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Evaluation Report

of the PhD Thesis entitled "From organic molecules to the nanoscale: The computational framework to design and improve functional materials"

PhD Thesis of Mr. Selvaraja Sengottiyana entitled: "From organic molecules to the nanoscale: The computational framework to design and improve functional materials" was prepared under the supervision of Prof. Tomasz Puzyn and the co-supervisor Dr. Alicja Mikołajczyk. It should be mentioned that QSARlab is a world class group that publish high impact papers and research within the area of Quantitative Structure-Activity Relationships (QSAR) modeling.
The submitted thesis was written on 80 pages; the results of this thesis are based in 4 original papers published in leading journals reflected in the total impact factor of 31.233 as detailed below:

1) Sengottiyan, Selvaraj, Kakoli Malakar, Arunkumar Kathiravan, Marappan Velusamy, Alicja Mikolajczyk, and Tomasz Puzyn. Integrated Approach to Interaction Studies of Pyrene Derivatives with Bovine Serum Albumin: Insights from Theory and Experiment. J. Phys. Chem. B 2022, 126, 3831−3843. (IF 2021 = 3.466; MNiSW 2022 =140)
2) Selvaraj Sengottiyan, Alicja Mikolajczyk, Tomasz Puzyn. How does the study MD of pH-dependent exposure of nanoparticles affect cellular uptake of anticancer drugs? Int. J. Mol. Sci. 2023, (IF 2022 = 6.208; ; MNiSW 2022 =140)
3) Selvaraj Sengottiyan, Alicja Mikolajczyk, Karolina Jagiełło, Marta Swirog, and Tomasz Puzyn. Core, coating, or corona? The importance of considering protein coronas in nano-QSPR modeling of zeta potential. ACS Nano. 2023, (IF 2022 = 18.027; MNiSW 2022 =200)
4) Kathiravan, Arunkumar, Selvaraj Sengottiyan, Tomasz Puzyn, Pushparathinam Gopinath, Kanagachidambaresan Ramasubramanian, Praveen Ayyappan Susila, and Mariadoss Asha Jhonsi. Rapid colorimetric discrimination of cyanide ions–mechanistic insights and applications. Analytical Methods. 2022, 14, 518-525. (IF 2022 = 3.532; MNiSW 2022 =70).

As general overview of this thesis, the author properly identify that the development of advanced chemicals and organic compound chemicals combined with nanoforms of the substance is necessary for the future of the European industry. However, experimental studies are time-consuming and costly, making it challenging to develop safe and sustainable chemicals. Computational frameworks combined with experimental validation have been proposed as a promising approach to develop new functional chemicals. Four case studies are presented in this thesis. The first case study (A) investigates the interaction and stability of BSA with newly synthesized potent pyrene derivatives using molecular dynamics and experimental validation. The second case study (B) applies molecular dynamics to investigate the influence of the physicochemical properties of the cancer drug methotrexate grafted with hydrophilic-γ-polyglutamic acid on its cellular uptake at different pH values. The third case study (C) presents the development of a set of complex descriptors describing the relationship between the value of the zeta potential, the core, the coating of NPs, and their PC fingerprints (the so-called nano-QSPR model) to predict and characterize the relationship between the physicochemical properties of NP and the formation of PC and biological outcomes in the medium at an early stage of experimentation. The zeta potential can potentially be used as a pre-indicator of cellular interactions with charged ions or NP molecules. Finally, case study (D) used TD-DFT

calculations, MESP maps, and experimental data to investigate the mechanism of charge variation in the nucleophilic addition of cyanide ions inside and outside the surface. The study aimed to develop new structures that could eliminate toxic ions, particularly in the bloodstream. The study also investigated the intermolecular interaction of malononitrile-functionalized DMN probe with cyanide ions using computational and spectroscopic methods. The results showed that the colorimetric response of the transition to the probe prevented intramolecular charge transfer when cyanide ions were added, leading to the development of a Michael channel.
The presented thesis provides fundamental insights into molecular dynamics and may be a starting point for the efficient and safe development of models critical for drug delivery and nanoparticle-based drug delivery systems to cancer cells. However, several factors, such as protein-particle interactions, agglomeration, diffusion, sedimentation, and toxic effects on humans and the environment, should be considered. Effective in silico methods based on machine learning and quantitative structure-property relationships (QSPRs) could help predict and characterize the relationship between the physicochemical properties of NP and the formation of PC and biological outcomes in the medium at an early stage of experimentation.

This thesis is well presented and has 6 sections including references that cites 175 papers.
The introduction discusses the potential of organic molecules and their derivatives in designing new functional properties, such as creating nanostructures and hybrid molecules for medical applications. It highlights the importance of developing new functional materials with micro-nanostructures of compounds and specific functional groups to determine certain specific properties. It also mentions the importance of developing new drugs to combat bacterial infections, including multidrug-resistant bacteria. Additionally, it discusses the limited knowledge about the factors affecting the physicochemical properties of drug-loaded nanoparticles and their interaction with biological systems, as well as the importance of designing safe and sustainable chemicals and detecting toxic ions like cyanide in various samples. It soundly suggests that computational methods including machine learning techniques can be efficiently used to predict the properties of nanoparticles and identify their toxicity. Considering this motivation, this section details the electronic-structure methods used in the theoretical studies at the level of quantum mechanics (QM) to compute the descriptors for the core (polymer) and coating (functional) groups. The workhorse QM method selected is density-functional theory (DFT) and time-depending (TD-DFT) to also consider exited states. The DFT method includes both hybrid and standard functionals. In addition, considering the size of the systems of interest, this work properly consider force field molecular dynamics to study the protein ligands/drug complexes. Moreover, machine-learning combined with nano-Quantitative Structure-Property Relationship is a novel and promising method to accelerate the prediction of properties.

In section three, the author formulates the main hypothesis. Section four presents the results and discussions of this work; it is properly divided in four sections related to the published papers:

Case Study 1. This case investigates the interaction of two newly synthesized pyrene derivatives, PS1 and PS2, with bovine serum albumin (BSA) protein using various physicochemical and computational methods. The experimental data indicate that BSA binds to the compounds through the static quenching mechanism, with PS2 exhibiting a higher binding affinity than PS1. Molecular docking studies and molecular dynamics simulations were used to predict the binding pathway and conformational alignment of the compounds within the BSA protein. The binding energy values derived from the computational simulation suggest that PS2 has a higher affinity than PS1, with the electrostatic interaction playing an important role in stabilizing the binding mode of BSA-PS2, while van der Waals interactions largely contribute to the stabilization of the binding site of BSA-PS1. The calculated binding energy values and FMO orbital transitions suggest that PS2 is more active than PS1. The results of the study provide useful insights into the mechanism of binding of compounds to BSA at the molecular level, which will be helpful in assessing the different mechanisms of their antibiotic action in the future. Cytotoxicity studies show that PS2 is more cytotoxic than PS1. The experimental values are in the micromolar range of the binding affinity of PS1 and PS2 for BSA, which correlate well with the predicted values.

Case study 2. This case discusses the problem of cell uptake by drug-loaded nanoparticles, comparing the permeation of the particles in different pH environments. The authors conducted molecular dynamics simulations and found that the tumor pH model is easier to uptake than the neutral model due to the nanoparticle's charge, structure, and energetics. The neutral model had less interaction with the headgroup region of the cell membrane, possibly due to less electrostatic force. The hydrogen bonding analysis and radial distribution function indicated that the tumor model has more hydrogen bonds with water and the membrane, making it more easily taken up by the cell membrane than the neutral model. The dipole moment and HOMO-LUMO analysis showed that the tumor pH has a high dipole moment, which affects the penetration forces within the cell membrane. The analysis suggests that all three models have high chemical reactivity, possibly exceeding the interaction of nanoparticles with the cell membrane, and that remarkable cytotoxicity is always hindered due to this high potency in acidic environments. The authors propose a mechanism of action for the penetration of drug-loaded nanoparticles through the cell membrane. They suggest that these methods can be useful for designing different modeled drug-loaded nanocarriers with different concentrations and pH values, which will be more useful for future research on cancer treatment. The findings are consistent with experimental methods, indicating that drug-loaded nanoparticles are highly capable of pH-dependent cellular uptake by the NP criteria of charge and geometry, and the energy-dependent internalization of the mechanism was observed.

Case Study 3. This case discusses the importance of identifying features of nanoparticles (NPs) that prevent or induce toxicity in order to produce safe and targeted nanosystems for industrial applications. The study characterizes 20 different NP structures using descriptors such as core, coating, and protein corona fingerprints to determine the quantitative relationship between the structure of the NP, protein corona formation, and the zeta potential (ζ). The developed nano-QSPR model shows that the zeta potential of the NP is determined by both intrinsic properties (core and coating descriptors) and extrinsic properties (corona descriptors) in a given medium. The knowledge of the original NP structure, surface functionalization, and protein corona formation can improve understanding of the relationship between particle surface and particle-cell interactions, ultimately leading to the development of predictive models that describe the stability and toxicity of NPs in real-time. The study concludes that reliable machine-learning-based predictions for the uptake of NPs and their potential risks in a real environment require consideration of core and coating features in addition to corona descriptors, which will enable the development of safe and sustainable nanomaterials with reduced toxicity.

Case Study 4. This case studied a colorimetric probe based on intramolecular charge transfer (ICT) used for the rapid and selective detection of cyanide ions in solution and water samples. The DMN probe showed a colorimetric response due to Michael adduct formation with cyanide, blocking the ICT channel between the ditolylaminothienyl moiety and dicyanovinylene. The sensing mechanism was investigated by spectroscopic and computational methods. The probe had a linear plot in the concentration range of 0.01-0.25 mM with a detection limit of 23 nM. A 3D printed portable accessory for smartphones and an open-source android application were developed for on-site work. In addition, a microfluidic paper-based analytical device (mPAD) was developed for selective detection of cyanide ions at very low concentrations. The greenish-blue colored DMN probe turned pink with 5 mM cyanide ions at the paper interface. The completely miniaturized mPADs sensing device offers portable, power-free, cost-effective, and safe detection in a cyanide-contaminated environment.

The document is well written in English with minor suggestions and typos as detailed below:

Page 29:
It says:
...solve the Schrodinger equation, numerous approximation..
It should be:
...solve the Schrödinger equation, numerous approximation..

It says:
“..application of computational models based on MD and MD, which are…”

MD is repeated twice, please correct this.

Page 31:
Methodology it says:
"The Hamiltonian of the single-electron system is as follows..."
and the equation looks wrong

Please change to:
"The Khon-Sham equations are given by:"

$$\left[-\frac{\hbar^2}{2m_e}\nabla^2 + V_n(\mathbf{r}) + V_H(\mathbf{r}) + V_{XC}(\mathbf{r})\right]\phi_i(\mathbf{r}) = \varepsilon\phi_i(\mathbf{r}),$$

where V_n, V_H and V_{XC} …

Page 33:
it says:
"The third potential is known as the exchange-correlation potential (XC), and it incorporates all interactions, including all the implications of quantum mechanics that are not taken into account in the other potentials..."

It should be:

The third potential is known as the exchange-correlation potential (XC), and it incorporates all the many-body interactions that are not taken into account in the other potentials...

Page 34:

Equation 5 was generated with different format as the other equations, please update using same format.

Final remarks and recommendations
I had the pleasure to read and review the Doctoral thesis of Mr. Selvaraja Sengottiyana, I have to stress that the main results of this work are of great interest for the scientific community; it properly addresses the needs to accelerate the discovery of new materials based in computational methods within the area of computational nanochemistry. The author combines nicely ab-initio DFT with MD force fields, QSAR and machine-learning methods to provide an novel and efficient path to design new compounds tailored to specific needs.
The high quality of the scientific work of Mr. Sengottiyana is clearly reflected in the high impact journal papers that he has already published during his PhD studies; for these reasons I recommend this thesis to be accepted and I support the application of the author for the degree of “Philosophy Doctor” (PhD).

Sincerely,

Henry P. Pinto.
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