Calorimetric Markers for Inflammation in *in vivo* Experimental Models

Avgustina Danailova[1], Sashka Krumova[1], Ivan Iliev[2], Lidia Gartcheva[3], Stefka G. Taneva[1], Svetla Todinova[1*]

[1]*Institute of Biophysics and Biomedical Engineering*
Bulgarian Academy of Sciences
Acad. G. Bonchev Str., Bl. 21, 1113 Sofia, Bulgaria
E-mails: avgustina_danailova@abv.bg, sashka@bio21.bas.bg, sgtaneva@gmail.com, todinova@abv.bg

[2]*Institute of Experimental Morphology, Pathology and Anthropology with Museum*
Bulgarian Academy of Sciences
Acad. G. Bonchev Str., Bl. 25, 1113 Sofia, Bulgaria
E-mail: taparsky@abv.bg

[3]*National Specialized Hospital for Active Treating of Haematological Diseases*
6 Plovdivsko pole Str., 1756 Sofia, Bulgaria
E-mail: lgartcheva@gmail.com

[*]*Corresponding author*

Received: January 15, 2019 **Accepted: March 26, 2019**

Published: December 31, 2019

Abstract: *In this work differential scanning calorimetry was applied to determine the specific calorimetric features of blood plasma proteome associated with immune response stimulation in experimental model (albino Wistar rats). The thermodynamic behavior of the blood plasma of male and female animals subjected to egg albumin (EA) treatment was investigated. The calorimetric profiles of blood plasma from EA treated rats exhibited reduced heat capacity of the albumin-assigned transition and up-shifted weighted average center of the thermogram as compared to healthy controls, the effect being more pronounced for male animals. Increase in the amplitude of the main transition at 70 °C was observed for female rats after EA treatment, which resulted in higher calorimetric enthalpy. Common feature of the thermograms of EA treated males and females was the broadening of the transitions above 75 °C and the appearance of exothermic transition above 90 °C due to protein aggregation.*

Our study clearly revealed gender-specific immune response in rats and contributes to better understanding of the correlation between the calorimetric features of blood plasma and the immunological conditions in the experimental animals.

Keywords: *Chronic inflammation, Animal model, Differential scanning calorimetry, Blood plasma, Egg albumin.*

Introduction

Inflammation is a protective body mechanism through which the immune system responds to pathogens, injuries and other damages of tissues. Although inflammation is essential for resistance to infections and for healing, long lasting and persistent inflammatory processes can become damaging and thus underlie the pathogenesis of many diseases including cancer. Over the past decade, new proofs have emerged confirming that chronic inflammatory responses facilitate cancer cell proliferation and tumor growth, from tumor promotion to

progression [3, 14]. For example, the risk of occurrence of colorectal carcinoma is increased for patients with chronic ulcerative colitis or Crohn's disease [20]. It is suggested that more than 50% of gastric adenocarcinomas and Lymphoma of gastric-mucosa-associated lymphatic tissue are due to infection with Helicobacter pylori [4, 26]. Hepatitis C virus causes acute and chronic infection and is one of the major causes for the development of hepatocellular carcinoma [1]. Chronic inflammation is also linked to the development of lung, breast and skin cancer [16, 21]. In many studies, a relationship between proinflammatory markers and the outcome of the disease in cancer patients has been established [5, 22]. Therefore, in-depth understanding of the inflammatory progression is important for the development of improved therapeutic strategies and to avoid unwanted consequences of the sustained inflammation.

Animal models are often utilized for investigation of the role of chronic inflammation for the promotion and development of cancer and other inflammation related diseases [2, 6, 13, 15, 24], as well as in pre-clinical studies in order to better understand the physiological and biochemical processes, associated with occurrence and progress of diseases. In this respect, it is crucial to develop tools that discriminate those different conditions.

Modern medicine thrives to develop non-invasive screening procedures that identify biomarkers for reliable and specific diagnosis of variety of diseases. In the last decade differential scanning calorimetry (DSC) was recognized as a method with high potential for disease diagnostics and monitoring since the thermal stability of the major blood plasma/serum proteins was shown to be altered in variety of diseases and conditions [9, 11, 17, 18, 25, 29, 30, 33]. However, so far only few studies apply DSC on blood serum derived from animal model [8, 23, 28]. Hence, the correlation between the calorimetric features of plasma/serum proteome and the health status of animal model still needs further in-depth studies.

The aim of this work is to determine the specific thermodynamic features of blood plasma proteins associated with the immune response stimulation in experimental rat models. We studied the thermodynamic behavior of the blood plasma proteome of male and female albino Wistar rats subjected to egg albumin treatment and the levels of the major serum proteins. Data clearly reveal gender-specific immune response in rats and therefore contribute to better understanding of the correlation between the calorimetric features of blood plasma and the immunological condition in *in vivo* experimental rat models.

Materials and methods

Experimental models

A total of 20 eight-month old albino Wistar rats (10 females and 10 males) were used as experimental animals in this work. The animals were obtained from the Experimental and breeding base for laboratory animals in Slivnica, and were kept in the vivarium in the Institute of Experimental Morphology, Pathology and Anthropology with Museum. The rats were divided into groups of 5 animals of the same sex and were housed in plastic cages in standard laboratory conditions (at a constant temperature of 23 ± 2 °C, humidity of 55% ± 5% and 12 h light/dark cycle), with free access to standard dry pellets and water *ad libitum*. The animals were fed with a standard diet throughout the entire duration of experiment. The treatment of the animals started after 7 days of acclimatization.

The experiments were approved by the ethical committee of the Institute of Experimental Morphology, Pathology and Anthropology with Museum and were in accordance with the ethical committee guidelines.

Immune response induction

Egg albumin (EA) was used as an antigen to induce an immune response in the model animals. 5 female and 5 male rats were injected subcutaneously with the antigen (150 μg/kg) 3 times during one month (i.e., every 10 days). First injection was carried out with 200 μl/rat immunological solution of EA in PBS buffer, $Al(OH)_3$ and Complete Freund's Adjuvant for sustaining the immune response. Incomplete Freund's adjuvant we used in the solution for the second injection. The solution for the third injection was prepared with only $Al(OH)_3$ in PBS and EA.

The remaining female and male rats were used as a control groups and were injected with sterile PBS buffer, pH 7.4.

Blood plasma preparation

All rats were sacrificed under deep anesthesia 10 days after the last injection. Blood was drawn by cardiac puncture in vacutainer tubes with anticoagulant (K_2EDTA). The whole blood was centrifuged for 15 minutes at 2800 rpm. The supernatant (blood plasma) was transferred into Eppendorf tubes and stored at –80 °C until thawed for measurement of antibody titers, protein fractions and DSC analysis.

Protein analysis

The total protein content in the blood plasma was determinate by the Biuret method [27]. The levels of the main plasma protein fractions – albumin, α_1, α_2, β_1, β_2 and γ-globulins were determined by Capillary electrophoresis (Capillarys 2, Sebia).

Indirect ELISA

Blood plasma of the experimental animals were assayed by indirect ELISA. 96-well ELISA plates (Greiner Bio-One) were coated with the antigen (egg albumin) in 50 mM carbonate buffer (pH 9.6) to concentration 10 μg/ml. The plate was incubated for 1 h at 37 °C, and than was blocked in 3% skimmed milk in PBS for 40 min. After washing three times for 5 minutes on a shaker with 0.05% Tween 20, the rat's plasma was added in dilution 1:1000 and was incubated for 2 hours at room temperature. The plate was washed with 0.05% Tween-20 three times, after which the secondary antibody Goat Anti-Rat IgG-HRP (Santa Cruz Biotechnology, USA) was added, diluted 1:5000 in PBS and was incubated for 2 hours at room temperature. After the final wash, color was developed by incubating with o-phenilenediamine (0.5 mg/mL in citrate buffer) and 5 μL of 30% H_2O_2 for 20 minutes. The reaction was stopped by adding 50 μL/well of 1M HCl. Optical density at 492 nm was measured with a microplate reader (TECAN, Austria).

Differential scanning calorimetry

DSC profiles were obtained using DASM 4 (Privalov, BioPribor)-built-in high sensitive microcalorimeter. Blood samples were diluted 8-10 times in PBS buffer (pH 7.4) and thermograms were recorded from 30 °C to 105 °C at 1 °C/min scanning rate. A constant pressure of 2 atm was maintained during the experiments. The baseline (buffer–buffer) scans were subtracted from the plasma scans that were further corrected applying a linear baseline fit. Thermograms were normalized to the total protein concentration to obtain the partial heat capacity as a function of temperature. The calorimetric profiles were analyzed using the ORIGIN 8 (MicroCal Software) program package and the following parameters were determined: enthalpy of the thermogram (ΔH), weighted average center of the thermogram (T_{FM}), denaturation temperature (T_m^{max}) and excess heat capacity (c_P^{max}) of the main transition.

Statistical analysis

Wilcoxon rank-sum test non-parametric analysis was used to compare the thermograms recorded for control and EA treated animals, the data were regarded as significantly different at $p < 0.05$.

Results and discussion

To probe the effect of EA induced inflammation on the experimental rat models we employed indirect ELISA analysis. Both female and male animals showed a significant IgG antibody response to the antigen challenge (Fig. 1). After the end of the treatment, the animals had high plasma IgG anti-EA titers, while control rats showed no anti-EA antibodies. Although the difference is not statistically distinct, it should be taken into account that the immune response is higher in male than in female rats.

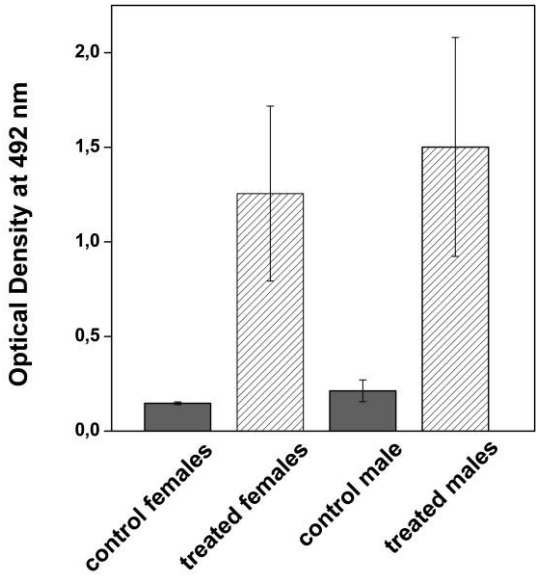

Fig. 1 Indirect ELISA test performed on blood plasma derived from male and female untreated controls and animals treated with egg albumin. Mean ± SD, $n = 5$.

Next we evaluated the effects of the triggered immune response on the levels and the thermodynamic properties of the major blood plasma proteins by means of capillary electrophoresis and DSC, respectively.

The obtained values for the major plasma proteins (see Table 1) are similar to those previously reported for rats [32] with the exception of the γ-globulins fraction that is higher in this study. No statistically significant difference was found between the untreated and treated animals of the same sex. The plasma protein content of control male rats was characterized with lower albumin content and higher level of β_2-globulin fraction than that of females (Table 1).

The calorimetric profiles of "healthy" and EA treated rats are presented in Fig. 2. The DSC scans of both female and male rats possess similar characteristics – a dominant transition at 70 °C and several smaller transitions at 51 °C, 62 °C, 81 °C and 89 °C (Fig. 2). On the basis of a number of studies performed on human blood plasma [10, 12, 31] and the data of Kosa et al. [7], demonstrating that albumin isolated from human and rat blood plasma exhibit a single sharp endothermic transition at ca 60 °C, the transitions resolved in the rats thermograms can be assigned to the melting of fibrinogen (51 °C), albumin (62 °C), globulins (70 °C), IgG and

transferrin (above 80 °C). The heat capacity ratio of albumun/globulin assigned transitions is less than 1 for control rats, in contrast to that of human plasma thermograms where it is 2.0 [31]. Most likely this is due to the lower percentage of albumin from the total protein content in rats (ca. 40%) than in humans (ca. 60%) (see Table 1 and [19]). The amplitude of the albumin transition in the thermograms of male rats is much lower than that for female ones and is visible only as a shoulder (Fig. 2) – this effect must be due to the lower albumin content in males than in females (Table 1).

Table 1. The levels of plasma proteins (albumin, α_1 and α_2, β_1- and β_2-, and γ-globulin fractions) determined by capillary electrophoresis for control and EA treated male and female rats. Mean ± SD, n = 5.

Groups	Albumin, [%]	α_1, [%]	α_2, [%]	β_1, [%]	β_2, [%]	γ, [%]
Control female	41.3±0.3	18.8±0.5	8.7±0.5	2.9±0.3	19.9±0.5	8.4±0.3
Female + EA	41.1±0.7	19.3±1.6	7.8±0.3	2.4±0.5	20.4±0.7	8.9±1.1
Control male	38.5±1.1*	18.5±1.5	9.6±0.4	1.8±0.7	22.0±1.1*	9.7±1.5
Male + EA	38.0±2.0**	19.0±1.5	9.9±0.8**	2.4±1.2	21.6±1.5	9.3±0.9

* indicates significant difference from control female animals
** stands for significant difference from treated female animals

The amplitude of the dominant transition, c_P^{max}, is slightly lower for female than for male control rats; however, no significant difference between males and females was observed for the enthalpy of the thermogram, the position of the dominant peak and the weighted average center of the thermogram (Table 2).

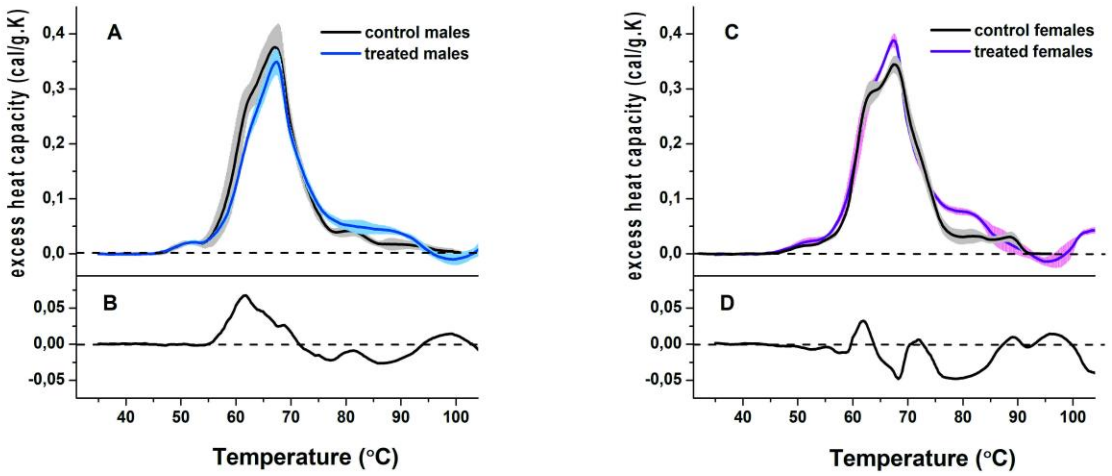

Fig. 2 DSC profiles (average – solid lines and standard deviations – shading) of blood plasma from controls and rats treated with egg albumin. Untreated male and female rats (black solid line and gray shading), A and C; treated male rats (blue solid line and cyan shading), A; treated female rats (violet solid line and magenta shading), C; difference thermograms obtained after subtraction of the averaged plasma denaturation profiles of control and treated rats, B and D.

The effect of EA treatment did not cause significant change in the levels of the major plasma protein fractions (Table 1), like for untreated rats the treated female animals had higher albumin and lower α_2 level as compared to treated male subjects (Table 1). The main effect of the applied treatment is on the amplitude of the albumin assigned transition at 62 °C that is

lower for treated male while higher for treated female rats compared to untreated ones (Table 2). The weighted average center of the thermograms, T_{FM}, is up-shifted by ca. 1 °C for treated males (Table 2) in comparison to untreated ones. For females the peak at 62 °C appears as a shoulder after EA treatment (Fig. 2B). In addition, a slight increase in the amplitude of the main transition at 70 °C was observed, which also resulted in higher enthalpy after EA treatment of female animals (Table 2).

Table 2. Thermodynamic parameters estimated from the calorimetric profiles of control treated male and female rats: enthalpy of the thermogram (ΔH), weighted average center of the thermogram (T_{FM}), denaturation temperature (T_m^{max}) and excess heat capacity (c_P^{max}) of the main transition. Mean ± SD, n = 5.

Groups	ΔH, [cal·g^{-1}]	T_{FM}, [°C]	T_m^{max}, [°C]	c_P^{max}, [cal·g^{-1}·K^{-1}]
Control females	4.6±0.3	66.9±0.3	67.6±0.2	0.35±0.02
Female rats + EA	5.2±0.3*	67.2±0.2	67.5±0.2	0.39±0.02*
Control males	4.8±0.3	66.3±0.4	67.0±0.6	0.38±0.05
Male rats + EA	4.6±0.2	67.4±0.3*	67.4±0.3	0.35±0.02*

* statistically significant difference from control animals, $p < 0.05$

A common feature between the thermograms of EA treated males and females is the broadening of the transitions above 75 °C and the appearance of an exothermic transition above 90 °C that must be due to protein aggregation, an effect that is gender-specific since the exotherm is located at 99 °C for males, while at 95 °C for females (Fig. 2). Similar effect was also observed by Michnik et al. [23] for rats receiving weakly 8 mg·kg$_{BM}^{-1}$ of testosterone enanthate; according to our data the hormonal treatment might induce immune response. The difference thermograms obtained after subtraction of the DSC profile of plasma derived from EA treated animals from that of control ones for males and females do not overlap in the range of the thermal scanning (Fig. 2B and D), revealing distinct response to the applied treatment for the two sexes. In addition, Wilcoxon non-parametric analysis reveals that the plasma thermograms of control and EA treated male rats differ significantly ($p < 0.01$) in the 57-68 °C and 74-90 °C temperature ranges, while those of females differ in much narrow temperature ranges, i.e., 64-69 °C and 74-85 °C. It should be noted that Garbett et al. [9] also found differences in the thermograms of patients with inflammatory disease (Systemic lupus erythematosus) related to their sex, that involved differences in the albumin assigned transition.

The presented data give insight on the effect of persistent inflammation on the DSC profiles of rat blood plasma which are somewhat similar to that of soft tissue inflammation in humans [31]. The excess heat capacities of albumin assigned transition decreased, whereas that of the globulin increased in humans with inflammation and treated female animals. Increased T_{FM} is also a common feature for the thermograms of patients with inflammation and treated male rats. We should note some differences between the "inflammation" thermograms of rats and of humans as compared to their respective controls: (*i*) the DSC profiles of plasma from patients with inflammation present the same number of successive transitions as the control one in contrast to rats plasma, where only one broad peak is depicted in the high temperature region (above 75 °C) of EA treated animals instead of two transitions in the respective controls; (*ii*) protein aggregation is observed for both treated animal groups, that is not occurring in the human profiles.

Conclusion

In summary our data revealed gender-related differences in the albumin content as well as in the calorimetric profiles of male and female rats involving the amplitude of the albumin assigned transition and its behavior after EA treatment. The presence of an exothermic transition appears as a specific feature of the induction of immune response in both males and female rats, its position was also found to be sex-dependent.

The model of chronic inflammation used in this study is valuable tool in exploring the potential of DSC analysis for the characterization of chronic, inflammatory responses in animal model.

The presented data prompt future studies on the effect of different diseases on the calorimetric profiles of rat blood plasma, as well as their gender specific features.

Acknowledgements

This work is supported by grant DFNP-17-133, Program for career development of young scientists and PhD students in Bulgarian Academy of Sciences, 2017 (A.D.).

References

1. Andrade L., A. D'Oliveira, R. Melo et al. (2009). Association between Hepatitis C and Hepatocellular Carcinoma, J Glob Infect Dis, 1(1), 33-37.
2. Balansky R., G. Ganchev, M. Iltcheva et al. (2018). Carcinogenic Response and Other Histopathological Alterations in Mice Exposed to Cigarette Smoke for Varying Time Periods after Birth, Carcinogenesis, 39(4), 580-587.
3. Balkwill F., A. Mantovani (2001). Inflammation and Cancer: Back to Virchow, Lancet, 357, 539-545.
4. Bayerdörffer E., B. Rudolpha, A. Neubauer et al. (1995). Regression of Primary Gastric Lymphoma of Mucosa-associated Lymphoid Tissue Type after Cure of Helicobacter Pylori Infection, Lancet, 345(8965), 1591-1594.
5. Cedrés S., D. Torrejon, A. Martínez et al. (2012). Neutrophil to Lymphocyte Ratio (NLR) as an Indicator of Poor Prognosis in Stage IV Non-small Cell Lung Cancer, Clin Transl Oncol, 14(11), 864-869.
6. Cekanova M., K. Rathore (2014). Animal Models and Therapeutic Molecular Targets of Cancer: Utility and Limitations, Drug Des Devel Ther, 8, 1911-1922.
7. Cosa T., T. Maruyama, M. Otarigi (1998). Spesies Differences of Serum Albumin: II. Chemical and Thermal Stability, Pharmaceutical Research, 15(3), 449-454.
8. Drzazga Z., K. Michalik, T. Halat, A. Michnik, H. Trzeciak (2010). Calorimetric and Spectroscopic Studies Characterization of Newborn Rat' Blood Serum after Maternal Administration of Cyclophosphamide, J Therm Anal Calorim, 102, 143-148.
9. Garbett N., G. Brock, J. Chaires et al. (2017). Characterization and Classification of Lupus Patients Based on Plasma Thermograms, PLoS One, 12(11), e0186398.
10. Garbett N., C. Mekmaysy, C. Helm, A. Jenson, J. Chaires (2009). Differential Scanning Calorimetry of Blood Plasma for Clinical Diagnosis and Monitoring, Exp Mol Pathol, 86, 186-191.
11. Garbett N., M. Merchant, C. Helm et al. (2014). Detection of Cervical Cancer Biomarker Patterns in Blood Plasma and Urine by Differential Scanning Calorimetry and Mass Spectrometry, PLoS One, 9(1), e84710.
12. Garbett N., J. Miller, A. Jenson, J. Chaires (2008). Calorimetry Outside the Box: A New Window into the Plasma Proteome, Biophys J, 94(4), 1377-1383.

13. Gianazza E., C. Sensi, I. Eberini et al. (2013). Inflammatory Serum Proteome Pattern in Mice Fed a High-fat Diet, Amino Acids, 44, 1001-1008.
14. Grivennikov S., F. Greten, M. Karin (2010). Immunity, Inflammation and Cancer, Cell, 140, 883-899.
15. Hakansson A., C. Branning, G. Molin et al. (2011). Colorectal Oncogenesis and Inflammation in a Rat Model Based on Chronic Inflammation Due to Cycling DSS Treatments, Gastroenterology Research and Practice, 2011, 924045.
16. Hughes J., H. Weill (1991). Asbestosis as a Precursor of Asbestos Related Lung Cancer: Results of a Prospective Mortality Study, Br J Ind Med, 48(4), 229-233.
17. Kędra-Królik K., I. Chmielewska, A. Michnik, P. Zarzycki (2017). Blood Serum Calorimetry Indicates the Chemotherapeutic Efficacy in Lung Cancer Treatment, Scientific Reports, 7(1), 16796-16796.
18. Krumova S., B. Rukova, S. Todinova et al. (2013). Calorimetric Monitoring of the Serum Proteome in Schizophrenia Patients, Thermochimica Acta, 572, 59-64.
19. Krumova S., S. Todinova, A. Danailova et al. (2015). Calorimetric Features of IgM Gammopathies, Implication for Patient's Diagnosis and Monitoring, Thermochimica Acta, 615, 23-29.
20. Lakatos P., L. Lakatos (2008). Risk for Colorectal Cancer in Ulcerative Colitis: Changes, Causes and Management Strategies, World J Gastroenterol, 14(25), 3937-3947.
21. Maru G., K. Gandhi, A. Ramchandani, G. Kumar (2014). The Role of Inflammation in Skin Cancer, Adv Exp Med Biol, 816, 437-69.
22. McMillan D. (2013). The Systemic Inflammation-based Glasgow Prognostic Score: A Decade of Experience in Patients with Cancer, Cancer Treat Rev, 39(5), 534-540.
23. Michnik A., Z. Drzazga, E. Sadowska-Krępa, B. Kłapcińska (2014). Calorimetric Monitoring of the Effect of Endurance Training and Testosterone Treatment on Rat Serum Denaturation Transition, J Therm Anal Calorim, 115(3), 2231-2237.
24. Mihaylova N., S. Bradyanova, P. Chipinski et al. (2017). Annexin A1 as a Target for Managing Murine Pristane-induced Systemic Lupus Erythematosus, Autoimmunity, 50(4), 257-268.
25. Nemsadze G., T. Lezhava, M. Gorgoshidze et al. (2016). Blood Plasma Main Proteins Stability of Patients with Ductal Carcinoma in Post-surgery Period, Int J Clin Exp Med, 9(2), 1338-1345.
26. Parsonnet J., G. Friedman, D. Vandersteen et al. (1991). Helicobacter Pylori Infection and the Risk of Gastric Carcinoma, N Engl J Med, 325, 1127-1131.
27. Ryan M., R. Chopra (1976). Paradoxical Effect of Fatty-acid on Steroid-albumin Interaction, Biochim Biophys Acta, 427, 337-349.
28. Tenchov B., S. Abarova, R. Koynova, L. Traikov, L. Tancheva (2017). Low-temperature Exothermic Transitions in Brain Proteome of Mice, Effect of Scopolamine, Thermochimica Acta, 650, 26-32.
29. Todinova S., S. Krumova, T. Andreeva et al. (2016). Unusual Thermal Transition in the Serum Calorimetric Profile of a Patient Diagnosed with Multiple Myeloma with Secretion of Monoclonal κ Free Light Chains: A Case Report, Cancer Research Frontiers, 2(3), 416-426.
30. Todinova S., S. Krumova, A. Danailova et al. (2018). Calorimetric Markers for Monitoring of Multiple Myeloma and Waldenström's Macroglobulinemia Patients, European Biophysics Journal, 47(5), 549-559.
31. Todinova S., S. Krumova, P. Kurtev et al. (2012). Calorimetry-based Profiling of Blood Plasma from Colorectal Cancer Patients, Biochim Biophys Acta-General Subjects, 1820(12), 1879-1885.

32. Zaias J., M. Mineau, C. Cray, D. Yoon, N. Altman (2009). Reference Values for Serum Proteins of Common Laboratory Rodent Strains, J Am Assoc Lab Anim Sci, 48(4), 387-390.
33. Zapf I., M. Moezzi, T. Fekecs et al. (2016). Influence of Oxidative Injury and Monitoring of Blood Plasma by DSC on Breast Cancer Patients, J Therm Anal Calorim, 123(3), 2029-2035.

Senior Assist. Prof. Avgustina Danailova, Ph.D.
E-mail: avgustina_danailova@abv.bg

Avgustina Danailova graduated from the Sofia University "St. Kliment Ohridski" where she received a B.Sc. Degree in Biology and M.Sc. Degree in Biophysics. She currently works in the Institute of Biophysics and Biomedical Engineering, Bulgarian Academy of Sciences, where she defended Ph.D. in Biophysics in 2018. Her area of research interest includes blood plasma proteome, biomolecular interactions, calorimetric markers for diagnosis and monitoring of diseases.

Prof. Sashka Krumova, Ph.D.
E-mail: sashka@bio21.bas.bg

Sashka Krumova presently works at Institute of Biophysics and Biomedical Engineering, Bulgarian Academy of Sciences, Sofia, Bulgaria. Her current research interests are in the fields of biomolecular interactions, biological membranes macroorganization and novel biophysical approaches for disease diagnostics and monitoring.

Assoc. Prof. Ivan Iliev, Ph.D.
E-mail: taparsky@abv.bg

Ivan Iliev is an Associate Professor at the Institute of Experimental Morphology, Pathology and Anthropology with Museum, Bulgarian Academy of Sciences, where he received Ph.D. degree in area of Immunology. His interests are in the field of immunology, biochemistry and cell biology.

Dr. Lidia Gartcheva, MD
E-mail: lgartcheva@gmail.com

Dr. Lidia Gartcheva graduated at Medical University – Sofia and gained a Degree in Transfusion Hematology. Her current research interests are in the fields of haematological malignancy, stem cells transplantation, immunological reconstitution after transplantation and multiple myeloma.

Prof. Stefka G. Taneva, D.Sc.
E-mail: sgtaneva@gmail.com

Stefka Taneva is a head of the Department of Biomacromolecules and Biomolecular Interactions at Institute of Biophysics and Biomedical Engineering, Bulgarian Academy of Sciences. She graduated Physics and Biophysics at Sofia University "St. Kliment Ohridski". She received Ph.D. degree in Physics from the Institute of Biophysics, Biological Research Center, Hungarian Academy of Sciences. Her main focus of research has been energetics of protein interactions, application of microcalorimetry in disease diagnostics and monitoring, mechanisms of light-energy transduction – bacteriorhodopsin, macroorganization of pigment-protein complexes, static and dynamic electric properties of biological membranes, biofunctionalization of polyelectrolyte multilayers for medical application.

Assoc. Prof. Svetla Todinova, Ph.D.
E-mail: todinova@abv.bg

Svetla Todinova graduated from Technical University, Sofia, with M.Sc. in Electronics. She received her Ph.D. degree in Biophysics at the Institute of Biophysics and Biomedical Engineering, Bulgarian Academy of Sciences. She is currently an Associate Professor at the same institute. Her areas of interests include biomedical science, biomolecular interactions, microcalorimetry, electronics, and circular dichroism.