REVIEW

by Prof. Maria Nikolova, MD, DSc
head of Immunology Department at the National Center of Infectious and Parasitic Diseases
member of the scientific jury,

appointed by order No. I-170 of 28.10.2025 of the Director of IMicB Stefan Angelov, BAS,
to evaluate a competition for the academic position "professor"
in the field of higher education 4. Natural Sciences, Mathematics and Informatics, professional field: 4.3. Biological Sciences, scientific specialty Immunology, for the needs of the Department of Immunology, Laboratory of Experimental Immunotherapy, IMikB - BAS, promulgated in the State Gazette, (SG, issue 84 of 10.10.2025)

1. ***Materials submitted for the competition***

Only one candidate has been admitted to the competition: assoc. prof. Anastas Dimitrov Pashov, MD, PhD. The submitted documentation is complete and arranged in accordance with the requirements of the Act on the Development of the Academic Staff in the Republic of Bulgaria (ADSRB), 2018 and the relevant Regulations of the Institute of Microbiology. The candidate has provided: administrative documents and diplomas, a complete list and copies of publications in specialized scientific journals and participation in international and national scientific forums that are relevant to the competition, information on participation and guidance of national and international scientific projects, a reference for citations; an author's reference for scientific contributions, a copy of a patent application as well as evidence of compliance with the minimal national requirements and the requirements of the Regulations for the implementation of the ADSB at the Institute of Microbiology.

2. Analysis of the candidate's professional and academic development

Dr. A. Pashov was born on 13.07.1962. He graduated from the First English Language School in Sofia. He graduated with honors in medicine from the Medical Academy, Sofia in 1989. During the period 1990 - 1993, he was a full-time postgraduate student in the scientific specialty "Immunology" at the National Center for Infectious and Parasitic Diseases in Sofia and in 1995 successfully defended his thesis entitled "Identification and quantitative characterization of the expression of two pan-leukocyte antigens recognized by newly obtained monoclonal antibodies CAF7 and 7E12". He continued his academic career at IBIR - BAS, successively as a research associate and senior research associate II degree (1998 - 2006). In 2010, he won a competition for associate professor at the Department of Immunology of the Institute of Microbiology, Stefan Angelov at the Bulgarian Academy of Sciences, where he currently works. Dr. Pashov has extensive international research experience, having held several post-doctoral positions at INSERM - France in the Laboratory of Prof. Srinivas Kaveri

(U430 in 1995 and 2002 and U872 in 2010, Cordelier Center, Paris), as well as at the University of Arkansas for Medical Sciences in the team of Prof. Thomas Keeber-Emmons (Little Rock, USA, 2003 - 2009).

The candidate masters highly specialized methods in the field of cellular and molecular immunology combined with unique experience and knowledge in the field of machine learning, bioinformatics and medical statistics. The candidate's total experience in the field of the competition is over 29 years.

2. *Evaluation of the candidate's scientific works during their overall academic development.*

Dr. Pashov has presented a general list of 94 scientific papers published in the period 1992 - 2025, four of which - in connection with the doctoral dissertation and 19 - included in the competition for associate professor. As an equivalent of habilitation work in this competition, **35 publications** from the period after obtaining PhD degree are presented, and another 26 are submitted under item D "articles outside the habilitation work". All 35 competition articles are **in foreign and Bulgarian scientific journals with assigned quartile, according to SJR**, as follows: 17 - Q1, 8 - Q2, 4 - Q3 and 6 - Q4, with the candidate being the first or corresponding author of 13 (37%) of them. Of the articles outside the habilitation work, 5 are with Q1, 3 - with Q2, 1 each with Q3 and Q4 and 4 - without an impact factor. Along with this, chapters from two monographs and one patent application are presented. The total **impact factor** of the candidate after the habilitation for associate professor is **283**. **The noted citations** of the reviewed works **are 1490, of which 302 citations for the last five years** (2021 -2025), all in specialized international publications, referenced in Web of Science or SCOPUS. According to Scopus, A. Pashov has an **H-index of 19**. A total of **24 participations in scientific forums** after the habilitation for associate professor are documented, including 5 in scientific events outside Bulgaria (France, Switzerland, USA) as the first or last author.

3. *Contributions of the scientific research activities*

The scientific research activity of Dr. Anastas Pashov impresses with several characteristics: theoretical depth of ideas, unusual for a contemporary researcher, absolute coherence of his entire work, and the difficult choice to move from the general to the particular, from the formulation of a theoretical postulate to its prooves in practice.

The candidate's overall scientific development is built around the idea of specificity and its relativity. During his first research years, as a full-time graduate student, he started by mastering the most specific diagnostic and therapeutic technology, that of monoclonal antibodies, to pass during his post-doctoral period through an in-depth study of the natural antibody repertoire. Thus, by logical accumulations, he has attained the freedom to formulate

and defend an unconventional, but, as it turns out, extremely practical thesis "polyreactivity as an evolutionarily established function of the immune system", which is also the quintessence of his habilitation works.

In fact, a number of well-known phenomena suggest that the paradigm of high affinity and exceptional specificity as hallmarks of adaptive immunity significantly limits the ideas about the capabilities of the immune system and its disorders in the context of complex pathologies such as tumorigenesis, autoimmunity and chronic inflammation. The candidate has set himself the ambitious task of arguing the thesis that polyreactivity is not “biological noise”, but an evolutionarily established characteristic of the immune system, by searching for the factors that shape and modulate it within the individual repertoire. Moreover, he proposes a methodology for characterizing at a systemic level the repertoire of antibody specificities, the so-called igome and its changes in the context of discrete pathological conditions such as tumors, neurodegenerative diseases, autoimmune conditions and infections.

The results of the articles presented as habilitation papers are thematically divided into three main areas: (1) The phenomenon of "antibody epitope mimicry" and its application, (2) Biophysical factors influencing the plasticity of antibodies, and (3) Algorithm for characterization of the repertoire at the system level and its clinical applicability.

The first area is focused on tumor-associated carbohydrate antigens (TABA), known for their overexpression at the expense of low immunogenicity. The candidate has proposed the concept of ***developing anti-tumor vaccines based on the so-called peptide mimotopes*** – structural analogues of TABA. Already in 2006 he validated the “reverse immunology” approach that has gained popularity in recent years – i.e. synthesis of immunogenic preparations based on preliminary analysis of natural structures. Unlike carbohydrates, specific peptide mimotopes induce T-cell-dependent responses, activating both CD4+ Th1 cells and cytotoxic T-lymphocytes (CTL). This approach connects T-independent and T-dependent antibody responses, as antibodies formed in response to a peptide immunogen cross-react with the carbohydrate tumor antigen. This direction includes publications N15 – 20 (according to the numbering of the bibliography of the Habilitation Reference and Contributions). Contributions of significant applied importance are the developed ***preclinical models of breast cancer and melanoma***, which demonstrated the efficacy and safety of vaccination with carbohydrate mimotope peptides (inhibition of tumor growth and metastasis), as well as its enhancement by inhibiting regulatory T-cells (Tregs). In connection with this effect, a ***hypothesis of quantitative tolerance*** based on antigen expression thresholds has been proposed. No less important is the critical distinction between antigenic mimicry (binding of the same antibody by two antigens) and immunogenic mimicry (two antigens inducing an equivalent response, regardless of the

genetic background. That gave the grounds to further develop the concept of ***multiple antigenic mimotopes (MAM***) – peptides that mimic different epitope variants and thus naturally increase the efficiency of the induced response.

A second central idea of the candidate's habilitation works is the idea that polyspecificity is subjected to modulation by environmental factors. These conclusions are the result of various structural and biophysical studies on human immunoglobulin preparations for intravenous administration (IVIg), (habilitation works 21 - 23). A theoretical contribution with direct applied significance is ***the concept of "cryptic polyspecificity***", according to which the latent ability of antibodies to bind to multiple antigens is revealed under stress conditions, in response to signals through agents that destabilize proteins, such as prooxidative iron ions (Fe2+) and heme. This induced polyspecificity has a distinct clinical significance. A multiple enhanced effect of binding of bacterial and viral pathogens by secretory IgA under acidic pH conditions and in the presence of heme has been demonstrated. In an experimental animal model of sepsis, modified IVIg has a significantly improved effect on survival, i.e. - extreme inflammation can only be controlled by polyspecific neutralizing antibodies. These studies justify improving the technology for manufacturing IVIg preparations by using a low pH step.

The candidate also explores the "dark" side of polyspecificity, related to the potential of natural antibodies for autoreactivity, and in this regard defines several new mechanisms of immune tolerance: idiotypic interactions of IgM, IgG and IgA in serum, as well as suppression of dendritic cell differentiation by natural antibodies (results summarized in habilitation papers 24 - 27).

Additional studies on the biophysical determinants of polyspecificity show the importance of solvation (participation of water molecules in the binding interface), antigen density - of particular importance for low-affinity antibodies, as well as the so-called "induced fit" reflected by the enthalpy and entropy of binding to different antigens of IVIg treated with different protein-destabilizing agents. As a methodological contribution of the candidate, the experiments with surface plasmon resonance should be noted (articles N 28 - 30).

The third pole of the developments leads to the analysis of the repertoire of antibody specificities, the so-called igome, at the systems level. This is not just the formulation of hypotheses, but the purposeful development of models based on the “reverse immunology” approach tested in the first thematic part, high-throughput antibody binding assays and phage display libraries. In practice, instead of the familiar sequencing of B–cell receptors, which does not provide real and complete information about antibody specificity, libraries of mimotopes obtained by scanning phage display of random peptides on entire repertoires are used. This approach further develops the already well-known methodology of J. Gershoni, through

additional bioinformatic analyses. One of the theoretical contributions of these studies is the study of the anti-carbohydrate repertoire within IVIg, which shows a conserved modular organization following a certain hierarchy: terminal groups, such as galactose and GalNAc, are highly immunogenic, while other groups, such as Neu5Ac, induce tolerance. Thus, the common exposure to antigens from the external, and from the internal environment defines the structure of the repertoire of "public" antibodies – specificities found in large groups or all individuals (article N35).

For me, the clinically-oriented developments of recent years are extremely valuable, in which, based on the already described concepts and methodological approach, the candidate investigates the repertoires at a systemic level in the context of various pathological conditions and shows that the public IgM repertoire (represented largely by natural antibodies) can be a source of biomarkers for various non-infectious diseases.

To my knowledge, these are the first high-throughput analyses of their kind in the field of clinical immunology using machine learning models and cross-reactivity graphs as potential biomarkers. Huge arrays of IgM epitopes or mimotopes (peptide microarrays) were analyzed, starting from the public IgM igome of healthy donors, to obtain igome graphs (mathematical structures modeling pairwise interactions (38, 39).

For example, in patients with brain tumors, a set of 4200 15-mer peptides from human tumor-associated antigens and viral epitopes was used andA classifier of 51 peptide reactivities was defined that differentiated patients with glioblastoma (GBM) from healthy controls and from patients with brain metastases based on IgM polyreactivities. The model also differentiated patients with GBM by gender. Most changes in patients were expressed in a loss of reactivity compared to healthy controls (article N38).

IgM and IgG antibody repertoires from patients with neurodegenerative diseases (Alzheimer's disease, AD and frontotemporal dementia , FTD, compared with the repertoire of public reactivities in healthy donors, show significant and distinct changes, with IgM reactivities to linear peptides best distinguishing AD and FTD groups (39). It was shown that in these diseases, IgM reactivities lose their idiotypic affinity, while IgG reactivities remain largely uncorrelated (N40). These results are consistent with the current understanding of the role of autoimmunity in AD and reveal changes in the idiotypic affinity as a part of the observed repertoire changes in neurodegenerative pathologies (N 41).

In patients with antiphospholipid syndrome (APS) as a model of autoimmune disease, igome analysis revealed the phenomenon of limited diversity of the public IgM repertoire. Patients with APS lose reactivity against linear epitopes of low complexity, associated with

impaired idiotypic connectivity and hence immune regulation. Thus, igome analysis proved to be a much more informative tool than classical immunochemical methods (42-44).

Mimotope analysis also finds application in the domain of infectious immunology. Two novel public linear IgM epitopes in the receptor-binding region of the SARS-CoV-2 spike protein: 922TTSTALG928 and 389VKGDDVR395, were proposed as possible target structures for vaccine development (N45). As mimotopes of public IgM antibodies, they reflect specificities of natural antibodies or of cross-reacting T-independent IgM responses. In the context of emerging pathogens, high-throughput antibody binding assays to large arrays of known epitopes of antigens from infectious agents were used to study the immunological history of bats (N46). A comprehensive picture of potential pathogens with epidemic potential for zoonoses and anthropozoonoses has been obtained. An additional contribution of the candidate is the analysis of data from the specificities of typical human pathogens such as HTLV1, EBV, dengue virus and Treponema pallidum.

In summary, the candidate's most significant theoretical contributions, each of which finds repeated practical confirmation in the presented publications, can be formulated as follows:

The immunological paradigm of “lock and key” is questioned. Monospecificity is seen as a simplification of the real-world interaction possibilities of antibodies, which are characterized by unique distribution of affinities over all possible structures. This property can be roughly characterized by the described libraries of mimotopes or epitopes.

The hypothesis of idiotypic networks has been reevaluated and it has been shown that idiotypic interactions are a real physiological phenomenon, which is confirmed by the presence of idiotopes correlating with specific pathological conditions.

The quality of Dr. Pashov's scientific activities is evidenced by the fact that during the period 2009 - 2025 he was the leader of two successful European projects (Marie Curie Reintegration Grants for the reintegration of Bulgarian scientists returning from abroad, and a bilateral Bulgarian-Norwegian project with the University Hospital in Oslo, funded under the BG09 EEA Grants program "Inter-institutional Cooperation"). Dr. Pashov is also the principal investigator of two projects funded by the National Science Foundation, one during the period 2017 - 2021, which ended with a very good assessment, and one ongoing for the period 2025 - 2028 ("The repertoire of specificities of B-lymphocytes infiltrating bone and brain metastases, as a source of diagnostic biomarkers"), which once again reveals the applied potential of the theoretical and methodological contributions formulated above.

4. ***Evaluation of the candidate's teaching activities***

An essential characteristic of the candidate's professionalism is the ability to convey special knowledge. Assoc. Prof. Pashov has been actively engaged in teaching activities since 1997. During the period 1997 – 2001 he lead the course "Tumor Cell / Tumor Immunology" as a part of the master's program of the Faculty of Biology, Sofia University Kl. Ohridski. During the period 2014 – 2015, within the framework of the Project "Fundamental and Applied Training of PhD Students, Postdoctoral Students, Specialists and Young Scientists in Interdisciplinary Biological Fields and Innovative Biotechnologies." BG051PO001-3.3.06-0059" he was developed and delivered the course "Challenges of Tumor Immunology"

He was the scientific supervisor of senior assistant Professor Shina Ivanova Pashova-Dimova (jointly with Assoc. Prof. Milena Murdjeva), who successfully defended her doctoral thesis entitled "Antigen-presenting and regulatory functions of B lymphocyte subpopulations", of Dr. Andrey Nikolaev Kenderov, who defended his thesis "Immunochemical studies on autoreactivity against HSP90 – 010623", as well as of three graduate students.

5. ***Overall assessment of the candidate's compliance with the mandatory requirements of ADSRB*** .

According to the submitted reference-declaration, supported by original evidence, Assoc. Prof. A. Pashov far exceeds the minimum national requirements and the requirements of the Institute of Microbiology and Biotechnology of the Bulgarian Academy of Sciences (which are higher than the national ones by 40 points) for holding the academic position of "professor" according to the individual groups of indicators, as follows: A (Dissertation for awarding a PhD degree 50/50, C. Scientific publications replacing habilitation work, in publications that are referenced and indexed in world-renowned databases **717**/100; D. Articles outside the habilitation work, chapters of monographs and patents. **322**/220, E. Citations or reviews in referenced scientific publications **2980** 120 and indicators from group E. (supervision of doctoral students, supervision of scientific projects and attracted funds) **454**/150. Thus, the overall assessment of the candidate's scientific and teaching activities amounts to **4523** points with 640 required by the regulations of IMik-BAS.

CONCLUSION

The assessment of the overall career development, scientific research and teaching activities of Anastas Dimitrov Pashov shows that the candidate fully meets and repeatedly exceeds the criteria of ADSRB and the requirements of the Regulations for the Implementation of ADSRB of the IMIkB-BAS for occupying the academic position of "professor" in the scientific specialty "Immunology". Assoc. Prof. Pashov is a rare phenomenon in contemporary Bulgarian immunology, and has won a prominent place on the international scientific scene. There are few examples of such bold theoretical ideas, proven through a respectable set of

modern high-tech approaches and whose practical value has been demonstrated in an extremely convincing manner. The candidate reminds us that immunology is very close to the probability theory and its mastering requires a solid knowledge in the field of higher mathematics, biophysics, biochemistry and informatics.

With pleasure and conviction, I propose to the esteemed jury to elect Assoc. Prof. Dr. Anastas Dimitrov Pashov, MD, to occupy the academic position of "professor" in the scientific specialty "immunology" for the needs of the Department of Immunology, Laboratory of Experimental Immunotherapy, IMIkB - BAS.

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Prof. Dr. Maria Nikolova, MD