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Data: Toruń, March 1st, 2013
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Opinion on Dissertation
submitted to the Institute of Physics Polish Academy of Sciences, Warsaw
for the degree of Doctor of Philosophy (Science)
by Man Hoan Viet

Computer physics has become well established branch of science in recent years. Fast and cheap computers allow for effective modeling of more and more physical phenomena. Particularly interesting and important are applications of numerical methods for simulations of biological objects. Scientists seek for new drugs and better ways to prevent illnesses. We do need methods having predictive power that may help do develop better medicines and to understand molecular foundations of pathological states. The dissertation by Mr **Man Hoan Viet, "Numerical Study of Protein-Ligand binding: implications for Alzheimer's disease and Infuenza virus"**, prepared under supervision of Prof. Man Suan Li, address important problems in this field. It uses advanced methodology of modern computer simulations in order to understand (on atomic resolution level) interactions related to a serious disease. I consider this topic to be justified, valid and important. The interactions between such molecules like peptides and proteins are abundant in the cell and govern the whole biological processes. Medical experiments have shown that numerous diseases are related to formation of amyloid plaques. This molecular process is observed also during development of Alzheimer's disease (AD). One of the best justified hypotheses relates AD to build up of fibrils made of so called β Amyloid peptides (βA). Oligomerization of proteins (or peptides) is perhaps a factor that triggers this disease. Numerous pharmaceutical labs search inhibitors of that fibril formation. Perspective drugs should stabilize monomers and destabilize complexes.

Mr **Man Hoan Viet** used in a quite intelligent way a bunch of protein structure and dynamics modeling techniques and estimated numerically interactions of thousands of prospective drugs with model β amyloid systems. Moreover, he used a non-standard Steered Molecular Dynamics (SMD) simulation method to estimate "medical" potential of many drug-like ligands of neuraminidase – an enzyme present in Swine Influeza A/H1N1 virus. This approach is original in many respects and has great potential for further applications.

In the following I will briefly describe content of the Thesis and will present my remarks.

The Thesis has 131 pages, is written in correct and understandable English. It consist of Introduction, 6 main chapters, a short Conclusions section, Appendix with a short list of acronyms and a very comprehensive list of references (279 positions). The content is organized in correct and logical way. It is based too large extent on 3 papers co-authored by the Supervisor and Binh Khanh Mai, Son Tung Ngo, Nguyen Sy Lam published in high-impact international journals (J. Chem. Inf. Model, J Phys. Chem B, J. Chem. Phys.) Mr Man Hoan Viet is co-author of one more paper, not related directly to the present Thesis, two other papers were submitted for publication.

In the Chapter 1 a brief account on main objectives of the Thesis project are presented, The author distinguish 7 main problems related to MD modeling of Aβ peptides and influenza viruses. Some of these topics are very detailed, but a general line of investigation is rather clearly outlined in this chapter.

The Chapter 2 (12 pages) is devoted to the literature review. This is very informative and well written chapter. The author gives broader perspective on the etiology of AD and a mechanism of influenza virus action. The problem of Aβ peptide aggregation is correctly described and major papers related to this are reviewed.

The Chapter 3 does not contain new knowledge: this is just a brief presentation of computational methods of classical MD simulations, a definition of protein-ligand binding affinity, docking methods, the MM-PBSA method and the Steered MD method. Computer codes and force fields are also shortly described here. It is not obvious to me whether this presentation is perfect: one can always argue that some descriptions are too short (for example SMD) or too simplified, but in

my opinion this chapter is a good compromise between necessary explanations and scientific brevity. However, some less popular concepts, such as P2 order parameter, should be defined here as well. I have noticed that in the formula (4) perhaps a summation sign is missing.

One promising strategy of preventing AD is inhibition of the aggregation of Aβ peptides. In the Chapter 4 (25 pages) a detailed study of inhibitory effects of two penatpeptides: KLVFF and LPFFD is presented. The hypothesis that the stronger is ligand binding to monomer of fibrils the better inhibitory effect is tested. Several standard modeling methods have been employed: docking using Autodock Vina, a series of some 400 ns MD simulations of dynamics using the Gromos force field and GROMACS code, MM-PBSA 200 ns calculations of free energy of binding, hydrogen bond distributions analysis, secondary structure content analysis. The inhibitory capacity is studied for dimers of Aβ (16-22) systems. P2 order parameters were useful measures of these dimer structure rearrangements. Binding free energies were calculated by MM-PBSA method for the equilibrated parts of the MD trajectories. The results show that LPFFD peptide slows down the oligomerization of fragments of toxic Aβ(16-20) better than KLVFF. Binding to an individual Aβ (1-40) peptides and to fragments of mature fibrils were studied as well. Also β content of shorter (40) and longer (42) Aβ peptides was calculated showing that, in accordance with the experiment, the longer amyloid is more prone to aggregation due to its higher beta structure content. This chapter contains a lot of interesting new data. However, the conclusions made here are of limited utility, since water solution structures of beta amyloids are not know yet. Fortunately, the author clearly underlines limitations of this approach. In my opinion the main achievement in this chapter is demonstration how one can use a set of predefined numerical calculations in order to evaluate inhibitory potential of a peptide. Some minor problems in this chapter drew my attention: on page 39 the author presents beta-sheet percentage with accuracy to 0.01 percentage points - that has no physical meaning to me. The criteria used to estimate where equilibrated part of the trajectory starts (fig. 12, page 40) are not presented nor explained. The Fig.17 caption is not correct. One general problem is not discussed in the chapter at all: how do we know, that stronger binding of a particular pentapeptide to an amyloidogenic monomer does not induce better (or faster) oligomerization (with multiple units, for example) instead of preventing it?

A very interesting observations on the role of Aβ 42 in AD disease comes from experiments: a monomer of this system is not toxic, but dimmers or oligomers are. Thus preventing oligomerizations of Aβ 42 is a promising strategy for AD prevention. In the Chapter 5 the question whether a shorter peptide A40 inhibits oligomerization of Aβ42 is studied. Using the MD method simulations are performed for an isolated Aβ40, Aβ42 and a mixed system Aβ 40+42. The focus is on the so called fibril-prone structure N* extracted from X-ray data. Simulations were performed in state-of-the art regime: using all-atom force field, SPC solvation boxes, numerous 500 ns trajectories. Results are encouraging: the presence of Aβ 40 reduces beta content in Aβ42 by 13 % percent. The author presents a good justification that this is not a marginal effect since that factor alone may increase characteristic time for fibrillation by 5 orders of magnitude. The calculated effect is clear but in my opinion such a study should be better based on much larger body of data then just 4 trajectories of Aβ40+42 di-peptide systems. Figures 41 and 42 show large scatter in side chain contact maps and hydrogen bond contact map thus structural basis of inhibitory effect can't be safely inferred from the numerical experiments presented in the Thesis.

Short tripetides have some therapeutic potential and thus numerous such systems are used or tested for medical applications. In the Chapter 6 (15 pages) a very comprehensive data for docking ALL possible 800 tripeptides to selected fragments of Aβ (9-40) peptide and Aβ (17-42) are presented. This unique data were carefully analyzed: it has been found that the binding strength correlate with a number of descriptors: a number of heavy atoms and carbon atoms, number of aromatic residues, mass, VdW volume and molar refractivity. These data, quite new for this particular molecular systems, may help to develop better peptide based inhibitors. The author estimated also computationally Blood-Brain-Barrier crossing factors for the tripeptides with the highest binding. Some peptides (WWW, WWP,WPW) have potential of crossing this barrier and have IC50 in micromolar-nanomolar range. I guess that despite this general and promising result the practical utility of these tripeptides will be limited: they don't satisfy the Lipinski rule (too many rings) and they will perhaps have low specificity. It has to be checked experimentally on animal models whether these hits are as active as predicted here. The author may consider setting up a WWW based database of all docked tripeptide structures, that the whole scientific community might perform further analysis of this valuable set of data.

Influenza poses a serious threat to humans and livestock. Existing anti-virial drugs have limited utility since the virus mutates quickly. Blockage of virus protein neuraminidase is only partially effective strategy. A few drug available in market (tamiflu, relenza, peramivir etc.) bind to the same charged pocket. In Chapter 7 very promising results of 27 top-hit hypothetic new drugs were used in virtual screening of its binding potential to H1H1 neuraminidase from influenza A virus. A novel approach using Steered MD was used to unbind drugs by force from the NA cavity. Numcrous force-time and force position profiles clearly show that the mechanical unbinding force depends on binding energy. For a model system of H5N1 and its selected mutants maximum forces correlate very well (R=0.99) with the experimentally determined free energies of binding (IC). For the studied systems this correlation can't be check due to the lack of experimental data, but correlation with Autodoc binding energy is rather weak (R=0.49). Nethertheless, it is shown that Fmax is a useful measure of binding affinity and may help to classify prospective drugs. Mr Man Hoan Viet recommended 4 best ligands for further studies as the most prominent new antivirial agents. The main advantage of this new application of SMD simulations is that it is not time consuming.

This chapter (7) was the most interesting to me. It opens also many new questions, the obvious one (already discussed by the author) is related to the choice of the pulling force vector. I think that it might be quite informative to study a bunch of related but randomly distributed force vectors for each ligand and to compare not just maxima, but distributions of the maxima. In that way one may check to what extent the observed correlation are accidental (or not). Another way of analysis should go towards checking the effect of pulling speed on the predicted order of ligands efficiency. I am quite curious whether the same order (Fig.75) would hold for a factor 10 high and lower speeds. These comments don't reduce my appreciation to laborious and impressive calculations presented in the Chapter 7.

The Thesis is written in rather good English. It contains impressive amount of computational data. The author does not present any new computational method, but shows his expertise in effective and illuminating applications of existing methods of computational biophysics to studies of critical medical phenomena and drug design. Figures and plots are of very good quality. Conclusions are based on the presented data. The author has shown his

excellent orientation in the scientific literature related to topics discussed in the Thesis. The literature is up-to-date, carefully selected, rather well edited and complete.

I have noticed some minor typographical errors listed in a separate page.

The extensive and original results obtained by Man Hoang Viet, related to current topics of biological physics, have been published (or submitted for publication) in 4 world class journals. One should note that in this Thesis there are parts of the material already published in J Chem Inf Moel (chap. 7) , J. Phys Chem. (Chap4) and J Chem Phys (Chap. 5).

I think that this is a very good thesis, it is fully compliant with Polish (and other, known to me, international) standards. Therefore, I recommend proceeding with further steps of the PhD degree procedure for Mr Man Hoang Viet.

Wiesław Nowak, Prof. zw.

Appendix:

1. Page 7 "Tamiu" -> Tamiflu
2. Page 8 "potentially more prominent"? (? Eng.?)
3. Pager 11, line t7, The → the
4. Page 13, Iron → iron
5. Page 13, reference to [44] is missing.
6. Page 18, last sentence –> bad grammar
7. Fig. 6 (d) , alternative definitions of improper angles are present in the literature
8. Page 27, line b3 "in two" → into
9. What about summation in formula (19) , page 29
10. Page 31 , line b7 "in this paper"???
11. Page 40, line t12, be -> by
12. Page 42 , Fig.3 → tab. 3
13. Page 46 , wrong fig. caption
14. Page 55, ologimerization → oligomerization
15. Page 63, table 4, nopoint to have 8 digits in <E2> !
16. Page 90 (see Movie 1…) ???? (copy and pase effect???)
17. Page 93 , line t14, Hung et al , but reference [249] has Nguen et al???
18. Page 109, table 109, Ruptures force 1769.64 pN (etc) – this accuracy has no sense!
19. Page 115 "precursor"→ precursor
20. Ref [47]
21. Ref [82] NSAID
22. Ref [109]
23. Ref [160]
24. Ref [250]
25. Ref [253]
26. Ref. [261] (209)