REVIEW

by

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Member of the Scientific Jury set to render a decision on a procedure for the acquisition of the educational and scientific degree "Doctor of Phylosophy" (PhD) in the field of higher education: 4. Natural Sciences, Mathematics and Informatics, professional field: 4.2 Chemical Sciences, scientific specialty: Polymers and Polymer Materials

Author: Assist. Prof. Eric Vassilev Dimitrov
Title: "Macromolecular Design and Synthetic Strategies for the Preparation of Polymers for Delivery of Biologically Active Substances and Oligonucleotides"
Scientific Supervisors: Prof. Stanislav Rangelov, DSc and Assoc. Prof. Natalia Toncheva-Moncheva, PhD

This review has been prepared in response to Order No. РД-09-54 of 07 April 2026 issued by the Director of the Institute of Polymers, Bulgarian Academy of Sciences (IP–BAS) and following the decision of the Scientific Jury meeting held on 22 April 2026. It has been prepared in accordance with the requirements of the Development of Academic Staff in the Republic of Bulgaria Act (DASRBA), the Regulations for the Application of the Development of Academic Staff in the Republic of Bulgaria Act, the Regulations of BAS and with the Regulations set at IP-BAS, for applying the Act aforementioned.

1. Biographical information about the candidate

Eric Vasilev Dimitrov completed his higher education at the Medical University of Sofia and graduated in 2024, obtaining a MS degree in Medicine and the professional qualification of Physician. Since 2019, he has been employed at the Institute of Polymers – BAS, initially holding the position of "Chemistry Technician" and, since 2024, the academic position of "Assistant Professor". In 2025, Eric Dimitrov was enrolled as a PhD student in an independent form of study at the Institute of Polymers – BAS, and was granted the right to defend his dissertation at the beginning of 2026. Since joining the Institute of Polymers, Eric Dimitrov has been actively involved in numerous research projects (a total of ten). He has presented the results of his research through poster and oral communications at a significant number of national and international scientific forums, with most of his presentations receiving awards.

Eric Dimitrov is the recipient of the Bulgarian Academy of Sciences' "Ivan Evstratiev Geshov" Award for the most outstanding young scientist under the age of 30 in the scientific field

of “Nanosciences, New Materials and Technologies” for 2025. He also received the “Prof. Ivan Schopov” Award of the Union of Chemists in Bulgaria for “Outstanding Young Scientist in the Field of Polymers” for 2024, as well as the Academician Ivan Yuhnovski Award for “Outstanding Young Scientist in the Field of Organic Chemistry” for 2024.

2. Assessment of compliance with the minimum requirements and quantitative criteria specified in the Regulations of the Institute of Polymers – BAS, according to Appendix 1

According to Article 6(1) of the DASRBA, Article 24(1) of its Implementing Regulations, Article 2(1) of the Regulations of the Bulgarian Academy of Sciences, and Article 28 of the Regulations on the Conditions and Procedure for Acquiring Academic Degrees and Holding Academic Positions at the Institute of Polymers – BAS, candidates for the acquisition of the educational and scientific degree “Doctor of Philosophy” are required to meet minimum requirements in two groups of indicators: Indicator A – submitted dissertation thesis (50 points) and Indicator D – scientific publications (30 points). Assist. Prof. Dimitrov has submitted a dissertation thesis based on five scientific publications in renowned international journals ranked in the first quartile (Q1) according to the metrics of Web of Science and/or Scopus. On this basis, he has accumulated a total of 175 points, significantly exceeding the minimum requirement of 80 points. Furthermore, Assist. Prof. Dimitrov has earned an additional 150 points under Indicators E and F, which are not mandatory for acquiring the educational and scientific degree “PhD”. These indicators reflect citations of the presented publications and the candidate’s participation in national and international research projects.

3. Review of the dissertation and analysis of the results

The research presented in the dissertation of Assist. Prof. Eric Dimitrov is within the contemporary and highly promising field of polymer science, focusing on the design and synthesis of amphiphilic copolymers with tailored composition, architecture, and properties applying modern synthetic and/or modification methodologies, and their subsequent use in the formation of nanoscale carriers of low-molecular-weight biologically active compounds or oligonucleotides.

The dissertation comprises 173 pages and contains 124 figures, 13 tables, and 54 unnumbered structural formulas and schemes. A total of 153 literature sources are cited, more than half of which have been published within the last fifteen years. The thesis is prepared in accordance with the requirements of the Regulations on the Conditions and Procedures for Acquiring Academic Degrees and Holding Academic Positions at the Institute of Polymers – BAS, and is structured into the following sections: Introduction, Literature Survey, Aim and Objectives, Experimental Part, Results and Discussion, Summary and Conclusions, Scientific Contributions, an Appendix containing lists of scientific publications, citations, participation in scientific forums and projects, awards, and a Bibliography.

The *Literature Survey* covers the fundamental concepts of (co)polymers and their architectures. The conditions governing both conventional and "living"/controlled polymerization processes are briefly discussed, together with their application to the synthesis of block copolymers. Particular attention is paid to the so-called "click" chemistry, which encompasses a group of highly efficient chemical reactions that enable the quantitative coupling of both low-molecular-weight compounds and polymer chains. The mechanisms of the most widely used types of "click" reactions are described. The survey further examines methods for the synthesis of biocompatible, biodegradable, thermo-responsive, and pH-responsive polymers, such as poly(ethylene glycol), polyglycidol, poly(ε-caprolactone), poly(2-alkyl-2-oxazolines), poly(acrylic acid), and others, as well as a synthetic analogue of phospholipids. All of them were subsequently used as building blocks in the macromolecular architectures developed within the dissertation research. The literature survey concludes with a summary and conclusions highlighting the expanded opportunities offered by modern controlled polymerization techniques and "click" chemistry reactions for the development of suitable synthetic strategies aimed at the preparation of novel nanocarriers of active compounds with improved performance characteristics.

The *Aim* of the dissertation is clearly defined, and its achievement is supported by the formulation of two groups of specific objectives related to the synthesis of various polymer architectures, the evaluation of their self-assembly behavior in aqueous media, and their potential application as nanocarriers of biologically active substances.

The *Experimental Part* describes the materials used, including reagents, monomers, and solvents, as well as the methods employed for the characterization of the (co)polymers and their assemblies. An impressive number of synthetic procedures (more than fifty) are presented for the preparation of monomers and (co)polymers, their functionalization, and the construction of various linear and branched macromolecular architectures through the application of appropriate "click" chemistry reactions.

The research performed by Assist. Prof. Dimitrov within the dissertation topic is systematically presented and discussed in the section entitled *Results and Discussion*. In accordance with the objectives derived from the stated research goal (aim), the results are organized into four main groups. The *first subsection* addresses the synthesis and detailed characterization of a series of amphiphilic polymer–lipid conjugates. Initially, the phospholipid analogue 1,3-dihexadecyloxypropan-2-ol (DHP) was synthesized. Its hydroxyl group was subsequently utilized for the introduction of terminal azide or alkyne functionalities, transforming it into a suitable reagent for modular coupling reactions. In the next stage, applying anionic, cationic, or controlled radical polymerization processes and/or appropriate post-polymerization modifications, a series of well-defined, low-dispersity polymers bearing terminal azide or alkyne groups were synthesized and characterized. These included alkyne-functionalized protected

polyglycidol and the following azide-functionalized polymers: methoxypoly(ethylene oxide), poly(2-isopropyl-2-oxazoline), poly(*tert*-butyl acrylate), and poly(ethoxytriethylene glycol acrylate) (PETEGA-N_3). The polymerizations were carried out in such a way to yield polymers with comparable molar masses, taking into account the subsequent removal of protecting groups from two of the polymers. In the following stage, the polymers were coupled with complementary functionalized DHP *via* copper-catalyzed azide–alkyne click reactions, for which optimal reaction conditions were established in each individual case. Particularly noteworthy is the doctoral candidate's ability to develop original solutions to difficulties encountered during the course of the work. For example, to achieve complete removal of excess DHP-hexynoate after its coupling reaction with PETEGA-N_3, Eric Dimitrov synthesized an alkyne-scavenging resin through the radical copolymerization of divinylbenzene and 4-(chloromethyl)styrene, followed by azidation of the cross-linked product. Following deprotection of the DHP–polyglycidol and DHP–poly(acrylic acid) conjugates, a series of amphiphilic polymer–lipid conjugates was obtained. These materials were shown to self-assemble in aqueous media, forming nanostructures of different morphologies depending on the length and topology of the polymer segment. The *second subsection* includes the development of synthetic approaches for the preparation of lipid-functionalized oligonucleotides, which are of significant interest with regard to the formation of liposomal spherical nucleic acids (SNA)s in aqueous media capable of delivering genetic material into the cells without using transfection agents. For this purpose, the synthesized phospholipid analogue DHP was extended with a short polyether spacer, after which the terminal hydroxyl group was converted into an azide functionality. The preparation of the nucleolipid was achieved *via* a metal-free azide–alkyne "click" reaction between the azide-functionalized DHP and an oligonucleotide bearing a terminal cyclic alkyne (DBCO) functionality. In a separate approach, the anionic polymerization of allyl glycidyl ether was initiated by the potassium alkoxide of DHP, introducing an average of three pendant allyl groups. In this case, the nucleolipid was obtained *via* a UV-induced thiol–ene "click reaction" with a thiol-end functionalized oligonucleotide. Liposomal SNAs were formed in an aqueous media through co-assembly of the obtained nucleolipids with phospholipids and cholesterol. The self-assembly in aquueoos media of the first type of nucleolipids resulted in the formation of vesicular SNAs with oligonucleotide chains densely arranged on their surface. The morphology of the resulting particles was investigated, and their cellular internalization was demonstrated. The concept of developing synthetic approaches for the preparation of spherical nucleic acids is further elaborated in the *third subsection* of the research presented in the dissertation of Assist. Prof. Eric Dimitrov. It includes the synthesis and characterization of graft polymer–oligonucleotide conjugates with different distributions of side chains, which are capable of self-assembling in aqueous media into spherical nucleic acids with polymeric cores. A homopolymer of 4-(chloromethyl)styrene and its block and statistical copolymers with styrene were obtained *via* controlled radical (co)polymerization. Subsequently, the pendant chloromethyl groups of the polymers were converted into azide functionalities through reaction with sodium azide. The resulting polyazide derivatives of the (co)polymers were then used for conjugation

with DBCO-end functionalized oligonucleotides *via* a metal-free "click" reaction. All three types of hybrid copolymers spontaneously form spherical nucleic acids in aqueous media. These structures were characterized using static, dynamic, and electrophoretic light scattering, as well as transmission electron microscopy. Initial *in vitro* studies were also performed to evaluate the systems' cytotoxicity, resistance to enzymatic degradation, and cellular internalization, demonstrating promising results. The *fourth subsection* of Assoc. Prof. Dimitrov's research results concerns the application of the modular approach of "click" chemistry for the preparation of well-defined amphiphilic linear and star-shaped block copolymers containing poly(ε-caprolactone) (PCL) and polyglycidol. The precursor of the hydrophilic block was obtained *via* "living" anionic polymerization of protected glycidol, terminated with several propylene oxide units. A terminal alkyne group was introduced through esterification with 4-pentynoic acid. To obtain the linear triblock copolymer, an α,ω-heterobifunctional PCL was used, whereas three- and four-armed PCL stars were employed to produce star-shaped block architectures. The terminal hydroxyl groups of these polymers were converted into azide groups, and the block architectures were formed *via* azide–alkyne "click" reactions. Finally, the protecting groups were removed from the polyglycidol blocks, rendering the polymer architectures amphiphilic. Subsequently, the copolymers were used to modify niosomes. Their potential application as nanocarriers of cannabidiol was also investigated.

4. Assessment of the scientific contributions

The scientific contributions of the dissertation can be summarized as follows: (i) Development and implementation of original synthetic approaches involving a combination of "living"/controlled polymerizations and highly efficient "click" reactions for the preparation of well-defined amphiphilic polymer architectures composed of biocompatible and/or biodegradable segments with predesigned molar-mass characteristics, topology, and functionality; (ii) Investigation of the self- or co-assembly of the obtained polymer architectures into various nanoaggregates and their potential application for the delivery of biologically active substances and oligonucleotides.

5. Evaluation of the quality of the abstract

The extended abstract accurately reflects all the results of the research presented in the dissertation.

6. Opinions, notes and recommendations

The dissertation of Assist. Prof. Eric Dimitrov covers a substantial amount of work, yet it is written in a concise manner. I believe that the presented research is the personal work of the doctoral candidate, who, in the course of its realization, has acquired new knowledge, experimental skills, and the ability to interpret results in depth by applying modern synthetic and

analytical methods. The candidate's ability to find appropriate solutions when problems arise during the research process makes an excellent impression.

I would like to point out some of the inaccuracies identified in the text: in Figure 1.46, a scheme of the polymerization of N,N-dimethylaminoethyl acrylate is presented, instesd of PDMAEMA, as stated in the figure caption; the structure of the star-shaped PCL on page 84 is not correctly presented; it would be more appropriate to describe the chloromethyl groups in the copolymers on page 132 as side groups rather than "chlorine atoms in the side chain"; potassium *tert*-butoxide (page 137) is an initiator and not a catalyst; the numbering of most figures in subsection III.4.2 does not correspond to that in the text; conclusion No. 3 is included in a preceding one.

I have the following questions:

- Is the obtained polymer–lipid conjugate DHP-PEEGE soluble in DMSO? When comparing the NMR spectra of the conjugates before and after removal of the protecting groups, it is preferable, whenever possible, to record them in the same deuterated solvent.
- For spherical nucleic acids to successfully perform their functions, a dense and radially arranged layer of oligonucleotide strands on the surface of the particles is required. In the presented preparation of liposomal SNAs, the synthesized nucleolipids constitute about 2 mol% of the liposome composition. Is this sufficient for the particles to exhibit the characteristic properties of SNAs? Were comparative studies performed (e.g., enzymatic degradation resistance and cellular internalization) between the liposomal SNAs and those obtained *via* self-assembly of the nucleolipid?

7. Conclusion

The dissertation of Eric Vasilev Dimitrov, in terms of the volume of the conducted research and the quality of the obtained scientific results, not only fully meets but significantly exceeds the requirements for the educational and scientific degree "Doctor of Philosophy" under the Development of Academic Staff in the Republic of Bulgaria Act and its implementing regulations, as well as the relevant regulations of the Bulgarian Academy of Sciences and the Regulations on the Conditions and Procedures for Acquiring Academic Degrees and Holding Academic Positions at IP – BAS. Therefore, I give my ***positive assessment*** and recommend to the members of the esteemed Scientific Jury to vote in favor of awarding Eric Vasilev Dimitrov the educational and scientific degree "Doctor of Philosophy."

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REVIEWER: Prof. Ivaylo Dimitrov, PhD