



Modeling and simulation in medical sciences: an overview of specific applications based on research experience in EMRI (Endocrinology and Metabolism Research Institute of Tehran University of Medical Sciences)

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Abstract

The concomitant use of various types of models (in silico, in vitro, and in vivo) has been exemplified here within the context of biomedical researches performed in the Endocrinology and Metabolism Research Institute (EMRI) of Tehran University of Medical Sciences. Two main research areas have been discussed: the search for new small molecules as therapeutics for diabetes and related metabolic conditions, and diseases related to protein aggregation. Due to their multidisciplinary nature, the majority of these studies have needed the collaboration of different specialties. In both cases, a brief overview of the subject is provided through literature examples, and sequential use of these methods is described.

Keywords Disease model · Alpha-amylase inhibitor · Amyloid · Insulin injection

Introduction

“Model” is a generic word, present in the majority (if not all) of scientific disciplines technical vocabulary, and could be represented by a simple diagram that is easily understood by a glance. It could also be a complex entity incorporating hundreds of thousands of interacting partners, that should be analyzed by an information processing system. Either simple or complex, a model is always an approximation, contains errors, and is usually a tool to be used alongside with others.

Even within a particular scientific branch, such as medical sciences, a “model” takes different forms. As an example, in the present context of COVID-19, there has been a worldwide effort to predict the spread and dynamics of the disease [1–4],

and a similar international endeavor toward finding potential drugs that would bind the virus proteins [5–7]. In both cases, sophisticated “models” were used, which have fundamentally different characteristics: one includes mathematical equations and the other is an atomistic representation of proteins.

The first modeling works that have been performed in EMRI had a mathematical nature and included modeling of angiogenesis in tumors (aiming at improving diagnostic) [8], and proposing a suitable time to test bone mass density in post-menopausal women receiving levothyroxine [9]. In the following years, different kinds of mathematical models have been used in numerous studies related with public health (e.g. [10]) and other clinically-oriented subjects such as osteoporosis (e.g. adjusting a predictive tool to assess fracture risk in osteoporotic patients for the Iranian population) [11].

In this condensed overview, examples of specific research subjects related to medical sciences are discussed with an emphasis on the use of in vivo, in vitro, and in silico models. These subjects have been investigated by students and researchers over the past twelve years in the Modeling and Simulation in Medical Sciences (MSMS) research group of EMRI with the help and contribution of numerous collaborators inside and outside EMRI. We believe that models are important tools that upon extensive development, could

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reduce the overall cost of research, and ideally replace the need for extended testing.

Glucose homeostasis and related metabolic conditions disorders

In a recent study concerning the global burden of diseases, increase of the mortality rate and total years of life lost (YLL) due to diabetes mellitus has been observed (around 31 and 25% respectively) between 2006 and 2016, while YLL itself had decreased (around 2%) [12]. A worldwide rise in the prevalence of diabetes is likely, estimated to reach 10.4% by 2040 [13], and justifies the search for novel therapies to control the disease and prevent its complications.

Various molecular targets have been proposed and explored in this regard, with the aim of improving insulin's effect (e.g. by influencing beta-cells function by modulating related receptors), as well as controlling oxidative stress and inflammation to counteract beta-cells apoptosis [14, 15]. A different approach consists of interfering with key carbohydrate digestive enzymes (glycosidases) that are responsible for the postprandial rise of blood glucose level, especially alpha-glucosidase [16], and alpha-amylase [17]. Inhibitors of these enzymes could also be potentially effective in obesity. Many studies are now reporting the glycosidase inhibitory potential of plants and their active components [18]; earlier studies had also demonstrated the efficacy of flavonoids in this regard [19, 20], and analyzed putative binding modes of these compounds to the enzyme by *in silico* techniques [21].

During *in vitro* experiments, with the use of pancreatic alpha-amylase, and based on the fact that the natural compounds flavonoids were known as amylase inhibitors [22], we found trans-chalcone (flavonoids precursor) to be an inhibitor of pancreatic amylase [23]. Further experiments showed it to be also effective *in vivo*, on a rodent diabetic model [24] and in a mice model of fatty-liver, where the compound was able to modulate lipid profile, leptin, and glucose levels, as well as improving liver steatosis [25]; these results were comparable to what we had seen in a mice model of obesity [26]. Trans-chalcone is actually considered as a potent scaffold, whose numerous derivatives span a wide range of therapeutic properties [27, 28].

The benzothiazole thioflavin T (ThT), with slighter inhibitory activity *in vitro*, was also found to be potentially anti-diabetic *in vivo* [29], as well as effective in weight reduction of a mice model of obesity [30]. ThT is a well-known probe for detection of amyloid structures [31], but interestingly, had also been found to extend the lifespan of *C. elegans* (as a model for studying ageing and lifespan) [32, 33], and seems to be an interesting therapeutic scaffold.

Synthetic aurones ((*Z*)-2-benzylidenebenzofuran-3-one derivatives), which bear structural similarity to flavonoids were

also tested *in vitro* against pancreatic amylase as inhibitors, and subsequent *in silico* tests (docking) suggested their putative binding modes into the enzyme [34]. Docking was done with the use of U-Dock 1.6 program [35], with the MMFF94s force field and on MOE (Molecular Operating Environment 2012.10) and the target was PDB entry 1OSE [36]. In this method, a series of ligands conformers were docked onto the target and poses with best energy were retrieved. This data could be of use for synthesizing more potent inhibitors. Recently, many natural extracts and synthetic new molecules have been investigated with regard to their alpha-amylase inhibitory properties [37–48] and this line of study seems to be promising with regard to finding effective anti-diabetic aids.

On the other hand, activators of the enzyme may potentially show an adverse effect in this regard, although hypothetically beneficial for digestive problems. Interestingly, the commercially available sweetener neohesperidin dihydrochalcone (from sweet orange), which has a chalcone structural component was found to activate alpha-amylase from different sources [49, 50]. Xanthine derivatives, pentoxifylline, theobromine and caffeine were also found to slightly (20–30%) increase enzyme activity [51]. To our knowledge, these should be the only small molecules that have been reported as activator of this enzyme.

In another context, sweet taste receptor (STR), a class C G-protein coupled receptor (C GPCR), has been suggested as a drug target for designing either new low-calorie sweeteners or drugs to control metabolic condition disorders such as type II diabetes mellitus [52, 53]. In order to design appropriate ligands for this receptor, a three-dimensional structure is needed, but since the human receptor structure is still not experimentally elucidated, *in silico* (molecular modeling) methods were used in this case. First, a new model of STR structure was developed [54]. Extensive docking experiments on the model revealed presence of carbohydrate binding modules as structural and functional motifs [55], which is applicable in further characterization of the binding site. Docking was performed on an ensemble of STR structures obtained by running a molecular dynamics simulation (MDS) experiment. MDS was run for 50 ns in YASARA program [56] using the YASARA forcefield [57]. The initial structure atoms were protonated at physiological pH, Particle Mesh Ewald (PME) was used, 8.0 Å cutoff for non-bonded interactions and 4 fs timestep were applied, hydrogen atoms were constrained and the system was at constant pressure and temperature (NPT). Based on carbon alpha RMSDs of the total MDS run, clusters were obtained based on minimum, median and maximum values. Docking was performed on these clusters with AutoDock Vina [58] on the interface present in YASARA which enabled computing H-bonds and HP-interactions, as well as minimization of the best poses to take water molecules into account. Finally, by studying interaction patterns of 316 sweet molecules as well as sweet proteins and antagonists of

the receptor, a few compounds were suggested as putative sweet molecules or agonists for the receptor [unpublished data]. Increasing our knowledge about this receptor structure/function and its agonists/antagonists would surely be of help in the current debate about the beneficial/detrimental effects of sweeteners [59–62].

Proteins aggregation: formation of pathogenic species

The integrity of proteins three-dimensional structures is a requirement for their normal functioning. Over disruption of their intramolecular interactions, “misfolded” proteins structures could be formed, which, depending on environmental conditions, may “aggregate” together and form stable clusters. Misfolding may happen upon mutations or other external causes, and lead to “protein diseases”. Aggregates may be structured (amyloid) or unstructured (amorphous), although various intermediate forms can also be observed [63].

Amyloid formation is suggested to have a pathogenic role in a range of disorders, including Alzheimer’s and Parkinson disease, metabolic diseases, and diabetes [64]. In recent years, formation of structured amyloid forms has been suggested to be a generic property of proteins, which means that any protein could be potentially driven toward forming amyloids [65], and as so, be used as a model to study the process in details. Inhibiting amyloid formation or disruption of amyloid structures by small ligands has been the subject of numerous studies in the past two decades, and based on these reports, there have been attempts to classify ligands and identify the structural prerequisites that make suitable anti-amyloid compounds [66–72].

One of the research subjects that could provide useful information for drug design is the study of structural changes that occur in proteins and result onto amyloid formation. Disruption of the native and functional architecture of the protein may start from specific locations, and aggregation-prone sites in proteins have an important role in triggering aggregates formation. Predicting those regions has been (and still is) the subject of multiple computational studies which have resulted onto design of various tools [73–83]. An alternative to these tools is the application of molecular dynamics simulation methods to proteins and monitor the changes that happen in their structures under various deleterious conditions. We have applied this method to insulin [84] and two forms of myoglobin (native and glycosylated) [85] and could spot specific regions that seem to be initiators in the structural change process. In both cases, high temperatures were employed in order to reach the unfolded state more easily [86]. For insulin, different conditions (presence and absence of KCl and NaCl) were tested, at high temperature and acidic pH (obtained by changing the protonation state of the protein).

For the actual run, MOE (Molecular Operating Environment MOE.2010.10), and the implemented MMFF94x force field were used for 15 ns runs of each condition. To check the conformational change over time, secondary structure content was analyzed by using various methods including DSSP [87], STRIDE [88], PALSSE [89], P-SEA [90], STICK[91], and XTLSSTR [92]. For myoglobin too, the same program (MOE.2010.10), and the implemented MMFF94x force field were used. In this case, in addition to secondary structure content (computed by DSSP), structures’ RMSDs were also compared in order to find the most stable regions. Our subsequent ongoing research projects focus on shorter proteins with the aim of pinpointing specific residues in well-defined secondary structure segments. These *in silico* methods could also provide a mean to screen potential stabilizing ligands that could counteract these structural changes to some extent [unpublished results].

In vitro methods provide the possibility to generate amyloid forms of various proteins, and to test potential anti-amyloid molecules. Phenolic, polyphenolic, and other cyclic compounds have been tested in our past (collaborative) studies on various proteins including polygalacturonase, albumin [93], myoglobin [94], and insulin [95].

Molecules that were found to inhibit proteins fibrillation, were then tested *in vivo*, on a rodent model of AD. In order to simulate part of the AD signs, abeta amyloid is injected to the animals hippocampus, which results into plaque formation and troubles in learning. Candidate ligands that were administered to the AD animal models, and could attenuate their symptoms to some extent included indole and trans-chalcone [96], sylimarin [97], metformin [98], thymol [99], and eugenol [100]. Based on our experience in generating amyloid structures in insulin, we have also proposed a modification in the generation of rodent AD-like model : instead of injecting abeta amyloids to the animals brains, we used insulin amyloids which are easier formed at a lower cost [101].

Insulin amyloid formation is a problem in patients who have to inject the protein subcutaneously to control their blood glucose levels. We found that chemical modification of insulin by anhydrides at its lysine residue could attenuate its amyloid formation, while insulin still remained functional with one of the modifiers (succinic anhydride) and with another, a delayed onset of insulin action was observed [102]. In another set of experiments, we simulated the condition that is observed in diabetic patients by injecting insulin amyloid to rodents. This resulted onto formation of masses composed of adipose cells in which amyloid deposits could be observed [103]. A subsequent test showed turmeric to be effective in reducing the size of those masses [104] and further studies are now conducted in order to further develop this model and check the effect of other compounds. Recently, our method was used by other researchers to assess the effect of serine proteases on amyloid fibrils [105].

Concluding Remarks

In the two main research subjects that have been discussed here, interdisciplinary work was needed, which is strongly dependent on extensive collaborations; whenever the projects included a higher diversity of specialty, more interesting viewpoints were found, and overall better results were obtained. In another set of researches (not discussed here), we had the opportunity to collaborate with clinicians and geneticists, where we could provide a “basic science” insight onto genetic diseases that were manifesting themselves at protein level. After a case was identified, the faulty gene was investigated, and we had a look at the (computed) protein structure of the mutated gene. This experience led us to define a new research section where we try to see whether our used in silico methods can provide more precise information on the proteins’ mutation effects on its structure.

While in silico and in vitro methods have serious limitations, and in vivo disease models are still far from the reality of pathogenic conditions in human bodies, it is still possible to relate (to some extent) the data obtained from these different methods. We are still a long way from the ideal situation where everything could be computed and there would be no need to perform other experiments, but in order to get closer to that aim, we need to gather more data, and continue to interact between disciplines.

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Compliance with ethical standards

Conflict of interest The authors declare to have no conflict of interests.

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