REVIEW

on the competition for the occupation of the academic position "Associate Professor"
at the Institute of organic chemistry with Centre of phytochemistry, Bulgarian Academy of Sciences
in the field of chemical sciences, code 4.2.
scientific speciality “Organic chemistry”
declared in the State Gazette, issue 91 from 02.11.2021

Candidate (sole): Assist. Prof. Dr. Atanas Atanasov Kurutos
Review: Prof. Dr. Vanya Bogdanova Kurteva, IOCCP-BAS; Member of the Scientific Jury, appointed by Order RD-09-269 from 17.12.2021 of the Director of IOCCP-BAS

Assist. Prof. Dr. Atanas Kurutos presented all required documents in hard copy and electronic form, which are in accordance with the Act for the Development of the Academic Staff in the Republic of Bulgaria (ADASRB), the Regulations for the application of the ADASRB, the Regulations for the terms and conditions for acquiring academic degrees and occupying academic positions of the Bulgarian Academy of Sciences and the Regulations for the terms and conditions for acquiring academic degrees and occupying academic positions of the IOCCP-BAS, related to the procedure for occupying the academic position “Associate Professor”. The documentation submitted has been prepared correctly, transparently and in accordance with all requirements and recommendations.

I. Personal and professional data of the applicant

Dr. Kurutos has nearly 10 years of experience in the specialty IOCCP-BAS and the Faculty of Chemistry and Pharmacy (FCP) of Sofia University "St. Cl. Ohridski". In 2016, FCP-SU awarded him the educational and scientific degree "Doctor" in the professional field 4.2. "Chemical Sciences", the scientific specialty Organic Chemistry. Since 2012 he has held the positions of chemist at IOCCP-BAS (2012-2013), full-time doctoral student at FCP-SU (2014-2016), assistant (2016-2018) and chief assistant (since 2018) in IOCCP-BAS. During the period 2015-2020 he completed 5 specializations at the Ruđer Bošković Institute in Zagreb, Croatia (2015, 1 month), Fribourg University, Switzerland (2017, 2 months), Roskilde University and

Copenhagen University, Denmark (2018, 1 month), and at Keyo University in Tokyo, Japan (2018-2020, 24 months).

Dr. Kurutos is the winner of the EUREKA Award for Achievements in Science in 2016 and with the First Prize for dissertation for the competition "High Scientific Achievements for 2016" of the Union of Scientists in Bulgaria. In 2020 he was awarded the "Academician Ivan Yukhnovski" award for outstanding young scientist in the field of organic chemistry.

II. General characteristics of scientific, applied and pedagogical activity and their reflection in the scientific literature

Dr. Kurutos presented for participation in the competition a list of scientific papers for his entire creative period, a list and copies of scientific papers with which he participated in this competition, and a habilitation thesis. A reference for fulfillment of the national requirements is presented. The distribution by indicators is as follows: indicator A - 50 points; indicator C - 100 points; indicator D - 277 points; indicator E - 100 points; and indicator G - Hirsch index 6. As can be clearly seen, the applicant's contributions fully cover the national requirements. His scientific output includes 34 scientific papers, of which 26 in refereed and indexed international journals (16 with rank Q1, 5 with rank Q2, 1 with rank Q3 and 4 with rank Q4), 1 in refereed but non-indexed international journal, 5 in unreferred international journals and 2 book chapters. Dr. Kurutos participated in the current competition with 20 scientific papers, of which 15 in refereed scientific journals (75%), 3 in non-refereed (15%) and 2 book chapters (10%). The distribution of scientific papers according to the rank of the peer-reviewed journal in which they are published is as follows: 11 in journals with rank Q1 (77.33%), 3 with rank Q2 (20.00%) and 1 with rank Q4 (6.67%). The data clearly show that a significant part of scientific reports (93.33%) have been published in journals in the two highest categories, Q1 and Q2.

According to indicator "B" Dr. Kurutos participates with 4 articles in journals with rank Q1, published in the last 4 years; 3 articles in *Dyes and Pigments* and 1 article in *New Journal of Chemistry*. It is important to note that in 3 of the 4 articles (75%) Dr. Kurutos is the corresponding author.

According to indicator "D" Dr. Kurutos participates with 13 articles in refereed and indexed journals, which are as follows: 7 articles in journals with rank Q1, 3 articles in journals with rank Q2, 1 article in a journal with rank Q4, and 2 book chapters. In 3 of the articles (23%), 2 in Q2 journals and 1 in Q4 journal, Dr. Kurutos is the corresponding author. An article in a non-

referred journal and 2 chapters of books that do not carry the candidate's points are also included.

Dr. Kurutos' articles have found a good response in international literature. Lists of noticed citations of all articles with the participation of Dr. Kurutos and the articles with which he participates in this competition are presented. Dr. Kurutos' scientific papers have been cited 110 times in the scientific literature, 50 of which are from the articles included in this competition. A detailed analysis shows that the observed 110 citations are from 19 of the candidate's articles (55.88%, 19 out of 34). On the 20 articles presented in the competition, 50 citations of 15 of the articles (75%) were noticed, i.e. the average citation of the articles in the competition is 2.5 (50/20), and of the cited articles is 3.33 (50/15). The Hirsch index is 6 according to the list exported from the SONIX system.

The report on scientific contributions, incorrectly called "habilitation work", is written concisely and clearly, despite some inaccuracies in the references to individual articles. The achieved results, published entirely in scientific communications, with which the candidate participates in the competition, are divided into four areas: 1. Fluorescent markers for nucleic acid labeling and application in confocal microscopy (articles 1, 2 and 4 of the submitted for the competition indicator C and articles 1, 3, 7, 8, 10, 11 and 13 of those submitted for the competition under indicator D); 2. Markers for insulin amyloid fibrils (articles 2, 4, 5, 6 and 16 of those submitted for the competition under indicator D); 3. Colorimetric pH-sensitive sensors as potential teranostic agents (Article 3 of those submitted for the competition under indicator C and Article 14 of those submitted for the competition under indicator D); and 4. Others (Article 15 of those submitted for the competition under indicator D).

Dr. Kurutos' scientific results have been reported with 35 oral and poster presentations at national and international scientific forums, 31 of which in the period 2014-2021. The latter include 13 oral presentations (42%) and 18 poster presentations (58%).

Dr. Kurutos has successfully led 2 research projects funded by the Research Fund of the Ministry of Education and Science on "New monomethine cyanine dyes as potential non-covalent biomarkers: synthesis, characterization and study of photophysical properties" (Competition for funding research for young scientists, 2016) and “New styrene and polymethine fluorophores as potential teranostic agents” (Competition for funding basic research of young scientists and postdoctoral students, 2021). He has participated in the implementation of tasks under 4 projects funded by the Research Fund at the Ministry of Education and Science, in 1 European project under the Scopes program, in 1 Center of

Competence (Clean technologies for sustainable environment – waters, waste, energy for circular economy) and in 1 scientific program ("Innovative low-toxic biologically active agents for precision medicine", BioActiveMed).

III. Basic scientific contributions

Assist. Prof. Dr. Atanas Kurutos is a distinct experimenter with scientific developments that fall into three main directions:

- ✓ Synthesis of heterocyclic compounds;
- ✓ Spectral characterization of heterocyclic compounds;
- ✓ Heterocyclic compounds with potential application in practice.

The main research achievements of Dr. Kurutos are in the area of the synthesis of ***fluorescent markers for nucleic acid labeling***. A series of lipophilic cationic dyes have been obtained as markers for recognizing the secondary structures of nucleic acids. Monomethyne cyanine dyes containing several positively charged fragments with increased affinity for polynucleotides were synthesized. Fluorophores have been found to interact mainly through intercalation with the DNA double helix. The localization of the dyes in the cells is also checked and the compounds are shown to target mitochondria and/or nucleons. Conjugates of monomethyne cyanine dyes with amino acids were obtained *via* copper(I)-catalyzed click-reaction. Low cytotoxicity, significant increase in fluorescent quantum yield in the presence of nucleic acids, efficient cell uptake and specific mitochondrial staining are found.

A series of mono-, di- and trication chlorine-containing monomethyne cyanine dyes have been synthesized. A modified eco-friendly synthetic protocol has been developed, overriding the disadvantages of the classical approach. High photostability of the dyes is established and their ability to mark DNA and RNA sequences is demonstrated. Many times lower cytotoxicity of the novel biosensors was measured compared to a commercial analogue and it is shown that they are suitable agents for marking eukaryotic cells and microbial organisms.

A series of low molecular weight compounds have been prepared as ***selective fluorescent markers for ribonucleic acids***. The dyes were found to form stable complexes with high sensitivity to RNA. Some of them have shown low cytotoxicity and promising preferential cytotoxicity to cancer cell lines. Oxazole yellow analogues with modified lipophilicity and high fluorescence yield, suitable for viral markers, have been synthesized.

A series of monomethyne cyanines with two positive charges is synthesized to ***visually distinguish living from apoptotic cells***. The compounds have been shown to complex with DNA, with the biocomplex having a significantly higher fluorescent quantum yield than the free dye. Effective marking of splenocytes and peritoneal mouse cells has been demonstrated and the possibility of studying the cell cycle has been evaluated.

A series of mono- and polymethine dyes are obtained as ***markers for insulin amyloid fibrils***. The spectral characteristics of the compounds alone and in the presence of non-fibrillar and fibrillar insulin are studied and the tendency to form dye-protein aggregates was confirmed. It is found that the increase in the intensity of the emission signal of the fluorophores in the presence of fibrillar insulin is strongly dependent on the length of the polymethine chain.

A variety of cyanine dyes have been synthesized as potential inhibitors of insulin amyloid fibril formation in patients with diabetes. Tri- and pentamethyne cyanine compounds have been shown to be most effective in physiological *in vitro* conditions and a plausible mechanism of action has been proposed.

It has been shown that the Förster resonance energy transfer between a standard amyloid marker as a donor and trimethyne cyanine dyes as an acceptor can be used to distinguish between non-fibrillar and fibrillar insulin states. Drastic quench of the marker fluorescence by the dye was observed only in the presence of fibrillar insulin and it was shown that the magnitude of the effect depends on the chemical structure of cyanine.

A series of ***pH-responsive heptametine cyanine dyes*** containing a piperazine moiety are synthesized. In order to fine-tune the optical characteristics, structural variations are introduced only with respect to the proton acceptor, while the cyanine skeleton remained unchanged. The applicability of the dyes for the detection of mono-dimensional protein aggregates is investigated by fluorescence spectroscopy.

A systematic study of the effect of the substituent at the terminal nitrogen atom in *meso*-amino-substituted sensors on fluorescent properties is performed. A combination of spectral and computational methods has been applied and the pKa values of the dyes have been shown to be directly related to their chemical structure. It is proposed that both piperazine ring nitrogen atoms are involved in the sensory process.

Arylhydrazone molecular switches have been synthesized. Spectral methods have shown that compounds exist in solution in various forms and their dynamic changes have been studied.

The topics in the future research of the candidate are described, which include synthesis and biological properties of new chromophore systems.

Dr. Kurutos' personal contribution to the 20 scientific papers presented in the competition is significant. He is the first author in 7 articles (35%), in 6 of which (30%) he is also the corresponding author. According to indicator "B" Dr. Kurutos participates with 4 collective articles, in which he is the first author, and in 3 of them he is also the corresponding author. According to indicator "D" he participates with 16 articles, in 3 of which he is in the first and the corresponding author.

IV. Critical remarks and suggestions

I also have some small critical remarks. There is some discrepancy in data in different documents. The CV contains several values for the Hirsch index, but none of them correspond to the data from the citations exported from the SONIX system. My main remarks concern the information on the scientific contributions of Dr. Kurutos. It is true that the design of compounds is made in search of particular properties, but the personal contribution of the candidate is mainly in the synthesis and spectral characteristics of the objects, so they should be emphasized, not their potential biological applications. As mentioned above, there are some inaccuracies in the references to individual articles in the information on scientific contributions, most likely due to incompetent handling of the EndNote program. Also, these references are to the list of all the candidate's publications, while it would be far more appropriate to be on the list of publications with which he participates in the competition, as only they are included in the contributions. These notes, however, do not in any way affect the overall positive impression of the scientific output presented.

V. CONCLUSION

It is undisputed for me that Assist. Prof. Dr. Atanas Kurutos is a productive researcher. Analysing the candidate's scientific achievements, the relevance and perspective of the topics and his personal qualities and skills, I think that the applicant meets all the requirements of the Act for the Development of the Academic Staff in the Republic of Bulgaria, the Regulations for the application of the ADASRB, the Regulations for the terms and conditions for acquiring academic degrees and occupying academic positions of the Bulgarian Academy of Sciences and the Regulations for the terms and conditions for acquiring academic degrees and occupying

academic positions of the IOCCP-BAS, related to the procedure for occupying the academic position “Associate Professor”, and I recommend that the Scientific Council of the IOCCP at BAS award to

Assistant Professor Dr. Atanas Atanasov Kurutos

the academic position of "Associate Professor" in the field of higher education 4. Natural sciences, mathematics and informatics, professional field 4.2. Chemical Sciences, scientific speciality “Organic chemistry”.

Sofia, March 07, 2022 Prepared the review

(Prof. Dr Vanya Kurteva)