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Review

of the PhD Thesis by Son Tung Ngo, MSc

"Interactions between small molecules and amyloid beta peptides: Implications for Alzheimer's disease"

submitted for the degree of Doctor of Philosophy of Physics, Institute of Physics, Polish Academy of Sciences (Warsaw, Poland)

In the XXIth century biology plays a central role in science. Biological molecules, i.e. those critical for the processes of life and health, are studied using physical methods. Among them computer physics and modeling have a big contribution to our understanding of the nature. The advantage of the theoretical modeling is in a precise control of "experimental'" conditions and in a relatively low price of the equipment required. The disadvantage comes from crude approximations made to construct physical models of molecules required to achieve reasonable simulation times.

The field of the computational modeling of biological molecules is booming, numerous journals are devoted solely to such research and insights from the modeling are published regularly in *Nature* or *Science*. The field got a new impetus after 2013 when the Nobel Prize has been awarded to M.Levitt, M.Karplus and A.Warshel – one of the founding fathers of this interdisciplinary science.

The PhD thesis of S.T. Ngo uses modern techniques of computer modeling, is devoted to studies of the urgent problem of Alzheimer's disease (AD) prevention and locates in the mainstream of computer/biological physics

research. Both the topic of the study and the methods applied are fully justified and correct. The title of the Thesis, prepared under the supervision of Prof. Mai Suan Li (at IF PAN, Warsaw) *"Interactions between small molecules and amyloid beta peptides: Implications for Alzheimer's disease",* correctly describes its content.

The Alzheimer's disease affects 35 000 000 people worldwide, some 250 000 in Poland, and its prevalence is 14% of a population of the age above 65 and 40% of those above 80 years old. Thus, it is a serious social and economic problem and searching for preventive medicines or chemical cure is of the high importance. The pathogenic aggregation of the amyloid β-peptide (Aβ) is considered a hallmark of the progression of Alzheimer's disease, the leading cause of senile dementia in the elderly people. In his thesis Son Thung Ngo uses a variety of computer methods to study numerous chemical compounds having potential for a strong interaction with toxic beta-amyloides. Interestingly, the effort is focused on substances present in Asiatic diet, for example Curcumin, or in traditional Vietnamese drugs. Both objects of the investigations and the methods applied are justified and interesting. Such strategy, at one hand, brings new data on important and widespread compounds and, on the other hand, has a high probability of hitting a new lead compound for the AD cure. As I wrote, the topic of the Thesis is very interesting and the whole research, in my opinion, addresses an important and timely problems in drug design science and in the computer physics.

The thesis has 132 pages, nearly 70 figures, and 7 Tables. It is divided into 6 basic chapters and 2 short chapters with Introduction and Conclusions. The list of references contains 274 positions. Bibliography is presented in quite meticulous way, though the order of references is not correlated with the appearance order in the text, thus it is somewhat random. The construction of the Thesis is correct. A separate list of acronyms used in the text is presented as an appendix, however, it is not complete (i.e. FMOC – page 89, MTT, HMP-page 89, MD page 93 etc.).

In this review I will discuss the content of all chapters and will give my general opinion in the summary part.

In a short Chapter 1 a brief note on current theories of AD ethology is presented. The main focus is on development of drugs which prevent Aβ aggregation. This discussion quickly focuses on compounds studied in the Thesis. The chapter contains a list of papers by S.T. Ngo (5 related to the Thesis + 5 others, total 85 citations, h=3 , current $h_{googlescholar}$=6).

The real review of the literature is presented in Chapter 2. Among few hypotheses on AD etiology (cholinergic, tau protein, amyloid cascade) amyloid beta peptides aggregation is studied here. In fact there is no real treatment of AD, but T.S. Ngo discusses many classes of potential inhibitors of the aggregation, especially those which are under regular pharmaceutical clinical studies (carvedilol - phase 4, curcumin - phase 2, nilvadipine - phase 3, etc.). The list is very long , literature is very extensive and this part clearly shows how important and difficult topic is addressed. This chapter contains also a review of selected attempts of modeling a drug-Aβ interactions. The presentation shows that the candidate studied the literature in depth and knows all major contributions to this particular area of research. My little criticism goes here to the presentation of the Aβ monomer in Fig. 2.5 – ligands shown in this cartoon should be in my opinion omitted.

In Chapter 3 computational methods are presented. First of all I want to stress that this Thesis doesn't contain any formal methodological developments. However, it contains very smart applications of variety of existing computational methods. Among them molecular docking is extensively used. One may always discuss what code/ target function is better, here the group has applied well established the Autodock Vina code and a typical target function. We use the same software for similar purposes, it has well established quality, good software support and is free for the academic users. Sure enough, details of setting docking protocols may affect results. The Author does not discuss this point too much, but I have noticed that he sticks to the same (or almost the same) docking conditions (protocols) for all compounds studied. This is a correct approach since, despite all limitations of the docking, a ranking for drug candidates may be created. The presentation of MD methods is standard, a little typo in description of formula (3.9) happened.

It would be good if the PhD candidate might justify why the charges for MD simulations with AMBER FF were generated in the described way (from AM1-BCC calculations). In the standard protein AMBER FF another method for charges estimates has been used, thus the presented method is not necessarily compatible with the rest of the simulated system (a peptide).

The presentation of free energy methods is correct (but perhaps in the formula 3.18 for vibrational entropy some summation sign is missing) as well as basic definition of measures used for data analysis. In my opinion for the completeness the RMSD definition should be presented as well in this sub-chapter.

The first new results are presented in Chapter 4. The Author presents large and well justified study showing that curcumin – ingredient common in food – binds to $A\beta_{1-40}$ peptides stronger that two perspective NSAID drugs ibuprofen and naproxen. In the simulations some measure (estimate) of the point where MD simulations are equilibrated is used, but I haven't found any explanation what were criteria for adopting this or other point for setting up this value. Was this purely subjective assessment or some more objective criteria have been used here? This point is not quite trivial since the selection of representative structures of Aβ peptides (monomers or oligomers) is important for proper calculations of free energy of binding. In some models we see that up to 250 ns are required before equilibrium is reached, but in other cases 100 ns (still quite a lot) is enough. (Two panels in Fig. 4.5 have incorrectly denoted time axes[10 000 ns], names of panels are also wrong – mode → model). In the second part of this chapter quite challenging systems of 6 Aβ9-40 fibrils are studied and even larger complexes (12 , 18 unites) are simulated. The detailed analysis of binding poses are presented together will informative discussion of hydrogen bonds and close contact between the ligands and the peptide aggregate. Conclusions from this part of simulations agree well with experimental finding. New results on binding places are presented. Interesting notion is on low role of electrostatic interactions in the mechanism of binding. Curcumin is apparently the best Aβ aggregates format on out of those 3 ligands studied. The data presented in this chapter has been already published in J Phys Chem B in 2012, and draw some attention of the scientific community. What bothers me here, is just only one general question: the ligand binds strongly to the Aβ

fiber, ok, but how do we know that its presence does not induce (or accelerate) further formation of aggregates (and ONLY inhibitory effect is present in the solution)? Is there any computational test of this hypothesis?

In Chapter 5 very appealing observation is computationally studied: is anti-arrithmic and FDA approved drug Propafenone good for AD prevention? T.S. Ngo shows his skills in free energy perturbation method (implemented in GROMACS). Out of 5 compounds studied the results of MD modeling show that Propafenone is the strongest binding to Aβ models, indeed. Moreover, the simulations have shown that this drug has some ability to destroy existing fibrils. This gives some hope for finding not only a preventive medicine, but an active compound that dissolves toxic amyloids.

The construction of the Chapter 5 follows a style of a normal paper, the manuscript perhaps has been submitted for publication. Unfortunately, in this chapter numerous new experimental results are presented as well, and I suppose that the data were obtained by the collaborators in other labs (cell viability, TEM). No information on the source of these data/figures/tables is presented in this chapter, thus I do not know to whom this achievements should be credited.

Data presented in Chapter 6 are even more exciting. I like very much the idea of scanning an original database with over 300 compounds derived from Vietnameese plans and looking for potential anti-AD drugs. The Author shows here his expertise in using QSAR techniques, PreADME software and commercial QuikProp drug design codes. The impressive amount of work is presented in this chapter: hundreds of compounds have been systematically docked to Aβ models, the best poses and candidates were selected in a multistep procedure and top 4 compounds were subject to the most advanced free energy calculations. At the end potential toxicity of the selected compounds has been computationally assessed and compared with the reference curcumin. Results are partially encouraging: Dracorubin and Taraxerol degrade mature fibrils. They are apparently toxic, but this has to be

carefully checked in experiments. The results of Chapter 6 has been published in Molecular Simulations journal in 2013.

In the last Chapter 7 in silico and in vitro characterization of vitamin K3 analogues with respect to anti-amyloidogenic activity is presented. Again, very interesting and important aspect of possible anti-AD role of new class of compounds is studied here. Vitamin K3 has flavone structure, its levels are compromised in AD patients serum, thus supplementation of this type of compounds may perhaps help in slowing down the AD progress. In this chapter, again, vast experimental data is presented (no information who had performed the experiments: cell viability, FTIR spectra etc.) as well as very new and insightful results of MM-PBSA simulations. Out of about 10 derivatives studied, only one VK3-9 protected cells against Aβ induced toxicity. In this group of compounds generation of reactive oxygen species (free radicals) plays an important role. Simulations can't address this problem but have clearly shown what binding modes of the VK3 analogues are preferred in the models of Aβ1-40. These modeling data may serve as a starting point for new synthetic efforts.

This chapter contains numerous useful data, however suffers from relatively high number of editorial/typo mistakes. Nether less it presents good science and very informative correlation between experimental and theoretical values.

To summarize, I have read the Thesis by T.S. Ngo with great interest. It is comprehensive, systematic, logical and well-presented study of important biochemical systems. Physical interactions in large molecular aggregates are studied using state of the art modeling methods. This work, for sure, illustrates expertise of Mr Ngo in computer modeling of biologically important systems, a good knowledge of some aspects of biophysics and practical skills in large part of rational drug design procedures. To large extent this Thesis has chemical, or even pharmacological, flavor. However, one should remember that the computational methods used were of pure physical character, based on classical or quantum physics, and the practical understanding of physics and computer science were critical in successful simulations. Thus, I have no doubts

that this Thesis meets all formal, legal and traditional conditions for a good PhD thesis in physics.

The editorial side of the Thesis is quite good, but not perfect. I list a number of noticed errors below:

Page 13 " the the"

Page 15 "Curcimin" > Curcumin

Page 18 "Wights"

Page 19 "CHARM" > CHARMM

Page 27 indices in (3.33)

Page 28 "ligand ligand"

Page 29 "suit"> suite

Page 30 AD > ? AD drugs

Page 35 all paargraphs MD simulations – very bad grammar

Page 42 "equilinrium"

Page 59 – description of panels is missing

Page 88 "APOE"> ApoE

Page 88 "amyloidogenic" > ? anti-amyloidogenic

Page 90 A > $A\beta_{1-40}$

Page 93 „microtiter"> microliter

Page 94 „Original"> Origin

Page 99 and others – references to non-existing Fig6 and Tables (2??), Figures 2B 4 B 5 B (page 101)

Page 101 ?A940?

Page 103 Figure captions not correct (4/6)

Page 104 "90capabel", "90which"

Page 105 "? Fig 7.12 B" – no such figure

Page 106 ? VK3-10 or rather VK3-9??

Page 107 Ibuprofen/ibuprofen – single convention should be used in names

In references perhaps some capital letters (atp> ATP , etc) should be introduced "by hand".

In summary, this is a very good Thesis. It is written in not perfect but acceptable scientific language. The presentation of formalism is limited to a necessary level. In the Thesis several vivid and non-trivial scientific problems are set and successfully solved. The text demonstrates very good computational capabilities of the PhD candidate. The majority of the results has been published in highly ranked international journals. Minor flaws indicated above, by no mean reduce my high rank of this Thesis.

Therefore I recommend that Son Tung Ngo, MSc should be granted the award of Doctor of Philosophy of Physics of the Institute of Physics, Polish Academy of Sciences.

My positive recommendation refers also to physical aspects of the Thesis. All legal { [Ustawa z dnia 14 marca 2003 r. o stopniach naukowych i tytule naukowym oraz o stopniach i tytule w zakresie sztuki (Dz. U. Nr 65, poz. 595, ze zm. w Dz. U. z 2005 r. Nr 164, poz. 1365 oraz w Dz. U. z 2011 r. Nr 84, poz. 455 z późn. zm.] } and customarily conditions are fulfilled, therefore I recommend that further steps leading to a PhD degree in physics should be undertaken.

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