Referee Report (RR) on Thesis "Organization and Fluctuations in Living Systems" Submitted for the Degree of Doctor of Philosophy by Gabriel Rochinha Morgado

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REPORT

The ph.d. thesis "Organization and Fluctuations in Living Systems" by Gabriel Rochinha Morgado describes an always intriguing interplay between fluctuations as disclosed by a stochastic part of the process, and the nonlinearities of the reaction-diffusion (RD) system, being signatures of the corresponding deterministic view of the process.

The Author has carefully uncovered the framework pertinent to the RD systems under study. First of all, it has been done in Chapter 1 which contains the methods. These are suitable analytical methods, characteristic of dynamical systems (with the stability analysis), in addition to chemical Langevin and master equations. The analytical methods are adequately complemented by numerical and computer-simulational methods with inclusion of the famous Gillespie algorithm and direct simulation monte carlo technique, first implemented by Bird.

Then, in Chapter 2 a stochastic approach is presented, leading to elimination of fast variables in stochastic nonlinear kinetics. In order to analyze the consequences of the elimination, the Author has considered a minimal chemical model, involving two species of variable concentrations, able to evolve into a Turing pattern. The two-variable model is assumed to reflect the reduction mechanism of two different three-variable models. The challenge here is to determine if the correlations of fluctuations in the three-variable models are correctly predicted by the two-variable model, right in the limit, where the reduction of deterministic dynamics appears to be the case. To achieve the goal, the Author has successfully introduced an analytical approach based on chemical Langevin equations linearized around the steady state of interest.

Next, in Chapter 3, mechanism(s) of termination as well as scaling procedure of the Turing pattern are disclosed. The termination of the Turing pattern goes roughly in a way that if the concentration is increased by a factor two, the characteristic length of the pattern is decreased by the same factor. The scaling manifests in a natural and logical way as well, namely, that the wavelength scales with a ratio of the square-root of diffusion coefficient of a species divided by the corresponding chemical reaction rate constant, as related to the deviation from a dilution limit.

In the last chapter (Chapter 4), a RD wave front is exploited in terms of the perennially alive Fisher, Kolmogorov, Piskunov and Petrovskii (FKPP) equation, with evidencing fluctuation effects on the wave front. This is mainly a stochastic approach to the FKPP equation, working for different diffusivities of the species.

The dissertation is based on five well-developed publications:

In Chap. 2: “Elimination of fast variables in stochastic nonlinear kinetics”, G. Morgado, B. Nowakowski, and A. Lemarchand, *Phys. Chem. Chem.Phys.*, **22**, 20801-20814 (2020).

In Chap. 3: “Termination mechanism of Turing patterns in growing systems”, G. Morgado, L. Signon, B. Nowakowski, and A. Lemarchand, Acta Phys. Pol. B, **50**, 1369 (2019); “Scaling of submicrometric Turing patterns in concentrated growing systems”, G. Morgado, B. Nowakowski, and A. Lemarchand, Phys. Rev. E, **98**, 032213 (2018).

In Chap. 4: “Fisher-Kolmogorov-Petroskii-Piskunov wave front as a sensor of perturbed diffusion in concentrated systems”, G. Morgado, B. Nowakowski, and A. Lemarchand, Phys. Rev. E, **99**, 022205 (2019); “Stochastic approach to Fisher and Kolmogorov, Petrovskii, and Piskunov wave fronts for species with different diffusivities in dilute and concentrated solutions”, G. Morgado, B. Nowakowski, and A. Lemarchand, Physica A, **558**, 124954 (2020).

The Author of the dissertation is the first author of all five publications. Thus, it can be concluded that his participation in the publishing of this principal ph.d. thesis set of papers is the most prevailing. It has been specified to a great extent in a separate file attached to the thesis, entitled “Declaration of contributions of Gabriel Morgado and co-authors to the five articles presented in the dissertation”.

As for the content of the PCCP (2020) paper on which a key part of Chap. 2 is based, the most valuable finding turns out to be a specification of the range of the RD two-dimensional dynamical system’s parameters as being sensitive to the size of the reaction chamber/confined space. In such a confined space, characteristic, for example, of fluorescence correlation spectroscopy or explosive media under confinement, a coupling between the system’s nonlinearities and fluctuations can be strong, changing the characteristics of he corresponding steady states.

As for the content of the Acta Phys. Pol. B (2019) paper on which the first part of Chap. 3 is rested, the effect of termination of a Turing pattern has been of concern. The termination has been achieved by means of adjusting the concentration of inhibitor in the system.

As for the content of the PRE (2018) paper on which the second part of Chap. 3 is based, scaling of submicrometric Turing pattern has been resolved by this study in which it was stated that doubling the concentration of the solute results in roughly decreasing pattern’s wavelength by the factor two. This finding can help design material’s properties on submicrometer scale.

As for the content of the PRE (2019) paper on which the first part of Chap. 4 is rested, a perturbation of diffusion in a concentrated solution has been studied, and a FKKP wave front appears to be a proper sensor of this perturbation. A shift between two profiles of the two concentration species serves for the quantitative measure of the perturbation to the diffusion process.

As for the content of the Physica (2020) paper on which the second part of Chap. 4 is rested, a stochastic approach to FKPP wave front in a form of master equation has been offered in it for two species with different diffusivities as being engaged in an autocatalytic reaction. The novelty of this study relies on exploring different concentration regimes of the species. Certain differences between stochastic and deterministic descriptions have been pointed out as leading to a better control of the autocatalytic process of interest.

The reception of the underlying dissertation by the present reviewer is decisively shaped by the limits of RD approaches employed by its Author. First, the meaning and usefulness of notion of mesoscale has to be under checking conditions, and which is a “distance” of it from the decisive micro- viz atomic scale, as well as from the macroscale (mesoscale dealt with as an in-between territory)? Second, how does really any RD approach, irrespective of the (meso)scale, reliably apply to living systems for which their function is of utmost concern? This quest becomes the more intriguing to the present reviewer the more the RD affected function does not undergo any justifiable physical description. And third, which are the physical and mathematical bounds of all RD approaches employed in the thesis, and how do they reveal step by step the organization and fluctuations in the modeled living systems (how, based on the description applied, the morphogenesis works from initial to final stage)?

As for the first general quest addressed, there is no precise procedure to specify the limits of the mesoscale. It is like in a gentleman agreement, namely, that we are confined to one social subspace (of *savoir vivre*, for instance) and do not enter the outer adjacent subspaces in which the rules are of different types with either ‘exaggerated’ *savoir vivre* conditions or with some distinctly violated ones. But exaggerating or violating the rules is sometimes more interesting and profitable than to follow the so-called normal rules that either may bother someone or they do not offer some excitement, or even a certain profit. Thus, there is a matter of pre-agreed convention to build a mesoscale framework in order to utilize its capacity to solve the problem, and this is at the expense of introducing too much atomic detail or if a continuum limit would be a dominating condition to apply. Introducing a random term into deterministic equation does not really imply that we are landing within the mesoscale, nor it is sufficient that we are touching upon its microscale (nanoscale) counterpart. For the present reviewer, a more personalized and distinguished address on mesoscale within the thesis presented would be of additional value.

As for the second, also general quest, pointed out by the present referee, it is fairly questionable to accept by the criterion of first-Turing-type modeling impression that by the underlying thesis the problem of organization and fluctuations in living systems has been solved completely, albeit certain examples such as embryo’s developmental morphogenesis, or others (toward somitogenesis) taken from appropriate literature studies, have been borrowed to justify the usefulness of the RD approaches applied. On the contrary, it rather first remained to justify that biology follows the physical rules and mechanisms that we are actually able to apply. Throwing a bullet from a suitable device that makes it underlying the free-fall conditions certainly implies that the bullet will land in an ultimate spot, yielding a hole in the ground. This is physics. But only “exceptional” bullets can drill a ‘biologically useful’ hole in which some reservoir of ground water under pressure will surprisingly appear as the overall bullet’s action can cause the water to pour out, making a porous-ground distribution over it as to reach the roots of plants for their further growth. This is not typically what the physical models can offer to make biology satisfied in order to be based on their rules, and with utilization of their basic mechanisms. The present reviewer would be pleased when certain reservations toward applicability of the modeling offered would be juxtaposed in a separate page of the thesis than to have them dispersed in certain parts of it.

Third, as argued by the present thesis, there exist bounds and limits of the RD approach, and therefore, the numerical and computer-simulational methods would become more efficient than their analytical counterparts. This would be a wish of the present reviewer to have included in the thesis in an even sketchy way a pathway of the morphogenesis based on a selected example, perhaps provided for readability in a form of an algorithm or similar. It would better illustrate the thesis subject based on five well-published papers but in a little bit closed (for experimenters) mathematical parlance. It would be an asset to have it incorporated to enlarge the potential number of readers and experimentally oriented followers, especially the ones seeking for certain biomimetic solutions, of relevance for future (bio)technologies.

Except of quite general quests addressed to the Author, which remains to be discussed, I cannot provide any special objections that can even slightly downgrade the overall very good quality of the dissertation written by Gabriel Morgado. By listing only two small, mostly technical points to indicate, namely that concerning the termination of structure-formation process on page 17 out of 142, i.e. the notion of partial equilibrium has to be united with local equilibrium, cf. Ref. [42], whereas on page 123 out of 142 "… the expansion can be truncated" should appear (but not 'troncated'), I can firmly state that the thesis are well-written and prepared very accurately (also in terms of the English quality) for evaluation. As a consequence thereof, my final reception of the thesis is fully affirmative and very positive.

In view of the above, let me very much recommend Mr. Gabriel Morgado's thesis for further procedural and formal steps to follow. It also enables to confirm that the reviewed dissertation fulfills the conditions of Polish Law of Science of July 20, 2018 (article 187) "Law of Higher Education and Science".

Bydgoszcz, June 15th, 2021.

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