastics, Molecular simulation, Nanoplastics, Adsorption, Adverse effects

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INTRODUCTION

Plastic materials are decisive in several aspects of human life because their physical and chemical properties result in high durability and strength, low production cost, and weight. Nonetheless, its high durability is not without negative effects; even with its great potential to be recycled, tons of plastics become polluting agents in the environment in the course of time. In 2019, approximately 368 million tons of plastics were produced worldwide (*Plastics Europe, 2020*), but only 6% to 26% were recycled (*Alimi et al., 2018*; *Li et al., 2021a*) and around 10% eventually ended up in the ocean (*Collard et al., 2017*; *Wang et al., 2019a*). As a result of these plastics being exposed to chemical, physical and biological agents, they begin to degrade into microplastics (size < 5 mm) and nanoplastics (size < 100 nm) (*Toussaint et al., 2019*). Moreover, some plastics have already been designed to be microplastics (MPs).

The COVID-19 pandemic has been an aggravating factor in this regard, and, since its beginning, the use of disposable plastics in the health sector has greatly increased. A recent study estimated that in 2020, 1.56 million masks might have ended up in the ocean (*Phelps Bondaroff & Cooke, 2020*; *Peng et al., 2021*). Among all the plastic disposals related to COVID-19, it was estimated that, from the beginning of the pandemic until August 2021, a total of approximately 25.9 tons had reached the oceans, including 12.3 tons of micro and nanoplastics (*Peng et al., 2021*).

Several studies prove the bioaccumulation of these particles in living marine life, indicating a direct route for contact with humans through the food chain as shown in the literature review conducted by *Toussaint et al. (2019)*. Other access routes would be ingestion or contact with contaminated water, exposure to aerosols containing nanoplastics (NPs) and contaminated air (associated mainly with textile industry), as well as contact with skincare products and cleaning products that have NPs in their composition (*Hüffer et al., 2017*; *Lehner et al., 2019*). It is already known that these NPs and MPs have a high surface/volume ratio and, consequently, great tendency to adsorb other substances. It is important to highlight that, depending on the polymer's glass transition temperature, the absorption of compounds can also occur. So, in addition to the presence of NPs and MPs, it is necessary to know how to deal with other types of pollutants adsorbed on its surface (*Li et al., 2021b*). Many experimental studies which have been conducted in recent years proved the sorption of antibiotics (*Guo, Liu & Wang, 2019*; *Chen et al., 2021*; *Li, Zhang & Zhang, 2018*), heavy metals (*Guo, Liu & Wang, 2020*; *Almeida, Manjate & Ramos, 2020*), pesticides (*Li et al., 2021a*), polycyclic aromatic hydrocarbons (PAH) (*Tan et al., 2019*; *Sørensen et al., 2020*), and other organic pollutants (*Wang & Wang, 2018*) in NP; these studies also showed how NPs may act as transport vehicles for these contaminants. Figure 1 represents a possible transport scheme of pollutants adsorbed on NPs through the food chain.

MPs and NPs with or without adsorbed pollutants, cause adverse effects to several species. *Reichert et al. (2018)*, *Okubo, Takahashi & Nakano (2018)* and *Okubo, Tamura-Nakano & Watanabe (2020)* demonstrated the negative effects in different corals when exposed to MPs. Some studies show that the combined exposure of MPs/NPs with

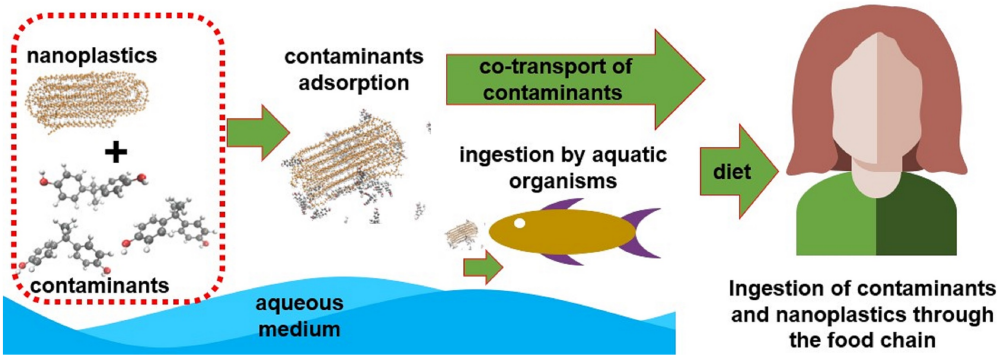

Figure 1 **A possible transport scheme of pollutants adsorbed on NPs through the food chain.**
Full-size DOI: 10.7717/peerj.13618/fig-1

adsorbed metals can heighten toxic effects in aquatic test organisms (*Lee et al., 2019*; *Qiao et al., 2019*). Similar evidence were observed in other experimental studies carried out with organic pollutants such as phthalates, PAH, pharmaceuticals, and flame retardants accumulated on the MP surface (*Li et al., 2020*; *Nobre et al., 2020*; *Pittura et al., 2018*). It is important to highlight that most experiments are carried out under laboratory conditions and employ high concentrations of MPs/NPs and pollutants, which are unrealistic conditions when compared to aquatic environments. Few studies indicate that the presence of pollutants adhered to the surface of MPs can lead to synergistic, antagonistic, and additive effects under real conditions (*Bhagat, Nishimura & Shimada, 2021*; *Rodrigues et al., 2019*). Regarding human exposure to MPs and NPs, there is a great concern in determining, first, effectively how sizeable the contamination is, and, second, the main contact mechanisms (*Lehner et al., 2019*; *Wright & Kelly, 2017*). However, the possible effects on human health tend to be estimated, as studies that prove the problems with exposure to nanoplastics have been materializing (*Gopinath et al., 2019*; *Domenech et al., 2021*).

The use of molecular simulation techniques, such as molecular dynamics and Monte Carlo simulations, is an alternative strategy to understand the possible health harms due to human exposure to NPs (*Hollóczki, 2021*). Molecular simulation is a powerful tool for studying phenomena at the nanometric scale and has been remarkably successful in predicting macroscopic thermodynamic and dynamic observables for various systems. It is continually growing as an option for describing system properties under conditions that experimental determinations are difficult to acquire (*Tuckerman, 2010*). It can also be applied together with experimental results to investigate the mechanisms behind a phenomenon of interest. Among these approaches, Monte Carlo and molecular dynamics simulations can describe particles by pairwise interaction potentials.

Molecular dynamics (MD) is a numerical simulation technique to calculate the thermodynamic and transport properties of many-body systems. The temporal evolution of a set of interacting particles is accompanied by the integration of classical equations of motion. The temporal averages of the trajectories and their fluctuations can be

correlated with the macroscopic properties of the studied system (*Tuckerman, 2010*). Another approach is called Monte Carlo (MC), which is a method that relies on randomly finding the lowest energy state. Simplistically, this is accomplished by changing the system by one move for each Monte Carlo step, calculating the system's free energy, and accepting or not the move based on that (*Frenkel & Smit, 2001*). Thus, MC is a simulation method that seeks to reduce the energy of the system in a stochastic way, while MD uses integration algorithms to solve the equations of motion of each particle following the system's dynamics.

Both MD and MC can be applied in understanding the adsorption of pollutants to NPs and studying the interaction mechanism between NPs and biomolecules, such as phospholipids, proteins, deoxyribonucleic acid (DNA), and ribonucleic acid (RNA). Knowing the mechanism involved in these interactions is crucial to predicting dangerous combinations and outlining action strategies that reduce negative impacts on the ecosystems.

Despite the potential of molecular simulation to describe the mechanism of interaction between molecules and predict system equilibrium and dynamic properties, it has only been used to analyze systems with NPs and MPs in recent years. Therefore, this review aims to examine how research is being conducted in this area, identify how nanoplastics are being characterized within the molecular dynamics methodology, and to identify and analyze gaps in knowledge. These discussions become important to point out the paths to be followed and the difficulties that need to be addressed more urgently, as well as to guide those who are starting to simulate NPs and MPs while providing an overview for those already working in the area. This review focuses on four points: (i) summarize the force fields conventionally used to describe NP by molecular simulations; (ii) discuss the effects of NPs in the structural and dynamical properties of biological membranes; (iii) evaluate how nanoplastics affect the folding of proteins; (iv) and discuss mechanisms by which NPs and MPs adsorb contaminants from the environment.

SURVEY METHODOLOGY

The combinations of keywords used in this research were: "nanoplastics molecular dynamics", "microplastics molecular dynamics", "nanoplastics Monte Carlo", and "microplastics Monte Carlo". To guarantee a thorough assessment of the literature, the keywords were searched in five different repositories: acs.org, Science.gov, sciencedirect.com, scopus.com, webofknowledge.com, and scienceresearch.com. In total, 706 results were found. Most of the articles were present in more than one of the repositories, thus guaranteeing an unbiased search. After analyzing the documents and excluding duplicates, 28 articles were considered with data that could be used in this review - basically, those that used molecular dynamics and/or Monte Carlo to study nanoplastics. It is interesting to highlight that, due to computational limitations, NPs are generally simulated rather than MPs. However, in many cases, the observations can be extrapolated to systems containing MPs. Meanwhile, there are situations in which the focus of interest is the NP itself.

GENERAL REMARKS OF THE SIMULATIONS AND FORCE FIELD ANALYSIS

The number of scientific articles using the molecular dynamics approach has been growing since 2011, with five published papers from 2011 to 2017 and 23 published papers from 2018 to 2022. Only one paper briefly reported Monte Carlo simulations. Before discussing each point proposed in the study, it is necessary to comment on how research is being conducted in this area, and on important differences between said studies. Initially, it is possible to identify two groups of studies, the first focused exclusively on molecular dynamics, and the second focused on molecular dynamics as a complement to an experimental study or with other models. Within the first group, one can see the time scale used is mainly in the nanosecond range, reaching the microsecond range when using coarse-grained force fields (*Bochicchio et al., 2017*; *Rossi, Barnoud & Monticelli, 2014*). Within the second group, there are studies that simulate systems at the nanosecond scale, but also other studies that simulate at the picosecond scale, the latter mostly following an experimental study (*Chen et al., 2021*; *Li et al., 2021b*; *Guo, Liu & Wang, 2020*; *Guo, Liu & Wang, 2019*; *Zhang, Zhao & Na, 2020*; *Qin et al., 2022*). Considering that all studies work with the same type of system, *i.e.*, a simulation box containing NP, a disparity between simulation times is noted, making a discussion about it worth having. This concern applies not only to studies of NPs through molecular dynamics, but also to any study that use this methodology. *Braun et al. (2019)*, in their practice guide for molecular dynamics methodology, highlight that for condensed systems, depending on the type of information desired, there may be a dependence of the properties with the fluctuations and correlations of movement between the molecules. It is even mentioned that systems with polymers and proteins have relevant scales in the range of nanoseconds to microseconds, depending on what information one wants to obtain.

As mentioned previously, Monte Carlo and molecular dynamics can describe particles by pairwise interaction potentials. These interaction potentials are associated with the positions of the particles but also with parameters related to the substance, the particle size, bonds, dihedrals, among others. This information is computed into force fields that can be defined as the functional forms used to describe the intramolecular and intermolecular potential energies of the system. Many are the force fields available in the literature and their modifications, such as OPLS-AA (Optimized Potentials for Liquid Simulations, All-Atom) (*Jorgensen, Maxwell & Tirado-Rives, 1996*; *Jorgensen & Tirado-Rives, 1988*); AMBER (Assisted Model Building and Energy Refinement) (*Ponder & Case, 2003*); CHARMM (Chemistry at Harvard Macromolecular Mechanics) (*Brooks et al., 2009*); GAFF (General AMBER Force Field) (*Wang et al., 2004*); and TraPPE (Transferable Potentials for Phase Equilibria) (*Martin & Siepmann, 1998*). They were developed based on quantum mechanical calculations or experimental data. When working with molecular simulation, one of the main concerns must be the selection of the force field. In this section, we discuss the main force fields used in the parameterization of nanoplastics, the software used, and the chosen water models.

We noticed that five studies used some version of the Material Studio program (*BIOVIA Dassault Systèmes, 2021*) with the COMPASS force field (*Sun, 1998*), which is already available in the simulator program. Among all the software mentioned, this is the only one that is paid and has a friendlier interface; however, the software used must not interfere with the result. These studies have both MD and experimental sections, and deal with the sorption of contaminants on NP. All the experiments, but one, use between 100 and 500 ps of simulation time before analyzing the results. As already mentioned, relevant time scales for these systems should be higher in magnitude. An interesting fact is that none of them considered the presence of water, even if implicit, neglecting the fact that the phenomenon of interest occurs in an aqueous medium.

The most used molecular dynamics simulator to study NP was Gromacs (*Lindahl et al., 2021*), with 13 studies using its versions. The force fields were more varied in this case, but seven of those studies chose to use the GROMOS (GROningen MOlecular Simulation) (*Scott et al., 1999*) in one of its versions, which is the force field from the same developer as for the Gromacs. Five investigations used the LAMMPS simulator (Large-scale Atomic/Molecular Massively Parallel Simulator) (*Plimpton, 1995*), while one used the Gabedit program (along with the AMBER force field) (*Allouche, 2011*) and another used the Amber molecular dynamic package (*Case et al., 2005*). Of the works that used LAMMPS, two used OPLS-AA (Optimized Potentials for Liquid Simulations—All Atom) with SPC/E (extended simple point charge model) water model (*Berendsen, Grigera & Straatsma, 1987*).

Regarding the force field only, two publications combined GAFF (*Wang et al., 2004*) and TIP3P water model (*MacKerell et al., 1998*). One publication used GAFF (*Wang et al., 2004*) for both NP and water-the studies that used GAFF obtained the partial charges from different methods of quantum calculations. One publication combined TIP3P to water and a version of GROMOS to the NP. Three publications used OPLS-UA (*Jorgensen & Tirado-Rives, 1988*).

A force field development was carried out for polystyrene and polypropylene. It was specially designed for the interaction between these plastics with lipids. In other words, this force field (MARTINI coarse-grained (CG)) (*Panizon et al., 2015*) is compatible with the popular MARTINI force field for lipids (*Marrink et al., 2007*), and it was used in four different publications for experiments with simulation time from at least 100 to 20,000 ns. Tables 1 and 2 summarize the number of published studies on nanoplastic simulations *via* molecular dynamics and the force fields used to model NP and water in each of them. *Hollóczki & Gehrke (2020)* used the Automated Topology Builder and Repository (ATB) version 3.0, a website that provides classical molecular force fields for novel compounds (*Malde et al., 2011*). This investigation of *Hollóczki & Gehrke (2020)* together with the studies from Table 1 are the 28 referred articles in this literature review.

THE EFFECTS OF NANOPLASTICS IN THE STRUCTURAL AND DYNAMIC PROPERTIES OF BIOLOGICAL MEMBRANES

Biological membranes are complex structures composed basically of proteins and lipids stabilized by dynamic cooperative non-covalent interactions (*Bogdanov & Dowhan, 2021*).

Table 1 Scientific publications applied to simulate nanoplastic *via* molecular dynamics or Monte Carlo and the force fields used to model the NP in each of them, identified by authors.

Force field	Number of articles	References
AMBER	1	*Cortés-Arriagada (2021)*
Compass	5	*Chen et al. (2021)*, *Li et al. (2021b)*, *Guo, Liu & Wang (2019)*, *Guo, Liu & Wang (2020)*, *Yang et al. (2021)*
GAFF	3	*Wang et al. (2019b)*, *Sarcletti et al. (2021)*, *Ramalho, Dordio & Carvalho (2022)*
GROMOS	7	*Feng et al. (2022)*, *Xue et al. (2011)*, *Tan et al. (2020)*, *Li et al. (2021a)*, *Qin et al. (2022)*, *Zhang, Zhao & Na (2020)*, *Lameh, Baseer & Ashiru (2022)*
MARTINI	5	*Dettmann, Kühn & Ahmed (2021)*, *Bochicchio et al. (2017)*, *Li et al. (2021c)*, *Panizon et al. (2015)*, *Rossi & Monticelli (2014)*
OPLS-AA	3	*Hollóczki & Gehrke (2019)*, *Hollóczki (2021)*, *Rossi & Monticelli (2014)*
OPLS-UA	3	*Bochicchio et al. (2017)*, *Bochicchio et al. (2022)*, *Panizon et al. (2015)*

Table 2 Scientific publications applied to simulate nanoplastic *via* molecular dynamics or Monte Carlo and the force fields used to model the water in each of them, identified by authors.

Water model	Number of articles	References
GAFF	1	*Sarcletti et al. (2021)*
MARTINI	1	*Li et al. (2021c)*
SPC	1	*Xue et al. (2011)*
SPC/E	2	*Hollóczki & Gehrke (2019)*, *Hollóczki (2021)*
TIP3P	3	*Wang et al. (2019b)*, *Ramalho, Dordio & Carvalho (2022)*, *Qin et al. (2022)*
TIP4P	1	*Toh et al. (2020)*

They are permeable protective barrier of the cells involved in relevant functions, namely sensing, transport, adhesion, and recognition processes. They consist of a bilayer of lipid molecules, and have functions such as the control of substances (*e.g.*, ions, nutrients, waste) into and/or out of cells, keeping toxic substances outside the cells as well as separating vital, and often incompatible processes inside the cells (*Watson, 2015*). Biological membranes are very complex and, in addition to the lipid layers that allow or prevent the diffusion of smaller molecules, some proteins form channels that can allow free diffusion into and out of the cell and channels that only allow specific passage of some compound (*Nelson & Cox, 2021*). The imbalance of these processes may be linked to diseases such as cancer, neurodegeneration, and muscular dystrophies (*Dias & Nylandsted, 2021*). The manipulation of membrane dynamics has also been associated with anesthetic effects (*Fábián et al., 2015*). Thus, understanding, at the molecular level, how the interaction mechanisms of membranes with the environment works can influence our comprehension in more than one field of knowledge. In this section, we discuss recent advances in studies to understand how lipid membranes behave in the presence of NP contaminants through molecular simulations techniques.

The simulations made by *Hollóczki & Gehrke (2020)* show that a membrane composed by 2 × 300 molecules of 1-palmitoyl-2-oleoyl-phosphatidylcholine (POPC) (*i.e.*,

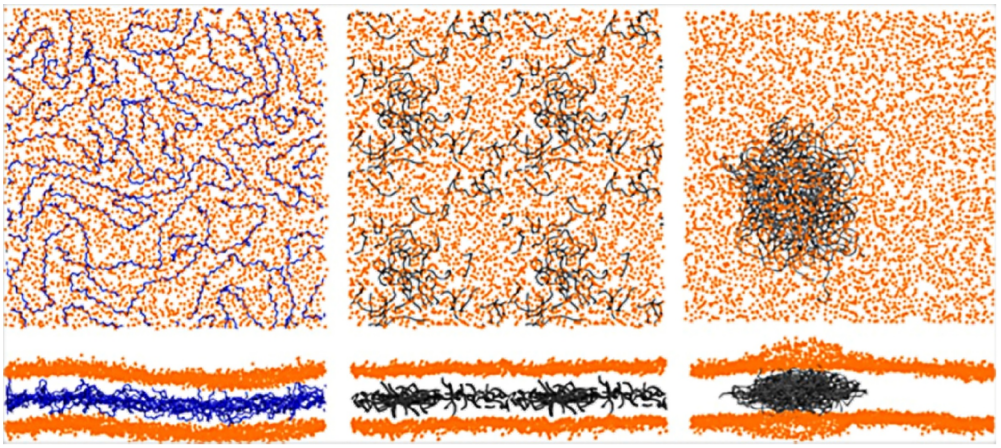

Figure 2 Typical distributions of the polymers inside pure POPC membranes (lipid:polymer mass ratio of 6.6%). Two views of the membrane (only head beads, in orange) are shown for each configuration: from the top and from the side. Left: long PP chains (in blue). Middle: short PE chains (in gray). Right: long PE chains (in gray). Reproduced from *Bochicchio et al. (2017)* - License CC BY 4.0 (http://creativecommons.org/licenses/by/4.0/). Full-size DOI: 10.7717/peerj.13618/fig-2

phospholipid commonly found in cell membranes) readjusts itself in the presence of nanoparticles of polyethylene with about 5 nm in diameter. The membrane rearranges to cover more of the NP, and the NP surface can rearrange itself to almost double in size in the presence of the membrane. The conformational changes gradually cause the membrane thickness to increase and the average area of each lipid to decrease during the 200 ns course of simulation time. In addition to structural changes, the results show that there are changes in dynamics as well since the presence of NP facilitates lipid movement within the membrane. Thus, it is suggested that the presence of NP has a significant effect on biological membranes (*Hollóczki & Gehrke, 2020*).

Bochicchio et al. (2017) conducted a study using coarse-grained simulations of polyethylene (PE), polystyrene (PS), and polypropylene (PP) with diameters around 7 nm interacting with lipid membranes of POPC. All NPs quickly entered the membrane and changed their behavior from solid to liquid at room temperature. Depending on the type of NPs and on their degrees of polymerization, one can observe different situations: polyethylene chains tend to aggregate when in a high polymerization degree, unlike the other two NPs whose chains tend to separate from each other, as shown in Fig. 2.
An interesting topic of this work is the study of heterogeneous membrane systems (those composed of ternary lipid mixture exhibiting liquid-ordered/liquid-disordered phase separation). These membranes are formed by more than one type of lipid, so it is possible to observe the dynamics of a more complex system. Once again, the result was related to the type of polymer: the separation of the lipid phase is disadvantaged by PP, while PS stabilizes the lipid phase, and PE modifies the boundary topology and causes cholesterol depletion from the liquid-ordered phase. The authors emphasize the need for further studies to better understand the toxicity of NPs that humans have come more and more in contact with recently. This study follows up a previous one (*Rossi, Barnoud & Monticelli, 2014*) which is simpler in the variety of compounds and deals with different

sizes of sets of polystyrene chains and a POPC membrane. Initially, they carried out an extensive study of the coarse-grained force field and, only after good agreement with the OPLS-UA force field, they performed the simulations with PS chains formed by 10, 20, and 100 monomers. In equilibrium, the set of shorter chains (with less than 100 monomers) were dispersed in the membrane, not aggregating, whereas the one with 100 monomers preferred to be concentrated in the center of the membrane. The results show that PS alters the properties of the membrane, significantly increasing its compressibility modulus and decreasing its bending modulus, which indicates a structural change in the membrane in addition to affecting its lateral organization.

In the wake of these last two investigations, a recent publication presented experimental and molecular dynamics results, introducing small chains (with 25 styrene monomers) in dipalmitoyl-phosphatidylcholine (DPPC) lipid bilayers (*Bochicchio et al., 2022*). The authors carried out the equilibration for 50 ns of three systems composed of the DPPC membrane and different mass ratios of PS. Because they use the OPLS-UA force field (*i.e.*, coarse grained-force field), it was possible to simulate up to 2 microseconds in the production stage. The experimental and molecular dynamics results complement each other and lead the authors to believe there is a critical concentration in partial segregation of PS chains within the membrane. Similarly to *Hollóczki & Gehrke (2020)*, *Bochicchio et al. (2022)* showed changes in membrane thickness. The authors reported a nonlinear increase in diffusion coefficients with the PS mass ratio (*Bochicchio et al., 2022*). For the bending modulus values, the behavior was inverse in the presence of PS. There was a substantial decrease in bending modulus compared to the total absence of PS. The results confirm that the effects of NPs on human health cannot be underestimated, and that concentration is a factor to be analyzed.

The last study to be covered by this section brings an exciting view of how treating water with chlorine can affect the MPs to the point of increasing the toxicity of these MPs (*Qin et al., 2022*). The authors conducted experiments, and, using MD in accordance to the methodology of *Bochicchio et al. (2022)*, they compared the system of pristine polystyrene MPs and chlorinated pristine polystyrene MPs (with chlorine- and oxygen-containing functional groups) in contact with bilayer membranes of phosphatidylcholine. Simulations showed that MPs interact differently with membranes and the authors speculate that the increased cell membrane permeability caused by chlorinated PS might be due to the presence of C-Cl bonds. Consequently, the presence of chlorinated pristine polystyrene MPs can cause even more damage at the cellular level (*Qin et al., 2022*).

All studies showed changes in lipid membranes when in contact with NPs or MPs. Considering the biological functions of membranes, it is plausible to conclude that these NPs/MPs somehow affect the cellular environment. All investigations suggested that further investments in this field are needed for proper assessment and control of the potential negative effects of NPs/MPs on environment and human health.

NANOPLASTICS AFFECTING THE FOLDING OF PROTEINS

Proteins play a substantial role in human health. They are critical for tissue growth and maintenance (*Yeung et al., 2017*), and for various biochemical reactions when proteins

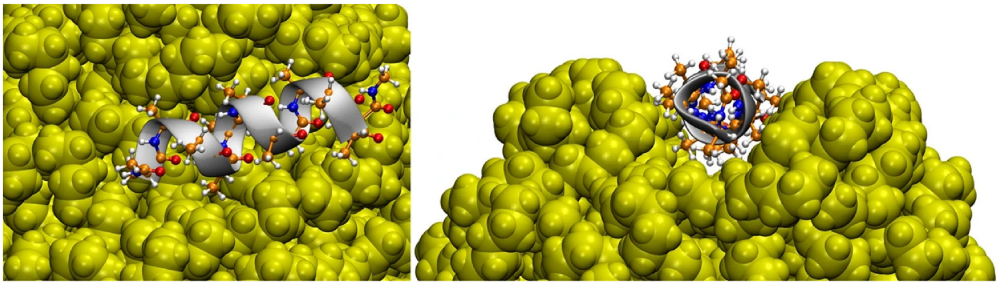

Figure 3 Three-dimensional structure of the helical peptide (composed of 12 alanine units) on thesurface of a PE nanoparticle (yellow) from two views. Reproduced from *Hollóczki & Gehrke (2019)* - License CC BY 4.0 (http://creativecommons.org/licenses/by/4.0/).
Full-size DOI: 10.7717/peerj.13618/fig-3

take the form of enzymes, with functions such as digestion, blood clotting, energy production, and muscle contraction (*Hatzimanikatis et al., 2004*). Equally important, proteins can also act as hormones that are chemical messengers responsible by the communication between cells, tissues, and organs (*Nussey & Whitehead, 2001*). Additionally, they are responsible for regulating the pH of body fluids (*Hamm, Nakhoul & Hering-Smith, 2015*), such as blood and stomach acid, and acting in the body's defense (*Li et al., 2007*). In recent years, studies that associate the presence of NPs in the human body with changes in protein structures began to emerge. The molecular dynamics methodology is currently used to understand the dimension of these changes and their possible effects on human health. It is proven that NPs tend to interact with proteins to the point of modifying their secondary structures, with the result being protein denaturation (*Hollóczki & Gehrke, 2019*).

Hollóczki & Gehrke (2019) studied four types of NPs interacting with a series of proteins, namely PE, PP, nylon-6,6, and polyethylene terephthalate (PET). They showed, for instance, that the amino acids polarity is a relevant factor in their adsorption on NPs. Non-polar amino acids such as phenylalanine and tryptophan tend to have such a high interaction with NPs that basically all amino acids in solution adsorb to NPs. They can form a micelle around NP, showing that the hydrophobicity of NPs can be masked by proteins, affecting their solubility and ability to aggregate (*Hollóczki & Gehrke, 2019*). It is interesting to highlight that the interaction between NPs and tryptophan zipper induced no significant changes in its overall structure, regardless of the adsorption of the peptide on the surface of the NP. However, the lack of structural rearrangement at the end of the simulation does not mean that the NP does not affect the protein structure because the time scale for this to happen could be greater than that available for the simulation. Moreover, a α-helix composed of 12 alanine adsorbed on hydrophobic surfaces of NP, and mainly for PE and PP, the nanoparticle rearranged to incorporate the peptide, affecting the conformation of the protein, as shown in Fig. 3. Another relevant point is that the results differ greatly depending on the NP, *i.e.*, the problems caused by exposure to NPs are different depending on their type. This was the first article that had

Hollóczi, notably the author of the most publications regarding the study of NPs with molecular simulations, published in said field.

Later, *Hollóczki (2021)* single-handedly published an article on the same topic. The study used, in addition to MD, the quantum chemistry theory. In order to overcome the limitations of MD regarding the time scale and the energetic barriers of the restructuring of compounds as proteins, which were pointed out even in the previous work (*Hollóczki & Gehrke, 2019*), *Hollóczki (2021)* discussed the use of simulated annealing (SA) strategy. In this methodology, one runs a simulation with the system at high temperatures allowing several conformations to be accessed, and then, the system is gradually cooled to, ideally, the minimum free energy level. *Hollóczki (2021)* used this methodology to find the best conformations for polyethylene and nylon-6,6, reaching the conclusion that the temperature range for carrying out the SA must be approximately between the condensation and freezing temperatures of the compound. This methodology proved to be quite efficient in revealing important structural information. Peptides' structures were, thus, optimized through quantum chemistry and then submitted to SA. The next step was to run SA for the peptide-nanoplastic pair. About 150 simulations were performed for each one of them. Further optimizations were performed using MD and quantum chemistry at the end of the simulations, and then the adsorption, interaction, and reorganization energies were defined and calculated. In this study, the two plastics influenced the stability of the secondary structure of the simulated peptide, corroborating previous results. The need for further studies to understand the consequences of these changes is also highlighted.

In a more recent study, *Li et al. (2021a)* performed an experimental investigation coupled with a simulation study of insulin fibrillation in the presence of nanoplastics and various contaminants. They chose insulin to represent the protein, because it is a model already widely used in the literature in the study of protein fibrillation and conformational changes. The research aimed to examine whether common organic contaminants (pyrene, bisphenol A (BPA), 2,2, 4,4-tetrabromodiphenyl ether (BDE47), 4,4-dihydroxydiphenylmethane (BPF), and 4-nonylphenol (4-NP)) enhance the abilities of NPs to accelerate insulin fibrillation as well as carry out a molecular study of the mechanism of action. By MD, the insulin, pyrene insulin and polystyrene insulin systems were studied. Although one of the goals was to examine the three dynamics together, the simulations were done in pairs. The presence of polystyrene had a more significant effect on the conformational transition of insulin than that which was observed with the presence of pyrene, which is consistent with the trends in the experimental results. It is possible to conclude that van der Waals forces predominate in insulin binding with PS or pyrene. These results complemented previous studies of the mechanism by which NPs promote insulin fibrillation.

Another study involving NPs and proteins deal with the effect of using PEG spacer on small peptides in a process commonly called PEGylation (*Xue et al., 2011*). This process is used to stabilize, immobilize or modify biomolecule properties. The authors sought to understand, at the molecular level, the structural effects caused on the peptides due to the PEGylation. The peptides were simulated under three conditions: free in solution,

attached to a PEG spacer, and attached to PEG spacer constrained to a two-dimensional lattice to mimic the display of a peptide on the surface of a microsphere. The results suggested that the no charged peptides in a PEGylation situation do not undergo a noticeable conformational change. However, peptides with high charges, both negative and positive, suffered conformational changes. These results show the need for specific studies and significant investment in this research field. Since we were unable to find additional reports focusing on this specific subject, it was not possible to compare results. This fact alone justifies the relevance of more investment to further develop the field. The mentioned study is presented here distinctly from the other works in this section due to its author discussing a process in which peptides are artificially changed through a PEG spacer while acting as a single unit, instead of examining the interaction between the protein and the microplastics separately.

In general, the study of interactions between NPs and proteins shows that a single type of nanoplastic cannot be used to understand the possible effects of contacting all types of nanoplastics with proteins. More than one of these studies discussed that polarity is fundamental in how NPs and proteins interact. It is also possible to conclude that this type of study is necessary to indicate new research routes in the subject. Different pairs, peptides, and NPs should be analyzed to understand the problem to a greater. The present number of investigations in this area is still insufficient to provide a general dimension of what must be approached.

SORPTION OF CONTAMINANTS ON NANOPLASTICS

Research has been conducted to investigate the effects of microplastic as a vector for POP (persistent organic pollutants) contamination in the aquatic environment (*Bhagat, Nishimura & Shimada, 2021*; *Rodrigues et al., 2019*; *Koelmans et al., 2016*). MPs and NPs present physicochemical properties, such as surface area, shape, chemical composition, functional groups, and surface charge, that promote their interactions with different organic and inorganic pollutants in the environment (*Zhang et al., 2020*; *González-Pleiter et al., 2021*; *Bhagat, Nishimura & Shimada, 2021*; *Wright, Thompson & Galloway, 2013*). Although some studies emphasize that the ingestion of microplastic contaminated with POP does not increase the risk for the marine animals when compared to the flux of POPs bioaccumulated from natural prey (*Koelmans et al., 2016*), others have focused on the potential harm of this combination, highlighting a possible existence of synergistic effects between MPs and POPs (*Bhagat, Nishimura & Shimada, 2021*; *Rodrigues et al., 2019*). However, it remains unclear whether this relationship actually exists under environmentally relevant conditions. More studies are needed to determine the actual capacity of NPs/MPs to transport associated pollutants that result in trophic transfer and bioaccumulation in the food chain. Experimental studies should be more widely developed based on actual conditions, mainly, concentration and nature of both MPs/NPs and contaminants commonly found in aquatic environments, combined with ecotoxicity assays with organisms of different trophic levels. While the experimental development continues to grow, simulation studies help indicate the paths to follow. In this field, in

terms of MPs, the most studied polymer types consist of PE, PP, PS, polyamide (PA), and polyvinyl chloride (PVC) (*Bakir, Rowland & Thompson, 2012*; *Fries et al., 2013*).

Organochlorine pesticides (OCP), polychlorinated biphenyls (PCB), polybrominated diphenyl ethers (PBDE), and PAH are some chemicals that can accumulate on MPs (*Bakir, Rowland & Thompson, 2012*; *Fries et al., 2013*; *Rodrigues et al., 2019*). In addition, MPs and NPs can release chemical monomers and additives with proven toxicity that are incorporated into materials during their manufacture, such as plasticizers, flame retardants, antimicrobial agents, and pigments (*Fries et al., 2013*). The sorption depends on the system properties (*e.g.*, temperature and pH) and the physical-chemical characteristics of the polymer and the contaminant.

The main sorption mechanisms between chemical compounds and MPs/NPs are hydrophobic interaction, electrostatic interaction, pore filling, van der Waals forces, hydrogen bonding, and $\pi-\pi$ interaction (*Tourinho et al., 2019*). Experimental investigations can provide evidence of the most likely type and mechanism of interaction; however, computer simulations are a valuable tool to predict and understand these processes. Moreover, the simulations can guide the experimental assays to look to the compounds with chemical characteristics that are harmful or interact more with a specific polymer.

Guo, Liu & Wang (2019) analyzed the sorption of sulfamethazine on PE, PS, PP, PA, PET, and PVC. To build the simulation box, only one polymer chain and one molecule of sulfamethazine were added in the vacuum for each system. The number of monomers varies from 100 to 300 depending on the polymer type. Besides, the simulation was performed in NVT ensemble at 298 K for 500 ps. The results suggested a dominance of electrostatic interactions in the sorption on PA, PS, PVC, and PP. In contrast, van der Waals interactions were dominant in the sorption on PE and PET. The computed adsorption energies decreased in the order PA > PET > PE > PVC > PS > PP.

Another antibiotic that was investigated was the tetracycline hydrochloride and its derivatives (chlortetracycline hydrochloride and oxytetracycline hydrochloride) (*Chen et al., 2021*). The simulation box consists of a PE chain with 300 monomers degree of polymerization, and one antibiotic molecule. The simulation was performed in NVT ensemble at 298 K. The interaction energy reveals that the adsorption capacity of tetracycline hydrochloride on PE is the weakest, whereas the chlortetracycline hydrochloride presents the highest adsorption capacity. *Chen et al. (2021)* also analyzed the radial distribution functions, which indicated a preference for the non-bond interaction between the carbon atoms of PE and the oxygens in the tetracycline molecules.

The adsorption of three pesticides (difenoconazole, buprofezin, and imidacloprid) on the PE molecular chain with polymerization degree equal to 160 was also evaluated, and interaction energies were found to be statistically the same among them (*Li et al., 2021b*). Besides, the MD simulation used the Grand Canonical Monte Carlo method without giving more details about the properties analyzed with this method (*Li et al., 2021b*).

Regarding PAH, *Yang et al. (2021)* analyzed the sorption of pyrene, 1-methylpyrene, 1-hydroxypyrene, 1-aminopyrene, and 1-pyrenecarboxylic acid on PS with polymerization

degree equal to 100, and found that said interaction had higher levels of energy when compared to sulfamethazine (*Guo, Liu & Wang, 2019*); however, the values for pyrene and its derivative were very close one another. Following the experimental evidence and the computational results, the pore-filling and the hydrophobic and $\pi-\pi$ interactions played an essential role in these adsorptions (*Yang et al., 2021*).

The sorption of $SrCl_2$ on PA, PS, and PP was also investigated, and the electrostatic interactions were the dominant mechanism (*Guo, Liu & Wang, 2020*). The simulation box was built following the methodology described by *Guo, Liu & Wang (2019)*. For $SrCl_2$, the adsorption energies followed the order PP > PS > PA, smaller than NPs and organic pollutants.

It is important to highlight that the simulations reported by *Guo, Liu & Wang, 2019*, *Guo, Liu & Wang (2020)*, *Li et al. (2021b)*, *Chen et al. (2021)*, and *Yang et al. (2021)* were conducted in the absence of water, even if implicit. The simulation boxes were composed of one polymer chain, one molecule of the pollutant and a vacuum layer. Although this kind of simulation requires less computational time, the validation is questionable when compared to experimental results in an aqueous medium, since the simulations were conducted in a vacuum.

Cortés-Arriagada (2021) studied the co-transport of BPA with PET using density functional theory (DFT) with B3LYP functional at def2-SVP basis sets. The solvent was considered implicitly, *i.e.*, a continuum medium characterized by the dielectric constant. To obtain an initial configuration of the PET nanoplastic, MD was performed by folding a single polymer chain described by AMBER force field using NVT ensemble, followed by energy minimization steps. The final nanoPET model was optimized *a posteriori* at the DFT level. Due to the nucleophilic nature of the outer surface of nanoPET and the hydrophobic characteristic of BPA, mass transfer and intraparticle diffusion of the pollutant into the nanoplastic were observed. An interplay between dispersion and electrostatics intermolecular interactions occurred, with the former dominating the inner surface adsorption, whereas the latter dominated the outer surface adsorption.

Feng et al. (2022) aimed to understand the process of aggregation of humic acid molecules (HA) with the contaminant benzo[a]pyrene (BaP) and heavy metal ions (Cu^{2+}) in an aqueous solution, as well as the influence of PS, PP, PVC, PET, and PE in these systems. Simulations with NP, HA, BaP, and Cu^{2+} show a competition between HA and BaP to adsorb on NP. When HA wins the competition and adsorbs on NP, it exposes carboxyl groups that offer interesting binding sites for Cu^{2+} adsorption. The results indicate that PS has the highest capacity of adsorbing BaPs. The motivation to study this system comes from the fact that environmental factors (such as dissolved organic matter - in the article represented by HA) can influence the interactions of NPs and contaminants. Hence the necessity to consider these factors in more detailed studies.

There are infinite combinations of pollutants and NPs that humans can contact, and each one can result in different consequences. However, the more studies in this field, the more it is possible to extrapolate behavioral trends. Thus, the investment of resources in this area becomes essential.

STUDIES THAT USED MOLECULAR SIMULATION AND DO NOT FIT INTO THE ABOVE SECTIONS

Although most MP/NP studies *via* molecular simulation can be fitted into the sections above, some address more specific issues. *Zhang, Zhao & Na (2020)* conducted combined research with molecular dynamics, ratio normalization, and molecular docking methods to understand biodegradation of phthalic acid esters (PAE) derivatives in marine and fresh-water environments. PAE are commonly present in plastics and confers their characteristics of malleability and plasticity; thus, they were chosen to serve as a model for the study of the biodegradability of plastics in marine environments. As we have already discussed, marine environment ends up being the final destination for tons of plastics every year, which is why the study is so important. As a result, five PAE derivatives were designed with excellent biodegradability as a goal, as well as functionality in mind.

Following the same train of thought, molecular dynamics and molecular docking appeared in *Lameh, Baseer & Ashiru (2022)* study about mutating existing enzymes to facilitate the biodegradation of PET and PP. The authors modified certain amino acids of the enzyme *Archaeoglobus fulgidus* (AFEST) and compared the changes in binding energy with plastics. They claimed that modifying an existing enzyme is the best approach, from the biotechnology viewpoint, to solve our plastic waste problem.

Moreover, *Wang et al. (2019b)* shared some exciting findings using molecular mechanics Poisson-Boltzmann surface area method and molecular dynamics simulations. Through prior knowledge that the hydrolase enzyme RPA1511 (obtainable from *Rhodopseudomonas palustris* bacterium) can efficiently depolymerize polylactic acid (PLA) plastic, they sought to understand which amino acids are responsible for this action. The binding affinity data showed that the RPA1511 could also degrade other polyester plastics. These results open doors for the study of more biodegradable plastics.

Another interesting research was that of *Ramalho, Dordio & Carvalho (2022)*. They carried out extensive molecular dynamic simulations of PE, nylon 6 and PET in an aqueous environment to achieve compression, mainly from their conformational behavior, of polymer chains after exposure to water. Over 200 ns of simulation, the three plastics had different responses, and, in the end, their chains equilibrated in the following ways: compacted and ordered, almost like a crystal, for PE, globular chains for nylon 6, and, for PET, tangled chains with the aromatic rings preferably oriented in parallel. Understanding how these plastics organize themselves when they are in such small particle form demonstrates how other contaminants can adsorb onto their surfaces and cause even more damage.

Since the presence of NPs in water is a reality, and, with research showing their potentially harmful effects, a line of study arises naturally: the search for methodologies for the removal of NPs from aqueous environments. *Sarcletti et al. (2021)* came up with the idea of removing NPs from water by applying an external magnetic field. They developed superparamagnetic iron oxide nanoparticles (SPION) that attract NP; they worked with both structural analysis, and molecular dynamics which supported the experimental results.

As far as we could track, the work by *Dettmann, Kühn & Ahmed (2021)* is the only one that applied MD to NPs found in soils. The authors used carbon nanotubes (with and without functional grouping) as a model for hydrophobic cavities and surfaces to represent an existing structure in organic soil particles. They carried out up to 500 ns of coarse-grained molecular dynamics simulations of hydrophilic and hydrophobic NP, carbon nanotubes, and water. NPs behave as expected concerning the hydrophobic carbon nanotubes according to their hydrophobicity. Regardless, the study stand out for being the first step in understanding processes in environments such as soils.

The work by *Li et al. (2021c)* about the impact of NP inhalation on the lungs is extremely interesting and pertinent. They investigated five types of NPs varying their sizes, surface charges, and molecular weights, and exposed them to interaction with lung surfactant (LS) film, both in the alveolar fluid and at the air-water interface. The authors pointed out that although the study does not yet represent an authentic environment in its complexity, the type of NP, its size, surface charge, and molecular weight were factors that modified the results in the interaction with LS. A study along the same lines (except that, rather than the concern about inhaling MPs and NPs, it deals with impacts on the human intestinal tract) is that of *Tan et al. (2020)*, which shows results on how five different types of MPs reduce lipid digestion when ingested with food. This work presents molecular dynamics and experimental results, with the former corroborating the latter's findings and going further in understanding interactions between NPs and lipids. The authors conclude that the interaction between lipid droplets and MP is expected to play an influential role in reducing lipid digestion.

Although we initially sectioned this review to manage the study in a more organized way, several other kinds of research were found that do not fit into any of our sections. We can highlight the studies that follow the line of seeking alternatives for removing NPs and MPs from the environment with which humans come into contact, mainly water. It is clear there are numerous ways to approach the problem of NPs and MPs.

CONCLUSIONS

The present literature review focus on molecular simulation methodologies to study MPs and NPs interactions with proteins, biological membranes, and other contaminants, the force fields used, and the main findings.

Most scientific publications are very recent, which strongly indicates that the subject is growing in importance. That is mainly because MPs/NPs released in very high quantities by human activities end up, mostly, in aquatic and marine environments. However, the interactions with and potential impacts on living organisms are largely unknown. All studies regarding the consequences of human contact with MPs/NPs have been hypothetical, while showing that MPs/NPs interact with their surroundings, fundamentally modifying their characteristics.

The MD simulation was the most used model out of the methodologies applied, and, based on the results, it fulfilled its objective in showing the interactions at the molecular level. An obstacle, however, is the level of simplification that is necessary during simulation, since natural systems, given their concrete complexities are still outside the

reality of investigations on a molecular level. Despite this, simulations can help a great deal to understand experimental data. The use of both experimental and computational approaches is in many scientific reports, and, in their conclusions, the authors have pointed out that they complement each other. As this is a fairly new field of study, there is no good methodology protocol to date on how to simulate NPs. Therefore, the steps in the methodological approaches vary considerably from study to study. Among these differences, one can highlight the simulation time, the force fields applied, the presence or absence of water models, and how the polymer chains are built to be considered NP particles. It is necessary to discuss the validity of certain practices within the molecular simulation to create a more mature protocol based on the information accumulated.
In addition to MD, another promising option is the MC methodology. Although many publications presented equilibrium properties that can be accessed through both MC and MD, only one investigation reported the use of MC methodology. Due to how the system reaches equilibrium, MC could be an alternative to achieve simulations with shorter computational times. Between the results obtained, it is interesting to highlight that the interactions between all NPs and the environment cannot be understood through a single nanoplastic model. Depending on the NP type, the interactions, whether with proteins, lipids, or contaminants, are expected to differ significantly. Thus, each NP may cause a different impact when in contact with humans and other living organisms, which makes further studies even more pressing.

In future perspectives, it would be also interesting to investigate the effect of NPs in DNA and RNA, and to include the effects of plastic additives on the molecular interactions of MPs and NPs with contaminants and biomolecules.

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Author Contributions

- Yamara Matos Oliveira conceived and designed the experiments, performed the experiments, analyzed the data, prepared figures and/or tables, authored or reviewed drafts of the article, and approved the final draft.
- Nathalia Salles Vernin conceived and designed the experiments, performed the experiments, analyzed the data, authored or reviewed drafts of the article, and approved the final draft.
- Daniele Maia Bila conceived and designed the experiments, performed the experiments, analyzed the data, authored or reviewed drafts of the article, and approved the final draft.
- Marcia Marques conceived and designed the experiments, analyzed the data, authored or reviewed drafts of the article, and approved the final draft.
- Frederico Tavares conceived and designed the experiments, analyzed the data, authored or reviewed drafts of the article, and approved the final draft.

Data Availability

The following information was supplied regarding data availability:

This is a literature review.

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