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Referee's report

on the PhD dissertation entitled:

A study of selected endocrine disrupting chemicals and their binding to host molecules with molecular modelling

by Anna Helena Mazurek

The PhD dissertation is composed of an outline on the pages No. 5-43 and the copies of the five publications [P1-P5] called here as the core of the PhD project.

In the outline (the list of contents is on the page 5) the Introduction presents the scientific merit of the dissertation and the review of the known mechanism of functioning of endocrine disrupting chemicals including estradiol (EST) , progesteron and bisphenol A. Cyclodextrin (CD) is treated as the host molecule able to bind and possibly transport the estradiol molecules across the human body. An attention is paid also on the estrogen receptor (ER) binding estradiol and initiating a cascade of numerous biochemical processes in the human cells. The theoretical modeling of the estradiol binding to cyclodextrin or to estradiol receptor is carried out with the molecular dynamics (MD). Selected details are mentioned here on the additive (CHARMM, AMBER) and polarizable (AMOEBA) force field used in the MD software. In parallel there were carried out quantum mechanical density functional calculations (B3LYP and M06-2X functionals) of selected systems in the solid state and in aqueous environment.

The core publications are:

Original articles (P1, P2, P3)

[P1] Mazurek, A.H., Thirion V., Szeleszczuk Ł., Piquemal J.-P., Clavaguera C., Simonson T. Polarizable models for selected Endocrine Disrupting Chemicals and their hosts. J. of Comp. Chem. SUBMITTED (in this document named as **Publication 2**)

[P2] Mazurek A.H., Szeleszczuk Ł., Bethanis K., Christoforides E., Dudek M.K., Zielińska-Pisklak M., Pisklak D.M. 17-β-Estradiol—β-Cyclodextrin Complex as Solid: Synthesis, Structural and Physicochemical Characterization. Molecules. 2023, 28, 3747. Doi: 10.3390/molecules28093747 (in this document named as **Publication 8**)

[P3] Mazurek A.H., Szeleszczuk Ł., Bethanis K., Christoforides E., Dudek M.K., Wielgus E., Pisklak D.M 17-β-Estradiol—β-Cyclodextrin Complex as an aqueous solution: Structural and Physicochemical Characterization supported by MM and QM calculations. J. of Mol. Structure XXXX (in this document named as **Publication 9**)

Review articles (P4, P5)

[P4]Mazurek A.H., Szeleszczuk Ł., Simonson T., Pisklak D.M. Application of Various Molecular Modelling Methods in the Study of Estrogens and Xenoestrogens. IJMS 2020, 21, 6411. DOI: 10.3390/ijms21176411 (in this document named as **Publication 1**)

[P5] Mazurek A.H., Szeleszczuk Ł. Current Status of Quantum Chemical Studies of Cyclodextrin Host–Guest Complexes. Molecules. 2022, 27, 3874. DOI: 10.3390/molecules27123874 (in this document named as **Publication 6**)

General characteristics of the dissertation

The subjects of the PhD dissertation cover impressive number of physico-chemical problems related to the structure of molecular complexes of biochemical and pharmacological interest. In particular, the complexes estradiol-cyclodextrin and estradiol-estradiol receptor attracted most of attention during the present work.

The investigations were conducted in various ways and almost all of them were completed in the form of the articles published in the scientific journals.

The paper P1 was submitted to the Journal of Computational Chemistry and it is under process of publishing („for peer review").

The papers P2 (2023) and P3 (2024) as well as the review papers P4 (2020) and P5 (2022) were already published in the international journals.

The PhD dissertation is supplied with the References section (180 entries, pages 30-41). Seven publications (2020-2023) co-authored by the PhD candidate were not included in the core (basis) of the PhD dissertation and they are named as the „Additional publications".

All the papers from the PhD dissertation are based on the teamwork of different groups of experienced scientists. The main contribution of the PhD candidate to those papers is evident. Such a dominant contribution of the PhD candidate is also reflected in the first place of Her name on the list of the co-authors.

The results published in the paper P2 and P3 constitute a complete study on the structure of the estradiol- β-cyclodextrin complex in the crystal and in the aqueous environment. My impression is that only the P2 and P3 papers with the reviews P4 and P5 can be a sufficient basis for completion of the PhD achievement.

The paper P1 is devoted to the molecular modeling of the estradiol-estradiol receptor (in monomeric form) and is considered by the PhD candidate as a proof of concept rather than a report of the complete study. Perhaps such an opinion is far too modest. I should underline at this moment huge work of the PhD candidate on preparation of the force field parameters needed to efficiently functioning of the software for molecular dynamics simulations must be underlined. Apart from impressive results this paper presents a clear overview of the future investigations. The paper P1 strengthens the quality of the PhD dissertation.

The formal scientific grades of the publications co-authored by the the PhD candidate are high :

For the P1-P5 publications the Polish Ministry of Science and Higher Education assignes 490 points.
For the P1-P5 publications the Impact Factor is equal to 18.6

For the overall publications of the PhD candidate the Polish Ministry of Science and Higher Education points are equal to 1428.
The overall Impact Factor is equal to 59.802

The Results (pages 25-41).

A very condensed presentation is given here for the basic papers, i.e. P1, P2, and P3. The results are outlined with a few sentences just to have an idea of what was done:

Paper **P1**:

There was carried out an elaboration of parameters (unknown earlier) of the AMOEBA polarizable force field for estradiol, progesteron and bisphenol A to be used in the MD software. A plausible comparison of estradiol (EST), progesteron and bisphenol A. Cyclodextrin (CD) molecular structure and electrostatic potential obtained with quantum mechanical Møller–Plesset perturbation theory and simulations with AMOEBA polariable force field was interesting. There was performed a stable MD simulation of the 10-ns run of the estradiol-estradiol receptor (half of its dimeric form) using the AMOEBA force field for EST. There was predicted the 3D structure of the receptor binding pocket where the terminal hydroxyl groups of the EST ligand interact with four amino acid residues of ER (three residues from A-ring of EST, i.e. the O3 oxygen, and one residue with the D-ring of EST, i.e. O17 oxygen).

Paper **P2**:

There was shown the synthesis and a exhaustive analysis of the estradiol-β-cyclodextrin complex in the crystalline and amouphous environment using the SCXRD, PXRD (powder X-ray diffraction), ^{13}C CP MAS ssNMR, FT-IR (Fourier transform infrared spectroscopy), TGA (thermogravimetric analysis), DSC (differential scanning calorimetry), Cryo-SEM experimental techniques. These

analyses were supported with the quantum mechanical periodic DFT calculations and the GIPAW NMR calculations.

Paper **P3**:

There was carried out the analysis of the estradiol- β-cyclodextrin complex in aqueous solution. A proof of the complex molar ratio of 1:2 (estradiol: β-cyclodextrin) was shown. There was predicted the complex stability constant. A series of exploratory calculations were performed with the quantum mechanical and semi-empirical methods concerning the thermodynamic properties of the complex in water environment.

The review papers **P4** and **P5** compared results of other laboratories on the cyclodextrin host-guest complexes.

The section „Conclusions and perspectives" (pages 26-29)

On the top of this section it is declared that the tasks planned at the beginning of the work were successfully completed. There are no doubts that it is true. The range of the admitted tasks overweighted those required for a typical PhD thesis.

The key achievents are :

--- the MD model of the binding pocket of the estradiol-human estradiol receptor (monomeric) complex

--- an extensive study of the physicochemical properties of the estradiol- β-cyclodextrin host-guest complex

-- the prove of the estradiol-cyclodextrin 1:2 ratio in the crystal

The results obtained here contribute essentially to the development of the molecular simulations of the complex systems and to the understanding of

molecular basis of the host-guest complexes. The analysis of the estradiol: β-cyclodextrin complex has been carried out for the first time. The elaborated new parametrization of the polarizable AMOEBA force field permits to start and develop further simulations on enzymes interacting with steroidal pharmaceuticals or cyclodextrin with pesticides.

Critical Referee's remarks:

[1] Formal remarks:

1.1 If a paper being a basis for the PhD thesis contain the Supplementary Material (SM) its copy should be attached at the same grounds as the copies of the original paper. For example, in the paper P1 there is attached only a fragment of SM, in P2 the SM is lacking, in P3 the SM is lacking, in P4 the SM is lacking. Although the PhD candidate discloses the corresponding links to the journal pages (the http:// etc.) it seems to be an oversimplified procedure. In principle, continuing of such a procedure of omitting SM one would replace the present copies of the „core" papers by the corresponding links to the journal sites.

1.2 Although this is rather a minor critical point but I thing it is worth to mention a discrepancy of the detailed presentation of the force field components when the bonded and non-bonded terms are shown explicitly (the AMOEBA forcefield, page 17, equations 4-11 up to the van der Waals interactions) while the electrostatic terms (permanent multipoles and the induction terms) are expressed in a few words only, not in terms of the formulae.

1.3 The reference No. [24] (page 24) in the Introduction section (24. Daulbayev C., Kaidar B., Sultanov F., Bakbolat B., Smagulova G., Mansurov Z. The recent progress in pitch derived carbon fibers applications. A Review. S. Afr. J. Chem. Eng. 2021, 38, 9–20, https://doi.org/10.1016/j.sajce.2021.07.001) does not seem to indicate the anhydrous form of the EST, as it was written on the page 8. A more suitable reference should be quoted.

[2] Some references are worth to mention:

The AMOEBA polarisable force field used in the present work belongs to the state of the art, perhaps the best in the world force fields applicable to the MD of complex molecular systems. Somewhat deeper discussion on the polarisable force field theory (e.g. transferability of electrostatic parameters) could include a series of recent papers co-authored by prof. Piotr Cieplak , e.g.

[Cieplak-1] PCMRESP: A Method for Polarizable Force Field Parameter Development and Transferability of the Polarizable Gaussian Multipole Models Across Multiple Solvents, Journal of Chemical Theory and Computation DOI: 10.1021/acs.jctc.4c00064 , Contributors: Yong Duan; Taoyu Niu; Junmei Wang; Piotr Cieplak; Ray Luo

[Cieplak-2] Assessment of Amino Acid Electrostatic Parametrizations of the Polarizable Gaussian Multipole Model, *Journal of Chemical Theory and Computation* , DOI: 10.1021/acs.jctc.3c01347, Contributors: Shiji Zhao; Piotr Cieplak; Yong Duan; Ray Luo

[Cieplak-3] Accurate Reproduction of Quantum Mechanical Many-Body Interactions in Peptide Main-Chain Hydrogen-Bonding Oligomers by the Polarizable Gaussian Multipole Model, *Journal of Chemical Theory and Computation*, DOI: 10.1021/acs.jctc.2c00710, Contributors: Shiji Zhao, Haixin Wei, Piotr Cieplak, Yong Duan, Ray Luo

[Cieplak-4] Transferability of the Electrostatic Parameters of the Polarizable Gaussian Multipole Model, *Journal of Chemical Theory and Computation*, DOI: 10.1021/acs.jctc.2c01048, Contributors: Shiji Zhao, Piotr Cieplak, Yong Duan, Ray Luo

[3] Some papers are not referenced:

Two references in the section „Additional publications" (page 42), i.e.
Szeleszczuk et al. IJMS (2021),22, 10100
and
Zielińska et al. J. Pharm. Biomed. Anal. (2022) 213, 114682
were not quoted in the body of the PhD dissertation.

These papers could be removed from the dissertation nevertheless they are an additional evidence of an intensive scientific activity of the PhD candidate.

[4] The Intermolecular interaction energies (QM) corrected with the BSSE:

Intermolecular interaction energies (QM) calculated in the present work with the B3LYP/cc-pVTZ method were obtained likely in a traditional way, i.e. as a difference between the total energy of a complex (e.g. water bound to EST, PRO, BPA, CD subunit) and the energy of individual components of the complex. From the Figure entitled „Interaction energy MM vs. QM" (in paper P1, part of the Supplementary Material, page 18 of 22) one can see that the calculated QM interaction energies are more negative (a stronger binding) than predicted with the MM approach. It is likely that the counterpoise correction (The BSSE – the basis set superposition error) applied to the QM interaction energy should bring the corrected QM values closer to the MM values.

The above critical remarks [1-4] under no cicumstances affect the essence of the PhD dissertation. They were expressed here to put the attention of the PhD candidate on some aspects of the investigations to be considered in the future scientific career.

The General conclusion of the Referee's Report:

The PhD candidate proved a deep understanding of the subject under investigations. She learned numerous experimental and theoretical methodologies and was able to use them in practice in the local and international laboratories (France, Greece). The results obtained here are impressive. The results contribute significantly to the development of the medical sciences and health sciences.

The presented PhD dissertation receives the highest possible score.

The final note is: *summa cum laude*

Andrzej Leś

/prof. dr hab. Andrzej Leś/

The COI declaration

I, the undersigned, confirm that I do not have any conflict of interest to declare in relation with this thesis.

Andrzej Leś

/prof. dr hab. Andrzej Leś/