R E V I E W

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as a member of the Academic Jury set to render a decision on a procedure for the obtaining academic position "Associate Professor" at the Institute of Organic Chemistry with Center for Phytochemistry (IOCCP), Bulgarian Academy of Sciences (BAS)

in the Professional Field 4.2. Chemical Sciences according to the Classifier of the Areas of Higher Education and the Professional Fields (Scientific Discipline "Organic Chemistry")

This Peer Review is prepared in response to Order № РД-09-269 of 17.12.2021 issued by the Director of the Institute of Organic Chemistry with Center for Phytochemistry, BAS, following the decision made by the Academic Jury that was held on 14.01.2022.

In the competition for the academic position "Associate Professor" announced in the Bulgarian State Official Journal, issue 91 of 02.11.2021 and on the website of IOCCP, BAS, as a candidate participates only Dr. Atanas Atanasov Kurutos from the Institute of Organic Chemistry with Center for Phytochemistry - BAS, Laboratory "Organic Synthesis and Stereochemistry".

1. General presentation of the received materials

The presented by Assistant Professor Dr. Atanas Atanasov Kurutos set of materials is in accordance with the the *Development of Academic Staff in the Republic of Bulgaria Act (DASRB)*, *the Rules for the Application of the Development of Academic Staff in the Republic of Bulgaria Act*, the *Rules of BAS* and meets the *Criteria of IOCCP-BAS* for the academic position of "Associate Professor".

The applicant Assistant Professor Dr. Atanas Atanasov Kurutos has submitted a total of 34 scientific papers, with 20 of which he participates in the procedure. Of the 20 scientific papers submitted for application in the competition, 18 are articles in scientific journals and 2 are chapters from books published by Nova Science Publishers. A list of 12 projects (without the need for a minimum condition for participation in the competition) is also presented, accompanied by evidence (official notes). Assistant Professor Dr. Atanas Kurutos is the leader of 3 projects, including 2 projects under the NSF of Bulgaria, and in the other 9 he is a team member. Presented for the participation in the competition is a habilitation thesis on "Synthesis and investigation on the photophysical properties of mono- and polycationic cyanine biosensors" under the form of a reference to the main scientific contributions and plans of the candidate. A list, accompanied by

evidence, of Dr. Kurutos' participation in 26 scientific forums in Bulgaria (5) and abroad (21) is presented with a total of 31 papers, 11 of which are oral presentations. No evidence is presented for the report under No 23. Participations with No 1-6 were used in the candidate's dissertation, and the report under No 11 of the list is published in full in a collection of the Conference for Young Scientists abroad and is not duplicated by publications from the presented list of scientific papers i.e. it can be added to the list of scientific papers for review.

Twenty-one scientific papers are accepted for the review, which are outside of the candidate's dissertation and the procedure for assistant professor at the IOHCF-BAS and are considered in the final evaluation, as well as 12 research projects, which, however, are not required as a minimum criterion for academic position "Associate Professor" (indicator F). 4 scientific papers on the dissertation and 10 scientific papers used in the taken of academic position of assistant professor are not reviewed. Of the 20 scientific papers accepted for review, 4 publications in journals with a high impact factor and Q1, bearing the required 100 points, participate in the competition as an equivalent number of articles of habilitation thesis (indicator C). The distribution of the remaining 16 scientific papers included in the indicator D, according to the respective Q factors is as follows: Q1 - 7 papers (175 points), Q2 - 3 papers (60 points), Q4 - 1 paper (12 points), 4 scientific papers, incl. 1 report in full text are without SJR or IF, and the other 2 are book chapters (20 points). The total number of points on indicator D is 277, which significantly exceeds the minimum threshold of 220 points.

2. Brief biographical information about the candidate

Atanas Atanasov Kurutos was born in 1988 in Sofia, Bulgaria. He graduated from the Bachelor's Degree in Chemistry in 2010 at Kingston University (UK). In 2013 he graduated with honors from the Master's Degree in Chemistry, in the Master's program "Modern Methods for Synthesis and Analysis of Organic Compounds" at the Faculty of Chemistry and Pharmacy of Sofia University "St. Kliment Ohridski", Bulgaria. In February 2014 he was enrolled as a full-time doctoral student with a 3-year term at the Department of Pharmaceutical and Applied Organic Chemistry at Sofia University "St. Kliment Ohridski" in the professional field 4.2. Chemical Sciences (Organic Chemistry) under the supervision of Prof. Deligeorgiev. In 2016 he received the educational and scientific degree "Doctor" after successfully defending dissertation "Synthesis of cyanine dyes and study of photophysical properties of some of them". During 2012 to 2013 he worked as a chemist, since 2016 as an assistant professor, and from 2018 until now as a senior assistant professor in the Laboratory of Organic Synthesis and Stereochemistry at the IOCCP-BAS.

In November 2015 he specialized in the field of photophysical research of cyanine dyes at the Ruđer Bošković Institute in Zagreb, Croatia. For the period 2017-2018 he conducted a series of short-term postdoctoral specializations at the University of Friborg, Switzerland and the Danish universities Roskilde University and the University of Copenhagen. From 2018 to 2020 he

was a postdoctoral fellow for 2 years at Keio University, Tokyo, Japan with a scholarship from the Japan Society for the Promotion of Science (JSPS).

He was awarded the EVRIKA Award of the "Evrika" Foundation for achievements in science for 2016, the First Prize for dissertation in the competition "High Scientific Achievements for 2016" of the Union of Scientists in Bulgaria, the "Academician Ivan Yuhnovsky" Award for a distinguished young scientist in the field of organic chemistry for 2020, the Award for best presentation at the Seventh International Conference "Environmental Engineering and Environment Protection" (EEEP'2021). He has reviewed a number of scientific articles for leading international journals published by Elsevier, Springer, Royal Society of Chemistry and MDPI (Multidisciplinary Digital Publishing Institute).

3. General characteristics of the candidate's activity

Evaluation of the scientific and scientifically applied activities of the candidate

Of the 20 scientific papers submitted for the competition, all written in English, 14 have been published in reputable international journals with a high impact factor – 4 in *Dyes and Pigments* (IF = 4.889), 3 in *Journal of Molecular Liquids* (IF = 6.165) and 1 paper each in *European Journal of Organic Chemistry* (IF = 3.021), *Journal of Inorganic Biochemistry* (IF = 4.155), *Journal of Photochemistry and Photobiology A: Chemistry* (IF = 4.291), *Journal of Molecular Structure* (IF = 3.193)), *Magnetic Resonance in Chemistry* (IF = 2,447), *New Journal of Chemistry* (IF = 3,591), *Chemical Physics Letters* (IF = 2,338). The total impact factor of these publications is **61.090** or an average of **4.364** per publication, which is a serious attestation for the quality of the scientific production of Dr. Kurutos. One of the presented articles was published in a journal only with Scopus SJR (*Key Engineering Materials*), and another 3 papers were published in a journal without impact factor (JCR) or SJR (*East European Journal of Physics*). Two chapters have also been published in books published by *Nova Science Publishers*, which are also without JCR or SJR. A paper was presented at the *International Young Scientists Forum on Applied Physics and Engineering* in 2016 in Kharkov, Ukraine, which was published in the conference proceedings in full text.

All 21 scientific papers of Dr. Kurutos are co-authored. Nine of the articles have 8 co-authors, 3 articles have 5 co-authors, 2 articles have 6, 7 and 10 co-authors, respectively, 1 article has 2 co-authors and 1 article has 16 co-authors. In 8 of the publications, Dr. Kurutos is the first author, and in 7 of them he is the corresponding author. He is the first author of the four publications participating in the competition as an equivalent number of articles on habilitation thesis (indicator C), and for 3 of them he is a corresponding author.

Evaluation of educational and pedagogical activity

No information about the textbooks issued by the candidate, conducting lecture courses, working with students, graduates and doctoral students.

Scientific contributions and citations

The scientific interests of Ch. Assistant Professor Dr. Atanas Kurutos are mainly in the field of synthesis of new mono- and polymethine dyes with one or more positive charges and study of the possibilities for their application as fluorescent biomarkers. The main scientific contributions in the publications of Dr. Kurutos, presented for participation in the competition, can be summarized as follows (I accept the distribution made by the candidate as appropriate):

1. *Synthesis and investigation of cyanine dyes and complexes as fluorescent markers for nucleic acid labeling and application in confocal microscopy.*

This is the largest group of the scientific production of Assistant Professor Kurutos, covered by 10 publications. A total of 31 cyanine dyes and their complexes have been synthesized and photophysically studied [publications 15, 16, 17, 19, 23, 24, 26, 28, 30 and 33]. Depending on their application, the dyes can be divided into the following 3 main groups:

- *Fluorescent markers for nucleic acids*

A series of lipophilic monomethincyanine dyes containing more than one quaternary ammonium atom has been synthesized [16]. Preferential marking of double-stranded helices was achieved, observed due to a drastic increase in the fluorescent intensity. In aqueous buffer solutions, the dyes showed a strong affinity for double-stranded DNA/RNA. The fluorophores studied were found to interact primarily through intercalation (reversible inclusion of a molecule between two other molecules) with the double-stranded DNA helix (ds-DNA). Using confocal microscopy it was found that 2 of the new compounds penetrated the cell wall and were localized in mitochondria and/or nucleoli.

Amino acid derivatives were obtained by copper(I)-catalyzed "click" reaction (azide-alkyne cycloaddition) with monomethine cyanine dyes [17]. The compounds are characterized by low cytotoxicity, large increase in quantum yield in the presence of nucleic acids, efficient cell uptake and specific staining of mitochondria, which defines them as promising biomarkers.

A modified and environmentally friendly concept for the synthesis of chlorinated analogues of commercial fluorophore *thiazole orange* has been proposed, in which the final products are obtained with higher yields and purity [26 and 30]. The synthesized compounds showed 100 times lower cytotoxicity compared to the commercial analogue, higher photostability and more stable complexation with biomacromolecules.

- *RNA-selective dyes*

New carbocyanine dyes with high photostability, low cytotoxicity and selective multiple amplification of their emission signal in the presence of ribonucleic acids have been synthesized [33]. The compounds also show a high antitumor potential, especially against difficult-to-treat colon cancer.

Oxazole Yellow analogues with halogen included in the benzoxazole heterocycle and increased lipophilicity have been synthesized for the first time by introducing an extended alkyl chain at the nitrogen atom of the quinoline residue and examined as potential markers for labeling MS2 bacteriophages [28]. The study is a prerequisite for the development of specific viral markers for future biomedical applications.

- *Fluorogenic substances for the visual discrimination between live/apoptotic cells and cell cycle analysis*

A series of new monomethine cyanine dyes with two positive charges was synthesized, with an emphasis on the introduction of a halogen atom into the quinoline fragment [15 and 26]. In the cell cycle study, a significant increase in the emission intensity of the compounds after DNA binding was observed, which defines them as a solid alternative to the widely used commercial biomarkers for antibodies in a number of cell assays.

2. Synthesis and investigation of markers for insulin amyloid fibrils

A number of new mono- and polymethine dyes with potential application as markers for pathogenic protein aggregates and amyloid fibrils have been obtained [18, 20, 21, 22, 29 and 34]. Six monocationic trimethine cyanine and two heptamethine cyanine dyes that form aggregates by interaction with the amyloidogenic protein lysozyme have been synthesized [18, 21]. A relationship between the structure of the dyes and their sensory action has been established, as well as the potential of the new compounds as agents complementing the classic amyloid markers *Thioflavin T* and *Congo Red*.

The potential of 23 newly synthesized cyanine dyes as inhibitors of insulin amyloid fibrils formation has been studied [22]. It has been found that tri- and pentamethincyanine dyes, which have specific structural features, most effectively inhibit *in vitro* the elongation of amyloid fibrils. Possible mechanisms for limiting the formation of insulin amyloid in the presence of cyanines have also been formulated.

FRET has been successfully used for the first time in a donor-acceptor combination of commercial *Thioflavin* and a series of trimethine cyanine dyes to distinguish between unfibrillated and fibrillar forms of insulin [34].

3. Synthesis and investigation of pH sensors

A series of symmetric heptamethine cyanine dyes containing mono-substituted piperazine as a pH-receptor fragment was synthesized for the first time [27]. Both piperazine nitrogen atoms have been found to be involved in the sensory process, with sensory efficiency attributed to geometric changes in the piperazine moiety depending on the pH of the medium rather than to intramolecular charge transfer (ICT) or another phenomenon that could be with high probability the photoinduced electron transfer at this type of receptors. Nevertheless, sensory activity, low

cytotoxicity and effective labeling of tumor cells demonstrate the high potential of the novel compounds as effective pH markers in biological environments.

Three benzothiazole derivatives of heptamethine cyanine dyes containing *meso*-positioned secondary nitrogen atom was synthesized [31]. The photophysical behavior of the novel compounds was also studied.

4. Others

Two of the candidate's works are related to topics other than the synthesis and application of polymethine dyes and their use as biomarkers, although some of the synthesized quaternary dye derivatives have been successfully used as corrosion inhibitors for galvanized steel [25]. Five new arylhydrazone switches have been synthesized and their conformation was studied in detail, as well as their configurational changes as a function of pH of the medium [32].

All contributions in the publications of Assistant Professor Dr. Atanas Kurutos can be qualified as scientific and scientifically applied and to be related to the demonstration with new instruments of significant new aspects of existing scientific areas and problems.

Assistant Professor Dr. Atanas Kurutos has presented a list of all 110 found citations on his scientific papers, 50 of which are selected for the current procedure without being used in other competitions for academic positions and scientific degrees. The 50 citations submitted for this competition come from authoritative international scientific journals with a high impact factor, which are referenced and indexed in the world-famous SCOPUS database, forming 100 points on indicator E, with a required minimum of 70 points. According to information from the SCOPUS database, the works of Dr. Kurutos have been cited a total of 75 times and have an *h-index* = 6, which satisfies the criterion of IOCCP-BAS for obtaining the academic position of "Associate Professor". All citations selected for the competition are by foreign authors, except for one source (No 9 from the list), in which the author's team is international with Bulgarian participation.

All citations participating in the competition are correctly presented after the exclusion of self-citations of all authors and are an excellent certificate for international recognition and high quality of the scientific production of Dr. Kurutos.

Implementation activity

There is no information about the candidate's implementation activities. No copyright certificates, patents or other documents for such activity have been submitted.

4. Assessment of the personal contribution of the candidate

The publications presented for participation in the competition by Dr. Kurutos are collective, and most of them have a significant number of co-authors, which is mainly due to the multidisciplinary nature of their content. Despite the large number of co-authors, the personal contribution of the candidate to the results obtained in scientific papers with his participation is

indisputable, as the subject of most of them is a kind of continuation of the theme of his dissertation under supervision of Prof. Deligeorgiev. In addition, for about half of the scientific publications submitted for participation in the competition, Assistant Professor Kurutos is a leading author and/or author for correspondence.

5. Critical remarks and recommendations

Most of the scientific works of Assistant Professor Atanas Kurutos participated in the competition are published in reputable international journals, known for its high demands on the quality of scientific products presented for publication. The works of Dr. Kurutos have been reviewed and evaluated by qualified specialists in the field from around the world, but nevertheless, they can be asked questions that should be accepted not as a challenge to the excellent results, but as recommendation for improving the future work of the candidate.

For example, Article No 16 commented that only two of the synthesized dyes target mitochondria and / or nucleoli. In this regard, the question arises, have studies been conducted to establish the relationship between structure and sensory activity of this class of dyes? A relationship between structure and activity has been observed in a number of publications, but it is not clear by what mechanism the different structural elements influence the sensory properties of the fluorophores. Also, is it clear by what mechanism the synthesized compounds mark (react with) DNA/RNA and what is the connection with the processes of their aggregation, planarization and hardening for the sharp increase in their fluorescent intensity?

Many publications have commented that the reason for the sharp (sensory) increase in the fluorescent intensity of the dyes is formation of aggregates. For example, in the Habilitation Thesis (p. 7, last paragraph) it is claimed that in the presence of fibrillary insulin cyanine fluorophores increase their emission signal hundreds of times due to the transformation of the structure of the dyes into H-aggregates. At the same time, Article No 34 (Results and Discussion, first paragraph) states the opposite statement that the decrease in fluorescent intensity of the dyes in the presence of fibrillar insulin is due to the formation of non-fluorescent H-aggregates! The question arises, what could be the reason for such a contradictory interpretation of the results? In addition, if the reason for the fluorescence of H-aggregates in the presence of fibrillar protein is a change in their geometrical characteristics and electronic structure, it should be clarified what exactly these changes are.

Studies for changes in the fluorescence intensity of compounds as a function of pH have shown a significant increase in the emission after transition to an acidic environment in compounds with secondary or methylated terminal piperazine nitrogen atom [27, Figure S37]. No possible photoinduced electron transfer (PET) has been observed or commented on in the publication, which could most likely be responsible for the sensory behavior of this type of structure, especially since the basicity of the piperazine nitrogen atoms is different! Furthermore, in similar structures, contrary to the conclusion in Article No 27, Article No 31 states that the

sensory properties of compounds are due not only to geometric changes but also to intramolecular charge transfer (ICT), which I find as a kind of contradiction.

6. Personal impressions

As a reviewer of the doctoral thesis of Assistant Professor Atanas Kurutos and a member of the committee for the "Academician Ivan Yuhnovsky" Award for Outstanding Young Scientist in Organic Chemistry, which he won in 2020, I have excellent impressions of his scientific activity and productivity. I have no other impressions outside of this competition.

Conclusion

The documents and materials submitted by Assistant Professor Dr. Atanas Kurutos for participation in the competition meet all the requirements of the *Law on the Development of Academic Staff in the Republic of Bulgaria (DASRB), Regulations for implementation of DASRB, Regulations for implementation of DASRB of BAS* and *Regulations of IOCCP-BAS*.

The candidate has submitted a sufficient number of scientific papers published after the materials used in his Doctoral Thesis and obtaining the academic position of "Assistant Professor". There are a number of original scientific and applied contributions in the works of the candidate, which have received international recognition. Most of his scientific works have been published in prestigious scientific journals issued by international academic publishers. His theoretical developments have a strong practical applicability in the area of medical diagnostics and biology. The acquired scientific qualification of Assistant Professor Dr. Atanas Kurutos is indisputable and cannot be questioned.

The research achievements of Dr. Kurutos fully comply with the specific requirements of the *Regulations of IOCCP-BAS* for application of the *Development of Academic Staff in the Republic of Bulgaria Act (DASRB)*.

After being acquainted with the materials and scientific papers presented in the competition, analysis of their significance and the scientific and applied contributions contained in them, I find it reasonable to give my positive assessment and recommend to the Scientific Jury to prepare a report to the Scientific Council of IOCCP-BAS for election of Assistant Professor Dr. Atanas Atanasov Kurutos for the academic position of "Associate Professor" at IOCCP-BAS in professional field 4.2. Chemical Sciences (Organic Chemistry).

18.02.2022 Reviewer:

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