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Integration of Molecular Docking Analysis and Molecular Dynamics Simulations for Studying Food Proteins and Bioactive Peptides

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ABSTRACT: *In silico* tools, such as molecular docking, are widely applied to study interactions and binding affinity of biological activity of proteins and peptides. However, restricted sampling of both ligand and receptor conformations and use of approximated scoring functions can produce results that do not correlate with actual experimental binding affinities. Molecular dynamics simulations (MDS) can provide valuable information in deciphering functional mechanisms of proteins/peptides and other biomolecules, overcoming the rigid sampling limitations in docking analysis. This review will discuss the information related to the traditional use of *in silico* models, such as molecular docking, and its application for studying food proteins and bioactive peptides, followed by an in-depth introduction to the theory of MDS and description of why these molecular simulation techniques are important in the theoretical prediction of structural and functional dynamics of food proteins and bioactive peptides. Applications, limitations, and future prospects of MDS will also be discussed.

KEYWORDS: *molecular docking, molecular dynamics simulations, protein and peptides, molecular interactions*

1. INTRODUCTION

In silico biology is a fast-growing field that encompasses the theory, programming, and application of computational methodologies to model, predict, and elucidate biological functions at the molecular level.[1] *In silico* methodologies are widely recognized as useful tools with specific goals, from gene and sequence identification,[2] genome, transcriptome, proteome, and metagenome assembly,[3] and *de novo* drug design.[4] In this regard, we can classify the *in silico* methodologies into two groups. The first group, also called bioinformatics, comprises the organization of the data for access to the information, development of tools that make the analysis statistically robust, and the use of the data and analysis to interpret and formulate the evolutionary hypothesis.[5−7] The second group, biomolecular structure methodologies, also called biomolecular simulations, are based on a fundamental physicochemical description of particles (atoms and molecules) with remarkable emphasis on how biomolecules, such as proteins and peptides, move, fluctuate, and physically interact.[8] Biomolecular simulations can supplement *in vitro* or *in vivo* experiments with a molecular-level understanding of biological processes because the simulated particles can be analyzed in atomic detail, therefore, adding a new level of understanding and interpretation of experimental data in terms of molecular interactions.[9−11]

Nowadays, there is an extensive diversity of biomolecular simulation methodologies applicable to a wide range of problems in structural biology, such as drug design. Tools like molecular docking are biomolecular simulation methodologies based on integrated bioinformatic analysis, which examine the interaction between molecules (e.g., proteins and peptides) and predict their binding modes and affinity at a molecular or atomic level through computer programming.[12,13] They have been widely applied as theoretical simulation strategies in drug discovery research and for virtual screening studies dedicated to find novel active biomolecules, such as bioactive peptides. However, instrumental methods capable of providing direct access to high-resolution molecular information are required for complex food systems, such as in the case of food emulsions with several interfaces in which the protein is responding differently to each local environment.[14] As a result of the dynamic nature of food proteins, it would be logical that using high-performance computing, like molecular dynamics simulations (MDS), could be applied to further analyze their conformation as well as the conformational rearrangement of the protein to changes in the external or surrounding environment.[15] In contrast to traditional bioinformatics and molecular docking tools, using high-performance computing has allowed for MDS to study the relationship between dynamics and functions. In other words, MDS serves as a powerful tool to deliver complementary information to experiments and allow for an enhanced interpretation of the changes to the secondary and tertiary structures of a protein stimulated by adsorption at an interface.[14] This promising tool is beginning to receive more attention from food scientists. For example, Chen et al.[16] recently indicated a favorable trend toward the use of MDS in the engineering of enzymes with

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Table 1. Examples of Different Tool Resources Employed for the *In Silico* Analysis of Bioactive Peptides[a]

online *in silico* tool	prediction function	webserver link	reference
Peptide Ranker	bioactivity potential scoring	http://distilldeep.ucd.ie/PeptideRanker	25
PreAIP	anti-inflammatory peptide screening	http://kurata14.bio.kyutech.ac.jp/PreAIP/	26
iDPPIV-SCM	DPP-IV inhibitor peptide	https://camt.pythonanywhere.com/iDPPIV-SCM	27
AntiAngioPred	anti-angiogenic peptide	http://crdd.osdd.net/raghava/antiangiopred/	28
AHTPIN	antihypertensive peptide	http://crdd.osdd.net/raghava/ahtpin/	29
HLP	intestinal stability	http://crdd.osdd.net/raghava/hlp/	30
PlifePred	plasma stability	https://webs.iiitd.edu.in/raghava/plifepred/	31
ToxinPred	toxicity screening	https://webs.iiitd.edu.in/raghava/toxinpred	32

[a]DPP-IV = dipeptidyl peptidase-IV.

specific properties that would allow for their industrial-scale application in the food industry.

This review will first look at the information related to the traditional use of biomolecular simulations applied for studying food proteins and bioactive peptides and how these methodologies have served as a bridge between *in silico* and *in vitro* analyses to deepen the study of the virtual structure of a protein when encountered with complex environments, such as those typically found in food matrices. We will also present an in-depth introduction to MDS theory and applications and explain why, moving forward, these molecular simulation techniques are necessary to help predict and explain the structural and functional dynamics of food proteins and bioactive peptides.

2. COMMONLY USED *IN SILICO* METHODS

Different *in silico* methodologies can be used to describe the potential use of foods and their bioactive compounds. If the main goal is to elucidate structure−activity relationships between bioactive molecules and their potential targets, both bioinformatic and biomolecular simulations can be supplemented with other methodologies. For example, chemoinformatic methodologies include the analysis of chemical information derived from structural information on biomolecules, such as secondary metabolites, peptides, and lipids, among others.[17] Some of these methodologies also make use of information deposited in databases to determine the frequency of putative bioactive peptides in the primary structure of food proteins.[18,19] Moreover, large databases of bioactive molecules can be used to study the chemical space or chemical similarities against well-known drugs. In addition, the use of artificial intelligence (AI) techniques in bioinformatics and biomolecular simulation exemplifies the integration of different fields under multivariate statistics, where the effect of many variables or chemical properties determine the bioactivity profile of specific targets.[20−22] Nevertheless, if the structure of target bioactive compounds is unknown, different models can be built based on the information on a molecular reference (i.e., ligands and substrates). Such methodologies are also known as ligand-based methods, with the most applied computational methods being the quantitative structure−activity relationship (QSAR), quantitative structure−property relationship (QSPR) analysis, and pharmacophore modeling.[23] Other methods, such as iBitter-SCM, are employed to determine the bitterness of peptides (bitterness peptide screening, https://camt.pythonanywhere.com/iBitter-SCM)[24] using the scoring card method (SCM). In addition, *in silico* screening methods are widely used to study toxins, food-borne pathogens, and trypsin inhibitors in foods at a molecular level.[33,34]

In foods for health research, *in silico* methods are used on proteins and bioactive peptides to determine different parameters, such as their affinity to bind specifically to their targets, their probability to be bioactive, their intestinal stability, and their ability to be retained in the circulatory system, among others. Table 1 lists a summary of commonly used software tools available for the *in silico* analysis of bioactive peptides. Of the many *in silico* methods described, molecular docking analysis is one of the most widely used tools in drug design research and virtual screening studies to find novel active molecules derived from natural sources (e.g., plants), where this type of biomolecular simulation is used to predict binding sites, elucidate the mechanism of molecular recognition by simulating the spontaneous binding process of biomolecules (e.g., proteins, carbohydrates, and lipids), and explain their intermolecular interactions.[13]

2.1. Overview of Molecular Docking Analysis in Food Proteins and Bioactive Peptides. In the area of bioactive peptides, molecular docking allows for characterization of the behavior of peptides in the binding site of target proteins. Because molecular docking is a structure-based method, it enables it to delineate the structure−activity relationship of peptides.[35] Overall, the molecular docking process includes predicting the molecular orientation of a ligand within a receptor and then calculating their complementarity interaction using a scoring function (i.e., binding affinity).[12] Figure 1 depicts the steps taken to carry out molecular docking analysis of a bioactive peptide. For example, once bioactive peptides have been successfully fractionated and identified (i.e., sequenced) and their bioactivity has been determined through *in vitro* or *in vivo* assays, they undergo structural preparation for docking. Next, the ligand is prepared for established target receptor−ligand complex structures using docking simulation software. Finally, the analysis of the data is performed by predicting the binding modes and affinities (i.e., scoring functions) of a small molecule (i.e., bioactive peptide) within the binding sites of target receptors (Figure 1).

In the case of food proteins, molecular docking has been used mainly to study the relationship between enzymes and substrates, which can help in the regulation of enzyme activity in foods, as well as to study antinutritive compounds, such as trypsin inhibitors.[13] For example, it was used to study the binding interaction between egg white ovalbumin and malachite green dye, a food additive with probable carcinogenic potential, showing that the interaction between ovalbumin and malachite occurred through hydrophobic and van der Waals interactions.[36] Similarly, molecular docking was used to determine that myrosinase, an enzyme found in broccoli (*Brassica oleracea* var. italica), was able to catalyze the conversion of glucosinolates to metabolites that possess health-

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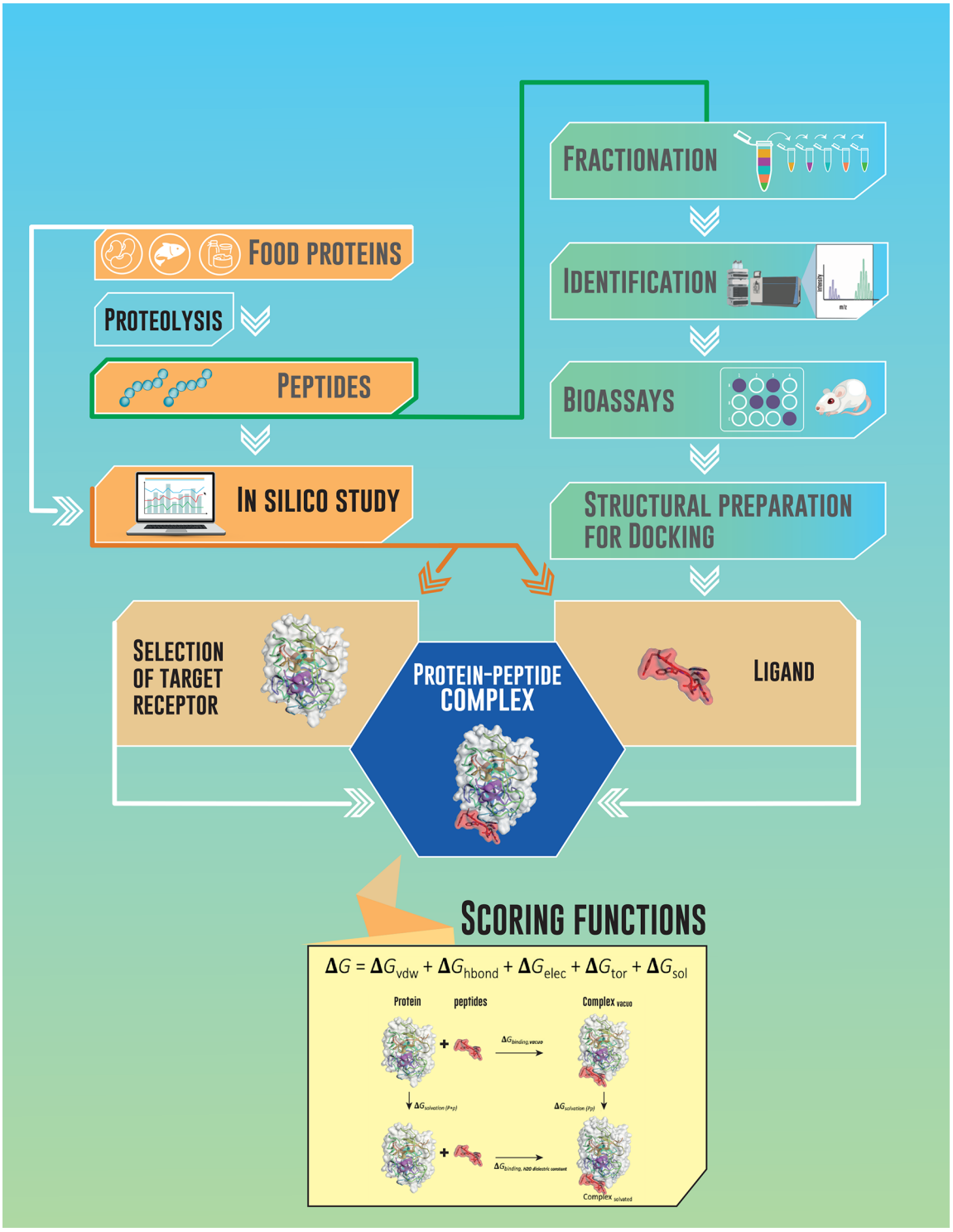

Figure 1. Steps required to conduct an *in silico* study of food peptides (ligand) and proteins (receptor).

promoting properties (i.e., isothiocyanates).[37] Cui et al.[38] applied molecular docking to study the mode of action of epigallocatechin gallate (EGCG) and their catechin isomers [epicatechin gallate (ECG), epicatechin (EC), and epigallocatechin (EGC)] against trypsin. They found that the binding affinity was in the order of GCG > ECG > EC > EGC, which suggests that the galloyl group can significantly enhance the binding affinity. Moreover, the interactions between catequin molecules and trypsin were by van der Waals and hydrogen bonding. Molecular docking was also used to reveal that, from a total of 127 compounds identified in rice (*Oryza sativa* Linn.), 8 of them possess α-amylase inhibitors.[39]

Overall, molecular docking in food proteins has been very useful in the study of nutritional components and food safety (Table 2). Nowadays, a considerable number of studies have also elucidated the mechanisms of action through molecular docking of different food-derived peptides that exhibited diverse biological activity toward hypertension.[40,41] Angiotensin-I-converting enzyme (ACE) seems to be the most common target protein used in docking analysis of bioactive peptides;

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Table 2. Examples of Docking Analysis of Bioactive Peptides toward Selected Targets[a]

protein source	peptide identified	main results	reference
Nile tilapia (*Oreochromis niloticus*)	GPEGPAGAR and GETGPAGPAGAAG-PAGPR	hydrogen bond, electrostatic, and hydrophobic interactions with the binding pocket of ACE	43
shortfin scad (*Decapterus macrosoma*)	RGVGPVPAA	hydrogen bonds and π interactions with the binding site cavity of ACE	41
Qula (an acid curd cheese-like product) casein	PFPGPIPN, KYIPIQ, and LPLPLL	binding energies of peptides with ACE were −7.08, −6.33, and −6.19 kcal/mol, respectively, better than a ACE inhibitor (captopril = −5.07 kcal/mol)	45
tilapia skin gelatin	VGLPNSR and QAGLSPVR	interactions with ACE through hydrogen bonds	44
yeast (*Kluyveromyces marxianus*)	LPESVHLDK and VLSTSFPPK	peptides interacted with the active site of ACE through hydrogen bonds	46
camel (*Camelus dromedarius*) milk	SHSPLAGFR, TLMPQWW, CLSPLQMR, and CLSPLQFR	hydrogen and hydrophobic bond interactions and, to a much lesser extent, π interactions	57
camel (*Camelus dromedarius*) whey protein	PAGNFLP and FCCLGPVPP	hydrogen, hydrophobic, and π interactions	58
rice (*Oryza sativa*)	YSK	interactions with ACE through hydrogen bonds	59
mollusk (*Cyclina sinensis*)	WPMGF	hydrogen bond and hydrophobic interactions	40
black soybean (*Glycine max* L. Merr.)	L/IVPK	peptide interacted with active sites of apoptosis-related key proteins (e.g., X-linked inhibitor of apoptosis protein, caspase-3, caspase-7, and Bcl-2)	47
chickpea (*Cicer arietinum* L.)	RQSHFANAQP	peptide increased the expression of the p53 level (key tumor suppressor protein)	48
casein	FQSEEQQQTEDELQDK	peptide interacted with the active site of thrombin	49
bean (*Phaseolus vulgaris*)	DFFLS, DFFL, FFL, LLSL, QQEG, NEGEAH, and AHTV	interaction with the active site of DPP-IV; NEGEAH and AHTV showed affinity with other residues of DPP-IV; for α-amylase, all peptides interacted with its active site	50
soybean	GSR and EAK	van der Waals, hydrogen bond, and π interactions in the active site of α-glucosidase	51
chia seed (*Salvia hispanica* L.)	APHWYTN, DQNPRSF, GDAHWAY, GDAHWTY, GDAHWVY, GFEWITF, and KKLKRVYV	enzyme−peptide pair interactions (including hydrogen bond), most interactions close or at least in the vicinity of the active site of elastase	52

[a]ACE, angiotensin-converting enzyme; DPP-IV, dipeptidyl peptidase-IV.

therefore, the antihypertensive or ACE inhibition activity is considered the most studied bioactivity through this *in silico* structure-based approach. For example, it has been employed for analyzing the interaction of bioactive peptides derived from smooth-hound (*Mustelus asterias*),[42] Nile tilapia (*Oreochromis niloticus*),[43,44] shortfin scad (*Decapterus macrosoma*),[41] Qula casein,[45] and yeast (*Kluyveromyces marxianus*) proteins.[46] In most studies, it was found that peptides interact with ACE through hydrophobic, hydrogen bond, van der Waals, and π interactions [i.e., a type of non-covalent interaction that involves π systems that make strong ($\pi-\pi$) aromatic stacking interactions]. Some studies observed a relationship between the binding energy of some peptides and their affinity with the ACE.[43,45] Thus, peptides with the lowest binding energy values (i.e., free energy) showed higher ACE inhibition. In addition, the inhibition pattern of bioactive peptides (i.e., non-competitive and competitive) toward ACE was in accordance with the results of molecular docking analysis, suggesting a correlation between the prediction of the mode of inhibition and their *in silico* data. Fox example, peptides with a non-competitive inhibition pattern against ACE usually established a hydrogen bond interaction[45] with the enzyme, while peptides with a competitive inhibition pattern commonly showed hydrophobic interactions.[44] Other valuable reports on the application of molecular docking analysis include applications in cancer,[47,48] thrombosis,[49] diabetes,[50,51] and skin-aging research.[52]

Although molecular docking has been very useful in the study of the structure−activity relationship of food proteins and peptides, it has some limitations that include the restricted sampling of both ligand and receptor conformations in pose prediction and the use of approximated scoring functions that produce results that may not correlate with experimental binding affinities.[35] Molecular docking is considered a rigid sampling method, where the protein structure is a static receptor, unable to respond to ligand perturbations and external molecules (e.g., ions and solvents) and environments (e.g., pH and electric field).[53] In molecular docking, the protein is handled in a rigid form; however, it can adopt many different conformations, depending upon the ligand to which it binds, because of the constant motion between different conformational states having similar energies.[54] Some intermolecular interactions cannot be accurately predicted, including solvation effects and entropy changes.[54,55] For example, Cheng et al.[56] performed a comparative assessment of 16 different scoring functions of 195 diverse protein−ligand complexes and found low correlation coefficients between the experimental binding constants and scores computed by those scoring functions. Molecular docking can be a very useful tool as a first approach to understand the interactions of peptides or target proteins. However, alternate strategies that can evaluate the virtual structure−activity relationships of these compounds in a more complex and realistic environment are still needed.

3. MOLECULAR DYNAMICS SIMULATIONS IN FOOD PROTEINS AND PEPTIDES FOR SOLVING BIOMOLECULAR INTERACTIONS

As described above, *in silico* tools, such as molecular docking, have been widely used in the study of structure−activity relationships of food proteins and peptides as well as in virtual screening studies to find novel active biomolecules. However, some limitations of molecular docking include the restricted sampling of both ligand and receptor conformations and the use of approximated scoring functions that can produce results that do not correlate with actual experimental binding affinities. MDS is a computer simulation method employed

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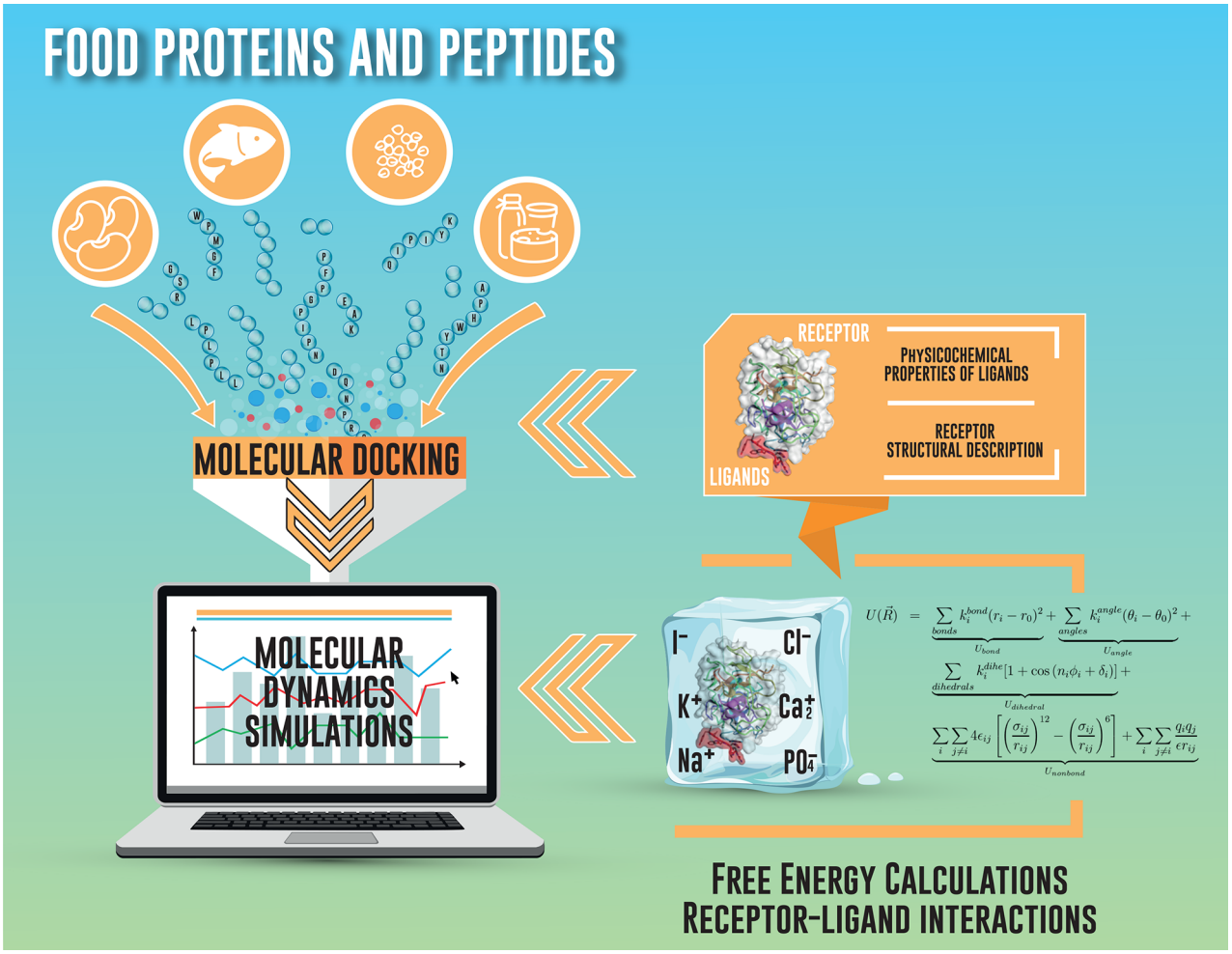

Figure 2. Development of an integrated *in silico* analysis strategy using molecular docking data obtained from food proteins and peptides, and refinement with molecular dynamics simulations to obtain theoretical receptor−ligand interactions and free energy within a complex environment.

in various engineering and science disciplines to calculate motion and equilibrium of each molecule, therefore providing information about the behavior of the complex protein−ligand in full atomic detail and at very fine temporal resolution. MDS was introduced in the late 1950s with the development of processing machines;[60] however, it was not until 1975 when the bovine pancreatic trypsin inhibitor protein simulation was first described by Levitt and Warshel.[61]

Our need to understand how the dynamic behavior of a biomolecule affects its functional behavior led to the development of these computer simulation techniques that allows us to predict the time evolution of a system consisting of interacting particles, i.e., atoms, molecules, and complexes. Thus, MDS allowed us to gain insights into the dynamic properties of biomolecular systems, such as transport coefficient simulations, protein folding and stability, ligand binding, and protein complexing, among others. For example, once specific peptide sequences have been identified, we can apply MDS to refine the several thousands of possible ligands obtained with molecular docking and discard false-positive molecules that act as decoys during the receptor−ligand complex formation. These simulations capture the behavior of proteins and other biomolecules in full atomic detail and at very fine temporal resolution to establish free energy values of the receptor with ligand interactions within a complex environment.[10] In the case of complex systems, such as interactions of bioactive peptides with target proteins, MDS is a very powerful technique that can solve the equations of motion of every particle on the system (e.g., atoms and molecules) using force field (FF) equations. Nowadays, MDS is able to precisely control the physical conditions of the experiment, from the temperature and ions around the peptides to the properties of the solvent where the system particles are embedded, such as the volume, geometry, and type of solvent, like water, organic solvents, or ionic liquids. Moreover, to compare to *in vitro* and *in silico* experimentation, MDS can impose physical and chemical restrains over the starting coordinates of the peptides, their protonation states, the surrounding particles as ions and membranes, the temperature, and other properties, like electric potentials, voltage gates, and potentials across membranes derived from protons or electrolyte concentrations (Figure 2).

3.1. Overview of Concepts Related to MDS. To understand MDS, we first need to define certain key concepts related to the physics behind molecular dynamics. The first term refers to molecular mechanic (MM) equations, which are the theoretical support of MDS; they comprise several numerical techniques applied to the study of physical−chemistry properties of biomolecular systems and are commonly based on classical mechanics. Classical mechanics are often referred to as Newtonian mechanics because the mathematical formalism addresses the resolution of Newton's

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equations to describe the change in position and acceleration of n particles in the system. MM is widely used for the prediction of physical properties, like the molecular structure or energy, and these properties are calculated by excluding the electrons and nuclei effect of the molecules.[8] The basic approach derives from the Born−Oppenheimer approximation, which remarks that, because electrons have much lower mass than the nuclei and as a result of their much greater velocity, they instantaneously move around the nuclei.[62] Thus, the properties of the electrons and the quantum aspects of the nuclear motion are ignored, deriving into parameters and an equation described by a FF.[63] Hence, atoms are the smallest unit of a MM system, modeled by the linear combination of nuclear properties and the average distribution of electrons on basal conditions. These non-explicit electron models are often denoted as "ball and spring" systems, where atoms are represented by point and soft spheres (nuclei), for which mass and charge (electrons) are defined by their relative atomic masses, connected by imaginary springs (chemical bonds). These numerical methods allow for the study of large systems, comprising hundreds and thousands of atoms, with few current computational resources. However, MM devises several limitations, like the resolution of problems on catalysis, where an explicit description of electron or chemical reactivity (bond breakage and formation) is needed. MM calculates the energy of a system on the basis of all nuclei coordinates, to produce a potential energy surface. Hence, the total potential energy of the system is given by the sum of all energies between the particles in the system.[8] Individual equations are used to model the covalent interaction terms, such as bonding, angles, and torsions, and non-covalent interaction terms to model van der Waals and electrostatic interaction contributions (Table 3). However, the practical use of these equations is limited, in part as a result of the continuous evaluation of the energy functions in a large number of particles and several times during a simple run. The most efficient solution is the use of empirical parameters, which are obtained by several experimental techniques or a higher level of theory, such as *ab initio* quantum chemical calculations.

Table 3. Energy Terms and Corresponding Equations Used To Model Bonding and Non-bonding Interactions in Molecular Dynamics Calculations[a]

energy term	equation
Bonding Interactions	
bonding and stretching	$\nabla l = \frac{1}{2}k_l(l - l_0)^2$
angle bending	$\nabla\theta = \frac{1}{2}k_\theta(\theta - \theta_0)^2$
torsional or dihedral bending	$\nabla\omega = \sum \frac{1}{2Vi}[1 + \cos(n\omega - \gamma)]$
Non-bonding Interactions	
van der Waals interactions	$\nabla \mathrm{vdW}(i, j) = 4\varepsilon_{ij}\left[\left(\frac{\sigma_{ij}}{r_{ij}}\right)^{12} - \left(\frac{\sigma_{ij}}{r_{ij}}\right)^{6}\right]$
electrostatic interactions	$\nabla_{el}(i, j) = \frac{1}{4\pi\varepsilon}\frac{qiqj}{ri, j}$

[a]All of the terms and equations describe the connectivity (bonding) and distant interactions (non-bonding) between the atoms of the systems and the neighbor atoms. Each group of terms incorporates all of the necessary equations to physically describe molecular models, from intramolecular bond formation to intermolecular interactions. Also, a potential of each term is calculated as the sum of individual contributions of each atom in the system. These potentials are well-defined in the force fields (FF); the FF data can also be transferable between molecular systems with similar molecular topologies.

With regards to FFs, most of them share a few essential terms that include a set of equations defining the potential energy, a series of atom types that depend upon the hybridization, charge, and types of atoms to which an atom is bonded, and a parameter set that defines force constants to relate atomic characteristics to energy components and structural data. All of the FFs formulate a common equation for the MM energy (E_{MM}) or potential energy resolution. The most predominant energy contributions found on atoms and molecules are shown in eq 1

$$E_{MM} = E\ \text{bonding}_{(\text{stretching}+\text{bending}+\text{torsional})} + E\ \text{non-bonding}_{(\text{electrostatic}+\text{vdW})} \quad (1)$$

where $E_{stretch}$ or bond stretching accounts for deformation of the bond distance between two atoms, E_{bend} or angle bending accounts for the cardinal variation in bond angles, E_{tor} or torsional accounts for a measure of the dihedral angle rotations, E_{vdW} or van der Waals (vdW) accounts for dispersive attractions and Pauli repulsions between nearby atoms, and E_{elect} or coulombic represents strong interactions resulting from the presence of atomic charges.

The first three terms relate to covalent bond modeling among atoms, whereas the last two terms (E_{vdW} and E_{elect}) are related to non-covalent interactions between nearby (vdW) and long-distance atoms (coulombic). Some FFs may include other terms to improve the results obtained, for example, polarization terms, cross terms, improper corrections, halide diffusive functions, and ring out-of-plane bending violation terms. However, the relation between the gain in accuracy and the increase in the computational cost must be considered.

As a result of the complicated nature of these potential energy calculations, there is no analytical solution to the equations of motion for systems with more than two interacting particles, and they must be solved numerically. Numerous algorithms have been developed for integrating Newton's equations of motion, and thus, it is possible to simulate enormous complexes of biomolecules, similar to those found in nutritional supplements, with in-house workstations or with supercomputer facilities. However, the common use of MDS can positively impact different research areas, such as peptide bioactivity, because the experimental work is commonly constrained by the difficulty or inability to directly probe and characterize the atomic interactions between bioactive peptides and their molecular targets. With the use of emergent sampling methods on MDS, we are able to sample intermolecular interactions between ligands and receptors in a very short time period (e.g., nanoseconds to microseconds).

3.2. Applications of MDS in Protein and Bioactive Peptide Research. MDS has been widely used in drug design,[64] physical and chemical characterization of complex motions between biomolecules,[65−67] and molecular design of advanced materials, such as bioelectrodes and biosensors.[68−70] For example, Ricci et al.[71] extensively evaluated physicochemical parameters of natural compounds (e.g., luteolin, ursolic acid, and carragenans) for their use as inhibitors of ubiquitin ligase of human papilloma virus (HPV E6) protein using

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MDS.[71] Santini et al. used MDS to probe the conformational flexibility of natural and synthetic aptamers in complex to cellular receptors as an alternative for drug delivery in emerging cancer.[72]

Although the use of MDS is well-accepted in several chemistry-related fields, in the food science discipline, it has begun to gain more attention primarily to engineer food enzymes, to better understand the thermostability of food proteins, and to evaluate the adsorption of a protein in the interface of a food emulsion. For example, MDS is being applied to study small compounds (e.g., secondary metabolites), full biomolecular interactions (e.g., proteins and peptides) capable of displaying effects on different enzymes, and ligand−receptor complexes that were not previously described for full proteins.[73] MDS is proposed as a more efficient tool for modeling protein denaturation during thermal processing and a support in mutation experiments to improve thermostability of several food enzymes (e.g., lipases, amylases, and β-galactosidase, among others).[74] In the context of food emulsions, MDS revealed the adsorption mechanisms of β-lactoglobulin at an oil−water (O/W) interface, a key aspect in dairy-based food formulations because most physicochemical properties of O/W emulsions stabilized by milk proteins are mainly determined by the structure and adsorption behavior occurring at the interface.[14,75] Others, like Barroso et al.,[76] applied MDS to capture the behavior of non-covalent binding compounds (i.e., tastants) to taste receptor sites in the tongue. Recent literature reports the application of MDS on food proteins and peptides, including some targeting food peptides that can be used to mitigate SARS-CoV-2.[77,78] In these studies, a combination of molecular simulation techniques allowed for the identification of fish peptides as possible inhibitors of the SARS-CoV-2 main protease, providing insights on the key intermolecular interactions of glycine residues of the receptor-binding domain.[78]

4. LIMITATIONS OF MDS AND FUTURE PROSPECTS

Despite the successes of MDS in tackling biochemical problems, it is still limited by two principal challenges. For example, the FFs used are in continuous refinement, and high computational demands prohibit routine simulations greater than dozens of microseconds in length, usually deriving in an inadequate sampling of conformational states. As an example of the high computational demands, MDS of a complex system of around 50 000 particles (including solvent water molecules, ions, ligands, and receptors) running in 64 central processor units (CPUs) can take several days to complete. However, with the implementation of graphic processor units (GPUs), the aforementioned system can be simulated in a few hours, draining the research resources to the acquisition of expensive GPU computer clusters.[79] Aside from challenges related to the high computational demands of MDS, the FFs are condensed approximations of the electronic behavior ruling the quantum world. While MDS accurately approaches the description of many molecular motions, these simulation techniques are poorly suited to systems where quantum phenomena are determinant, for example, biomolecular systems with transition metal atoms as photosystems or respiratory enzymes.

To tackle these methodological limitations, some researchers introduced quantum mechanical (QM) calculations into classic MDS FFs. For example, the motions and reactions of enzymatic active sites are described to the electronic level of theory, and the larger system motions, such as domain displacements and side-chain rotations, are approximated using MM.[80] Additionally, to neglect QM electronic effects, MDS is also limited by short time simulated scales, which range from hundreds to a few thousands of nanoseconds. To describe thermodynamic properties and reproduce coupling formation, such as those observed in drug binding, all of the possible conformational states of the protein must be explored during simulation time. Unfortunately, like in many biochemical processes, these events occur on time scales that are much longer than those handled by MDS. A number of solutions to this challenge have been developed; however, these kinds of enhanced sampling techniques are in strong dependence of the previous knowledge of biomolecular systems.

In the last couple of decades, the pharmaceutical industry has focused in licensing of peptide-based drugs for therapeutic applications.[81,82] Therefore, a deep understanding of the molecular motions in the receptor−ligand (e.g., protein−peptide recognition) can lead to clinical applications in pharmaceutical science, biomedical, and therapeutic food industries. Current sampling methods in MDS (e.g., metadynamics) replicate exchange techniques and free energy calculations that are highly dependent upon collective variables or reaction coordinates that are not very clear to define.[9] Unconstrained methods, such as accelerated molecular dynamics (aMD), comprise the addition of boost potentials that flood the energy depths and derive in the smoothing of the potential energy surface. This technique allows for the crossing of the energy barriers of biomolecular systems, like those found on protein−peptide complexes. These techniques derive in a direct acceleration by orders of magnitude, in a description of the process that occurs in longer chemical simulation times.[83,84] Furthermore, aMD provides unbiased sampling without selection of collective variables related to the complex biochemical behavior of the systems. The application of boost potentials that follows a Gaussian distribution allow for the recovery of the free energy surface through cumulated expansion to the second order.[85−87] The improvements on the sampling problem of binding that underlie MDS have allowed Gaussian accelerated molecular dynamics (GaMD) to capture in atomistic detail processes, such as the conformational changes essential to enzyme function,[88] the folding of proteins and peptides to their functional structures, the transport of drugs and other small molecules across membrane receptors,[89] and the binding/unbinding of drugs to their therapeutic targets.[85,87,90,91]

Other potential applications of MDS include assessing interactions of food bioactive peptides with enzymes related to chronic disease (e.g., ACE and DPP-IV) for the development of functional foods and/or nutraceutical products. In these situations, MDS can provide valuable information in deciphering functional mechanisms of proteins/peptides and other biomolecules, overcoming the rigid sampling limitations in docking analysis. As more food scientists adopt MDS in their research, this valuable tool will also serve as a platform to better understand the structure and function relationships of food proteins and peptides within food systems.

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Notes

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