Abstracts of selected publications of Assoc. Prof. Svetla Todinova for participation in the concourse for the academic position "Professor"

1. **Todinova S.,** Krumova S., Danailova A., Petkova V., Margarita Guenova, Mihaylov G., Gartcheva L., Taneva S.G.. Calorimetric markers for monitoring of multiple myeloma and Waldenström's macroglobulinemia patients. Eur Biophys J., **2018**, 47(5), 549-559. doi:10.1007/s00249-018-1277-3. SJR (Scopus):0.672, **IF: 2.527 (WebS), Q2**

The blood proteome has been studied extensively for identification of novel reliable disease biomarkers. In recent years, differential scanning calorimetry has emerged as a new tool for characterization of the thermodynamic properties of the major serum/plasma proteins and for the establishment of calorimetric markers for a variety of diseases. Here we applied calorimetry to monitor the effect of treatment of patients diagnosed with multiple myeloma and Waldenström's macroglobulinemia on the calorimetric profiles of patients' blood sera. The parameters derived from the calorimetric profiles were compared with the primary serum biomarkers, monoclonal immunoglobulin (M protein) concentration, and κ/λ free light chain ratio. For the secretory cases, the calorimetric parameters thermogram's shape similarity and weighted average center strongly depended on the M protein level but had lower sensitivity and specificity. By contrast, for non-secretory cases, the calorimetric parameters did not depend on the κ/λ free light chains ratio and exhibited significantly higher sensitivity and specificity than M protein levels. A combination of the immunological and calorimetric tests was found to greatly improve the sensitivity and specificity of the clinical status evaluation. The pronounced differences in blood sera thermograms before and during monitoring reflected the individual patients' response to treatment received and showed maintenance of heterogeneity during the disease course.

2. **Todinova S.,** Krumova S., Gartcheva L., Dimitrova K., Petkova V., Taneva S.G.. Calorimetric manifestation of IgA monoclonal immunoglobulins in multiple myeloma sera. Thermochimica Acta, **2018**, 666, 208-211. DOI: 10.1016/j.tca.2018.07.005. **IF:2.251(WebS), Q2**

Multiple myeloma (MM) with secretion of monoclonal immunoglobulin A (IgA) is among the common myeloma types. The diagnosis of IgA MM is based on a panel of clinical and paraclinical markers, the primary one being the IgA paraprotein level. One of the drawbacks of IgA MM diagnostics and monitoring, especially at low IgA levels, is the migration of monoclonal IgA in the β-globulins region of the serum protein electrophoresis profile where it overlaps with "healthy" β-globulin proteins and is thus not clearly resolved. The present study explores the manifestation of IgA monoclonal immunoglobulins in the thermograms of multiple myeloma sera. We show that the electrophoretic mobility of IgA paraproteins is related to altered intermolecular interactions, plausibly the formation of IgA oligomers and/or albumin-IgA complexes. We demonstrate that high IgA levels exhibit specific calorimetric features that discriminate IgA MM from other MM subtypes.

3. Danailova A., Krumova S., Iliev I., Gartcheva L., Taneva S.G., **Todinova S.** Calorimetric Markers for Inflammation in in vivo Experimental Models. INT.J. BIOAUTOMATION, **2019**, 23(4), 479-488. DOI:doi: 10.7546/ijba.2019.23.4.000645**. SJR (Scopus):0.267**, **Q3**

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.

4. Krumova, S., Balansky, R., Danailova, A., Ganchev, G., Djongov, L., Gartcheva, L., Taneva, S.G., **Todinova, S.** Calorimetric assay to follow colorectal cancer development in experimental rat models. Thermochimica Acta, **2020**, 691(2), 178723. doi:10.1016/j.tca.2020.178723. SJR (Scopus):0.558, **IF**:**4.626 (WebS), Q2**

Gastrointestinal tract cancers are among the most common malignancies that still lack reliable and highly specific biomarkers. Differential scanning calorimetry has been widely explored in the last decade for the detection of disease- and stage-specific changes in temperature-induced conformational transitions in blood plasma proteins. The goal of the present study was to explore the potential of calorimetry to follow colorectal cancer tumor development in rats treated with 1,2-dimethylhydrazine. The plasma calorimetric features are related to the sex, age and occurrence of proliferative lesions and tumor formations in the intestinal tract of treated animals. The process of tumor induction led to both stabilization of the plasma proteome and decreased similarity between the plasma calorimetric curves of healthy and diseased rats; therefore, these parameters might be regarded as indicators of malignant transformation of intestinal cells and progression of tumor formation.

5. Langari A., Danailova A., Krumova S., Komsa-Penkova R., Golemanov G., Giosheva I., Gartchev E., Taneva S.G., **Todinova S.** Aging-related changes in the calorimetric profile of red blood cells from women with miscarriages. Journal of Thermal Analysis and Calorimetry volume, **2020**, 142, 1919-1926. https://doi.org/10.1007/s10973-020-10112-3. SJR (Scopus):0.415**, IF**:**4.626 (WebS)**, **Q2**

Miscarriages are one of the most common problems of pregnancy abnormalities; however, in most cases the etiology of this pathology is unknown. Herein, we applied differential scanning calorimetry to study the aging process of red blood cells (RBCs) derived from women with miscarriages as compared to healthy non-pregnant and pregnant women of reproductive age, with the aim to identify specific calorimetric features associated with high-risk pregnancy. The calorimetric profiles of RBCs derived from healthy pregnant and non-pregnant women along the cells aging path are characterized with: (1) slow reduction

in the hemoglobin heat capacity and (2) downshift of the thermal transitions of hemoglobin, Band 3 and Band 2.1, 4.1 and 4.2 proteins. A large population (71%) of the studied cases with miscarriage are associated with faster aging of RBCs, i.e., faster thermal destabilization of hemoglobin and Band 3 transitions compared to healthy non-pregnant and pregnant women. The results suggest that the accelerated temperature-induced destabilization of RBCs from the majority of women that underwent miscarriages along cells aging is an additional criterion for estimation of the risk of miscarriage.

6. **Todinova S.,** Krumova S., Bogdanova D., Danailova A., Zlatareva E., Kalaydzhiev N., Langari A., Milanov I., Taneva S.G. Red Blood Cells' Thermodynamic Behavior in Neurodegenerative Pathologies and Aging. Biomolecules 2021, 11(10), 1500. https://doi.org/10.3390/biom11101500, **IF**:**4.879 (WebS), Q1,** не оглавява ранглистата

The main trend of current research in neurodegenerative diseases (NDDs) is directed towards the discovery of novel biomarkers for disease diagnostics and progression. The pathological features of NDDs suggest that diagnostic markers can be found in peripheral fluids and cells. Herein, we investigated the thermodynamic behavior of the peripheral red blood cells (RBCs) derived from patients diagnosed with three common NDDs—Parkinson's disease (PD), Alzheimer's disease (AD), and amyotrophic lateral sclerosis (ALS) and compared it with that of healthy individuals, evaluating both fresh and aged RBCs. We established that NDDs can be differentiated from the normal healthy state on the basis of the variation in the thermodynamic parameters of the unfolding of major RBCs proteins—the cytoplasmic hemoglobin (Hb) and the membrane Band 3 (B3) protein. A common feature of NDDs is the higher thermal stability of both Hb and B3 proteins along with the RBCs aging, while the calorimetric enthalpy can distinguish PD from ALS and AD. Our data provide insights into the RBCs thermodynamic behavior in two complex and tightly related phenomena—neurodegenerative pathologies and aging, and it suggests that the determined thermodynamic parameters are fingerprints of the altered conformation of Hb and B3 protein and modified RBCs' aging in the studied NDDs

7. **Todinova S**., Raynova Y., Idakieva K. Irreversible thermal denaturation of Helix aspersa maxima hemocyanin. J Therm Anal Calorim, **2018,** 132, 777–786. DOI:doi.org/10.1007/s10973-018-6959-7, **IF:2.471(WebS), Q2**

The thermal unfolding of hemocyanin, purified from garden snails Helix aspersa maxima (gastropod) (*HaH*), was investigated by differential scanning calorimetry (DSC), far-UV circular dichroism (CD) and UV–Vis spectroscopy. Denaturation of *HaH* was found to be an irreversible process. One transition, with an apparent transition temperature (T_m) at 79.8 °C, was detected by DSC in the thermogram of HaH in 20 mM HEPES buffer, containing 0.1 M NaCl, 5 mM CaCl2 and 5 mM MgCl2, pH 7.0, using scan rate of 1.0 °C min^{-1}. The scan rate dependence of the calorimetric profiles indicated that the thermal unfolding of investigated HaH was kinetically controlled. The Tm and calorimetric enthalpy values (ΔH_{cal}) for the thermal denaturation of HaH were found to be independent of the protein concentration, indicating that the dissociation of the hemocyanin into subunits does not take place before the rate-determining step of the process of thermal unfolding started. The calorimetrically observed thermal transition showed excellent coincidence with the unfolding transition monitored by CD measurements. The thermal

denaturation of *HaH* was described by the two-state irreversible model, and parameters of the Arrhenius equation were calculated.

8. **Todinova S**., Raynova Y., Idakieva K. Calorimetric Study of Helix aspersa Maxima Hemocyanin Isoforms. Journal of Analytical Methods in Chemistry, **2018**, Hindawi, Article ID 8450792, 8 pages, ISSN:2090-8865, DOI:doi.org/10.1155/2018/8450792, **IF:1.589 (WebS), Q3**

The thermal unfolding of hemocyanin isoforms, β-HaH, and αD+N-HaH, isolated from the hemolymph of garden snails Helix aspersa maxima, was studied by means of differential scanning calorimetry (DSC). One transition, with an apparent transition temperature (Tm) at 79.88°C, was detected in the thermogram of β-HaH in 20 mM HEPES buffer, containing 0.1 M NaCl, 5 mM CaCl2, and 5 mM MgCl2, pH 7.0, at a scan rate of 1.0°C min−1. By means of successive annealing procedure, two individual transitions were identified in the thermogram of αD+N-HaH. Denaturation of both hemocyanins was found to be an irreversible process. The scan-rate dependence of the calorimetric profiles indicated that the thermal unfolding of investigated hemocyanins was kinetically controlled. The thermal denaturation of the isoforms β-HaH and αD+N-HaH was described by the two-state irreversible model, and parameters of the Arrhenius equation were calculated.

9. Idakieva K., **Todinova S.,** Dolashki A., Velkova L., Raynova Y., Dolashka P. Biophysical characterization of the structural stability of Helix lucorum hemocyanin. Biotechnology & Biotechnological Equipment, Taylor and Francis Ltd., 2021, 35(1), 18-28. DOI:https://doi.org/10.1080/13102818.2020.1837010, **SJR (Scopus):0.376, IF**:**1.785 (WebS), Q3**

The structural stability of the hemocyanin purified from the hemolymph of garden snails Helix lucorum (HlH) was investigated by means of far-UV circular dichroism (CD), differential scanning calorimetry (DSC) and transmission electron microscopy (TEM). For the first time, TEM analyses showed the presence of tubular polymers in hemocyanins after three-day dialysis against a stabilizing buffer containing high concentrations of Ca2+ and Mg2+ ions (100 mmol L^{-1}). The conformational stability study of native HlH by means of CD in a wide pH range (2.5-11.5) defined the pH stability region of HlH at pH 6.5 – 8.0. DSC analyses demonstrated the thermal stability of this hemocyanin. One transition, with an apparent transition temperature (Tm) at 82.3 °C, was detected in the heat capacity curve of HlH in 50-mmol L^{-1} Tris-HCl buffer, pH 7.2, at a heating rate of 1.0 °C min−1. The calorimetrically observed thermal transition correlates well with the unfolding transition monitored by CD measurements. The two-state kinetic model was used to analyse the process of irreversible thermal denaturation of HlH; Ea of 451 ± 4 kJ mol^{-1} was calculated. The obtained results on the conformational stability of HlH will facilitate the further investigation of the properties and potential biomedical applications of this respiratory protein.

II. Publications under Indicator **Г7**

Abstracts of selected publications of Assoc. Prof. Svetla Todinova for participation in the concourse for the academic position "Professor"

1. Guncheva M., **Todinova S**., Yancheva D., Raynova Y., Idakieva K. Thermal stability and secondary structure of feruloylated Rapana thomasiana hemocyanin. J Therm Anal Calorim, **2019**, DOI:https://doi.org/10.1007/s10973-019-08373-8, 2715-2720. SJR (Scopus):0.587, **IF: 2.731 (WebS), Q2**

This is the first report on the conjugation of hemocyanin from Rapana Thomasina (RtH) with ferulic acid, which itself exhibits an immunostimulatory activity. In a two-step reaction, 20 to 180 ferulic acid residues were covalently linked to one molecule RtH. The effect of modification of RtH with ferulic acid on the thermal unfolding was evaluated using differential scanning calorimetry (DSC). The DSC curves of the feruloylated-RtHs characterize with an asymmetric shape, which is an indication for the existence of more than one structural unit in the analyzed samples. The protein thermal stability is not affected by the modification; however, the profiles of the DSC curves of the feruloylated-RtH and the native RtH differ, which implies the reorganization in the protein molecule and is in correlation with the secondary structure analyses. The conformational changes in the RtH molecules that are due to the feruloylation were followed in the Amide I region (1600–1700 cm−1) of the ATR-FTIR spectra. In all prepared samples, we observed a rearrangement in protein molecule, a decrease in α-helices and coiled structures in favor of the β-structures, and no aggregation in comparison with the native RtH.

2. Guncheva, M., Idakieva, K., **Todinova, S.,** Stoyanova, S., Yancheva, D.. Biophysical Properties and Cytotoxicity of Feruloylated Helix Lucorum Hemocyanin. Acta Chimica Slovenica, **2020**, 67(1), 253-259. ISSN:1580-3155, DOI:DOI: 10.17344/acsi.2019.5400, 253-259. SJR (Scopus):0.297**, IF:1.735 (WebS), Q3**

For the first time Helix lucorum hemocyanin (HlH) has been feruloylated. Two HlH conjugates with 40- and 120- ferulic acid residues were prepared, denoted as FA-HlH-1 and FA-HlH-2. Expectedly, the feruloylation of HlH induced a rearrangement of the protein molecule, a decrease in the α-helical structure at the expense of β-structures was observed. Besides, the FA-HlH conjugates were more prone to aggregation, which is probably due to the stabilization of the partially unfolded protein molecules by non-covalent bonding. Interestingly, the thermal stability of HlH was not affected by the modification. The native and feruloylated HlH were not toxic to normal fibroblasts (BJ cells). We observed a decrease in cell viability of breast cancer MCF-7 cells to about 66% after a 48h exposure to 70 μg/well of FA-HlH-2.

3. Guncheva, M., **Todinova, S**., Yancheva, D., Idakieva, K.. Rosmarinic acid-conjugated hemocyanins: synthesis and stability. J Therm Anal Calorim, **2020,** 142, 1903–1909. DOI:https://doi.org/10.1007/s10973-020-09738-0, **JCR- IF:4.626 (WebS), Q2**

Two oxygen-transporting proteins known for their immunostimulating and anticancer properties, namely hemocyanin from Helix lucorum (HlH) and hemocyanin from Rapana thomasiana (RtH), have been modified for the first time with rosmarinic acid (RA). We prepared two conjugates RA–HlH, and RA–RtH containing 47 and 50 rosmarinic acid residues, respectively. The conformational analysis showed that the secondary structure of RA–HlH is less ordered than that of the native HlH, and for the modified protein we observed a decrease in α-helical structures in the favor of random coils, unordered structures, and aggregates. Calorimetric studies showed an increase in the thermal stability of RA–HlH in comparison with the native HlH which is in agreement with the observed structural changes. On the other hand, the conformation of RA–RtH was very similar to that of the native RtH although some rearrangements in the molecule were found. Interestingly, the modification of RtH with RA resulted in a decrease in its thermal

stability. A 48-h in vitro experiment showed that tested in concentrations up to 0.7 mg mL^{-1} the two modified hemocyanins did not inhibit the cell growth of human fibroblasts.

4. Guncheva M., Idakieva K., **Todinova S.,** Stoyanova E., Yancheva D.. Folate-conjugated Helix lucorum hemocyanin – preparation, stability, and cytotoxicity. Z Naturforsch C J Biosci., **2020,** 75, (1-2), DOI:https://doi.org/10.1515/znc-2019-0144, 23-30. SJR (Scopus):0.261, **IF:1.469 (WebS), Q3**

This is the first report on the modification of a hemocyanin from Helix lucorum (HlH), a large molluscan respiratory protein, with folic acid (*FA*). In a two-step synthetic reaction, we prepared samples of HlH conjugated with 20 and 50 *FA* residues denoted as *FA-HlH-1* and *FA-HlH-2*, respectively. Comparison of the attenuated total reflectance-Fourier transform infrared spectra in the amide I band region showed a structural rearrangement in the HlH that is due to FA conjugation. The changes in the secondary structure were more noticeable for FA-HlH-2. The thermal stability of HlH was not significantly affected by the FA modification, which is consistent with the observed structural similarities with the native protein. Preliminary cytotoxicity assays showed that FA-HlH-1 and FA-HlH-2 stimulate fibroblast proliferation when applied in concentrations of 50 and 100 µg/well. A negligible reduction of fibroblast growth was observed only for FA-HlH-1 and FA-HlH-2, exposed to 200 µg/well for 48 h. We found that FA-HlH-2 exhibits a low to moderate cytotoxic effect on two breast cancer cell lines, which express folate receptors, a hormone-dependent (MCF-7) and a hormone-independent (MDA-MB-231). FA-HlH-2 protects nontransformed cells and affects only neoplastic cells, which could be an advantage, and the protein could have potential in combination with other chemotherapeutics.

5. Guncheva M., Idakieva K., **Todinova S**., Yancheva D., Paunova-Krasteva T., Ossowicz P., Janus E. Structural, Thermal, and Storage Stability of Rapana Thomasiana Hemocyanin in the Presence of Cholinium-Amino Acid-Based Ionic Liquids. Molecules, MDPI, **2021**, 26(6), 1714. DOI:10.3390/molecules26061714, SJR (Scopus):0.7, **JCR- IF:4.412 (WebS), Q1,** не оглавява ранглистата

Novel biocompatible compounds that stabilize proteins in solution are in demand for biomedical and/or biotechnological applications. Here, we evaluated the effect of six ionic liquids, containing mono- or dicholinium [Chol]1or2 cation and anions of charged amino acids such as lysine [Lys], arginine [Arg], aspartic acid [Asp], or glutamic acid [Glu], on the structure, thermal, and storage stability of the Rapana thomasiana hemocyanin (RtH). RtH is a protein with huge biomedicinal potential due to its therapeutic, drug carrier, and adjuvant properties. Overall, the ionic liquids (ILs) induce changes in the secondary structure of RtH. However, the structure near the Cu-active site seems unaltered and the oxygen-binding capacity of the protein is preserved. The ILs showed weak antibacterial activity when tested against three Gram-negative and three Gram-positive bacterial strains. On the contrary, [Chol][Arg] and [Chol][Lys] exhibited high anti-biofilm activity against E. coli 25213 and S. aureus 29213 strains. In addition, the two ILs were able to protect RtH from chemical and microbiological degradation. Maintained or enhanced thermal stability of RtH was observed in the presence of all ILs tested, except for RtH-[Chol]2[Glu].

6. Guncheva M., **Todinova S.,** Uzunova V., Idakieva K., Raynova Y., Ossowicz P., Janus E., Tzoneva R. Destabilization of β-Hemocyanin from Helix pomatia in Presence of Choline Amino Acids Results in Improved Cell Specificity and Cytotoxicity against Human Breast Cancer. Chemistry Select, John Wiley

& Sons, Ltd, **2019**, 4(39), 11460-11466. DOI:https://doi.org/10.1002/slct.201902464. JCR- **IF :1.811, Q2**

Many hemocyanins, the oxygen-transporting proteins in some invertebrates, are studied in depth in view of their immunostimulatory and vaccine adjuvant properties. However, their direct cytotoxic effect on breast cancer cell lines is poorly investigated. Here, we evaluated the effect of native β-hemocyanin from Helix pomatia (β-HpH) and its complexes with choline amino acids ([Chol][AA]) on the viability and morphology of estrogen-dependent (MCF-7) and estrogen-independent (MDA-MB-231) breast cancer cells. In the tested concentration range, the native ß-HpH has no effect on the cytotoxicity and migratory capacity of the two tested cell lines. [Chol][AA] induce rearrangement in the ß-HpH molecule. The complexes characterize with decreased thermal stability and altered cytotoxicity. Their cytotoxicity toward MDA-MB-231 cells is preserved or enhanced. At the highest assayed concentration, β-HpH-[Chol][Trp] produced the same cytotoxic effect against MDA-MB-231 cells as that reported for doxorubicin. Oppositely, the native ß-HpH, significantly stabilizes MCF-7 cells and their metastatic potential was enhanced..

7. Guncheva M., Ossowicz P., Janus E., **Todinova S.,** Yancheva D. Elucidation of the effect of some cholinium amino acid ionic liquids on the thermal and the conformational stability of insulin. Journal of Molecular Liquids, **2019**, 283(2), 257-262. ISSN:0167-7322, DOI:doi.org/10.1016/j.molliq.2019.03.074, 257-262. **SJR (Scopus):0.849, JCR- IF:5.056 (WebS), Q1,** не оглавява ранглистата

The major concerns about protein-based drugs are their stability i.e. maintaining the protein in the folded state throughout processing and storage, as well as the preparation of novel formulation. Stabilization of the monomeric form of insulin (*In*) under the condition of low pH has been a recent challenge. In our earlier investigation, we found that 1-butyl-3-methylimidazolium-based ionic liquids (ILs) containing acetate, trifluoroacetate or dicyanamide anions enhance *In* thermal stability and prevent protein aggregation. In the present study, six non-toxic ILs containing biocompatible cholinium cation [Chol] and an anion charged amino acid (asparginyl (Asp), glutaminyl (Glu), lysinyl (Lys) and arginyl (Arg)) were synthesized using a two-step procedure. Their effect of the ILs on the *In* secondary structures was evaluated using FTIR spectroscopy. Rearrangement in the protein molecules, an increase in the beta-structures on behalf of the α-helices, partial denaturation but no aggregation was observed in all In solutions containing ILs. Differential scanning calorimetry was applied to elucidate the effect of ILs on thermal stability of In. Interestingly, in presence of [Chol][Glu] and [Chol] 2 [Asp] the denaturation temperature of the In was shifted to higher temperatures with 9.6 and 4.1 °C, respectively.

8. Kardaleva P., Guncheva M., **Todinova S.**, Angelov I., Ossowicz P., Janus E. Effect of ketoprofen-based ionic liquids on secondary structure and thermal stability of human serum albumin. J Therm Anal Calorim, **2020,** 142(5), 1911-1917. DOI:https://doi.org/10.1007/s10973-020-10111-4, 1911-1917. **SJR (Scopus):0.42, JCR- IF:2.731 (WebS), Q2**

This is the first study on the interactions of ionic liquids (ILs) composed of cations amino acid esters and anion ketoprofen (KETO) with human serum albumin (HSA). The effect of the ILs on the thermal unfolding of the HSA was negligible in comparison with that of the KETO. For the IL series, the denaturation transitions tend to shift to lower temperatures by 1–3 °C. The secondary structure analyses have shown that KETO, [L-LeuOEt][KETO], and [L-ValOBu][KETO] cause folding, and HSA molecule becomes more

coiled. In the presence of [L-ValOPr][KETO] and [L-ValOiPr][KETO], HSA structure although partially unfolded seemed very close to that of the native protein. In contrast, [L-ValOEt][KETO] induces the same conformational changes of HSA as ketoprofen. For the latter, we performed binding assays and we found that the affinity of the HSA to bind the IL is comparable to that for the ketoprofen. The estimated binding constants were 3.1×10^4 and 1.4×10^4 L mol^{-1} for ketoprofen and [L-ValOEt][KETO], respectively. This study on KETO-ILs could be a basis to the development of a new drug formulation.

9. Taneva S.G., Krumova S., Bogár F., Kincses A., Stoichev S., **Todinova S.J**., Danailova A., Horvath J., Násztor Z., Kelemen L., Dér A. Insights into graphene oxide interaction with human serum albumin in isolated state and in blood plasma. International Journal of Biological Macromolecules, Elsevier, **2021**, 175, 19-29. DOI:10.1016/j.ijbiomac.2021.01.151, SJR (Scopus):0.97, **JCR- IF:5.953 (WebS), Q1,** не оглавява ранглистата

The interactions of graphene oxide (GO), a 2-dimensional nanomaterial with hydrophilic edges, hydrophobic basal plane and large flat surfaces, with biological macromolecules, are of key importance for the development of novel nanomaterials for biomedical applications. To gain more insight into the interaction of GO flakes with human serum albumin (HSA), we examined GO binding to HSA in its isolated state and in blood plasma. Calorimetric data reveal that GO strongly stabilizes free isolated HSA against a thermal challenge at low ionic strength, indicating strong binding interactions, confirmed by the drop in ζ-potential of the HSA/GO assemblies compared to bare GO flakes. However, calorimetry also revealed that the HSA-GO molecular interaction is hampered in blood plasma, the ionic strength being particularly important for the interactions. Molecular modelling calculations are in full concert with these experimental findings, indicating a considerably higher binding affinity for HSA to GO in its partially unfolded state, characteristic to low-ionic-strength environment, than for the native protein conformation, observed under physiological conditions. Therefore, for the first time we demonstrate an impeded interaction between HSA and GO nanoflakes in blood plasma, and suggest that the protein is protected from the plausible toxic effects of GO under native conditions.

10. Todinova S., Nikolova B., Iliev I., Semkova S., Krumova S., Taneva S.G. Thermodynamic behavior of breast cancer cell lines after miltefosine and cisplatin treatment. J Therm Anal Calorim, **2021**, DOI:https://doi.org/10.1007/s10973-021-11094-6, **IF:4.626 (WebS), Q2**

Breast cancers exhibit a different response to drug treatment. In this work, we analyze and compare the effect of two anticancer drugs differing in their primary action, miltefosine and cisplatin (cis-Pt), on two different breast cancer (the low—(MCF-7) and high—(MDA-MB-231) metastatic) cell lines, and one normal epithelial (MCF-10A) breast cell lines. The effect of cip-Pt and miltefosine on the thermodynamic behavior of the cancer cell lines was analyzed by differential scanning calorimetry, the cell morphology and viability were determined by optical microscopy and MTT test. We revealed distinct effects of miltefosine and cis-Pt on the thermodynamic behavior and viability of cancer and normal cells. Importantly, the normal MCF-10A cells were drastically affected by miltefosine, while not by cis-Pt. MDA-MB-231 cell line, on the other hand, is more susceptible to cis-Pt than MCF-7 cells, while both cancer cell lines are equally affected by miltefosine. The drug-associated alteration of the thermal unfolding of the cells constituents correlated with the changes in the cell viability. The altered thermodynamic behavior of the cancer cells upon the drug treatment strongly indicates altered conformations of the proteins in cancer cell membrane and cellular matrix, and the DNA-containing structures.

11. Gospodinova Z., Zupko I., Noémi B., Manova V., Georgieva M., **Todinova S. J.,** Taneva S.G., Ocsovszki I., Krasteva M. Cotinus coggygria Scop. induces cell cycle arrest, apoptosis, genotoxic effects, thermodynamic and epigenetic events in MCF7 breast cancer cells. Z Naturforsch C J Biosci. **2020**, 76(3-4), 129–140. doi: 10.1515/znc-2020-0087. SJR (Scopus):0.36**, JCR- IF:1.649 (WebS), Q3**

Current plant-derived anticancer therapeutics aim to reach higher effectiveness, to potentiate chemosensitivity and minimize the toxic side effects compared to conventional chemotherapy. Cotinus coggygria Scop. is a herb with high pharmacological potential, widely applied in traditional phytotherapy. Our previous study revealed that leaf aqueous ethanolic extract from C. coggygria exerts in vitro anticancer activity on human breast, ovarian and cervical cancer cell lines. The objective of the present research was to investigate possible molecular mechanisms and targets of the antitumor activity of the extract in breast cancer MCF7 cells through analysis of cell cycle and apoptosis, clonogenic ability assessment, evaluation of the extract genotoxic capacity, characterization of cells thermodynamic properties, and analysis on the expression of genes involved in cellular epigenetic processes. The obtained results indicated that in MCF7 cells C. coggygria extract causes S phase cell cycle arrest and triggers apoptosis, reduces colony formation, induces DNA damage, affects cellular thermodynamic parameters, and tends to inhibit the relative expression of DNMT1, DNMT3a, MBD3, and p300. Further studies on the targeted molecules and the extract anti-breast cancer potential on animal experimental model system, need to be performed in the future.

12. Todinova S., Komsa-Penkova R., Krumova S., Taneva S.G., G. Golemanov, Georgieva G., Tonchev P., Tsankov B., Beshev L., Balashev K., Andreeva T.D. PlA2 polymorphism in glycoprotein IIb/IIIa modulates the morphology and nanomechanics of platelets. Clinical and Applied Thrombosis/Hemostasis, **2017,** 23(8), 951-960. ISSN:1938-2723, DOI:10.1177/1076029616687847, 951-960. **ISI IF: 1.852, Q2**

Glycoprotein IIb/IIIa (GPIIb/IIIa) is the most abundant platelet surface receptor for fibrinogen and von Willebrand factor. Polymorphism PlA1/A2 in the gene of GPIIb/IIIa is among the risk factors for the development of arterial and venous thrombosis. The aim of this study is to evaluate the effect of the carriage of PlA1/A2 on the size, topographic features, and membrane stiffness of platelets from healthy controls and patients with deep venous thrombosis (DVT). Atomic force microscopy (AFM) imaging and nanoindentation (force-distance curves) were applied to investigate the morphological and nanomechanical properties (Young's modulus) of platelets immobilized on glass surface. The surface roughness (Ra) and height (h) of platelets from patients with DVT, carriers of mutant allele PlA2 (Ra = 30.2 ± 6 nm; h = 766 ± 182 nm) and noncarriers (Ra = 28.6 ± 6 nm; h = 865 ± 290 nm), were lower than those of healthy carriers of allele PlA2 (Ra = 48.1 ± 12 nm; h = 1072 ± 338 nm) and healthy noncarriers (Ra = 49.7 ± 14 nm; h = 1021 ± 433 nm), respectively. Platelets isolated from patients with DVT, both carriers and noncarriers, exhibit a much higher degree of stiffness at the stage of spreading (E = 327 ± 85 kPa and 341 ± 102 kPa, respectively) compared to healthy noncarriers (E = 198 ± 50 kPa). In addition, a more pronounced level of platelet activation was found in polymorphism carriers. In conclusion, the carriage of PlA2 allele modulates the activation state, morphology, and membrane elasticity of platelets.

13. Andreeva T., Komsa-Penkova R., Langari A., Krumova S., Golemanov G., Georgieva G.B., Taneva S.G., Giosheva I., Mihaylova N., Tchorbanov A., **Todinova S**. Morphometric and Nanomechanical Features of Platelets from Women with Early Pregnancy Loss Provide New Evidence of the Impact of Inherited

Thrombophilia. Int. J. Mol. Sci., **2021**, 22, 7778. DOI:https://doi.org/10.3390/ijms22157778, SJR (Scopus):1.46, JCR- **IF**:**5.924(WebS), Q1**, не оглавява ранглистата

Pregnancy is associated with hypercoagulation states and increased thrombotic risk, especially in women with thrombophilia. We combine atomic force microscopy (AFM) and flow cytometry to examine the morphology and nanomechanics of platelets derived from women with early pregnancy loss (EPL) and control pregnant (CP) and non-pregnant (CNP) women. Both control groups exhibit similar morphometric parameters (height and surface roughness) and membrane stiffness of platelets. EPL patients' platelets, on the other hand, are more activated than the control groups, with prominent cytoskeletal rearrangement. In particular, reduced membrane roughness (22.9 ± 6 nm vs. 39.1 ± 8 nm) ($p < 0.05$) and height (692 ± 128 nm vs. 1090 ± 131 nm) ($p < 0.05$), strong alteration in the membrane Young modulus, increased production of platelets' microparticles, and higher expression of procoagulant surface markers, as well as increased occurrence of thrombophilia (FVL, FII20210A, PLA1/A2, MTHFR C677T or 4G/5G PAI-1) polymorphisms were found. We suggest that the carriage of thrombophilic mutations triggers structural and nanomechanical abnormalities in platelets, resulting in their increased activation. The activation state of platelets can be well characterized by AFM, and the morphometric and nanomechanical characteristics might serve as a new criterion for evaluation of the cause of miscarriage and offer the prospect of an innovative approach serving for diagnostic purposes.

14. Komsa-Penkova R., **Todinova S.,** Andreeva T., Krumova S., Taneva S.G., Golemanov G., Georgieva G., Mihaylova N., Tchorbanov A., Tonchev P. Alterations in platelet activity and elasticity modulus of healthy subjects, carriers of G20210A polymorphism in the prothrombin gene. J Biomed Clin Res, **2016**, 9(1), 72-79. DOI: https://doi.org/10.1515/jbcr-2016-0011

Platelet activation is a complex process in which platelet reorganization takes place associated with changes in the cell shape, topology, membrane elasticity, and microparticle production. The aim of this study was to investigate the changes/aberrations in the platelet activity, elasticity, and morphology in healthy subjects, carriers of A allele of prothrombin G20210A polymorphism. Blood samples from 18 healthy subjects were used for platelet analysis by force-mode atomic force microscopy. Restriction analysis was used to investigate the carriage of G20210A polymorphism in the prothrombin gene. Flow cytometry was applied to evaluate platelet activation. Young's modulus of the plasma membranes of platelets derived from healthy subjects, carriers of variant A allele of prothrombin 20210G>A polymorphism (407±69 kPa) is two times higher than the one determined for non-carriers (195.4±48.7 kPa; $p<0.05$). The background activity of platelets measured as an interrelation of Cd41/Cd61 and CD62 by flow cytometry was also higher in carriers of variant A allele of prothrombin 20210G>A polymorphism (5.0%) than in non-carriers (1.3%). Platelets isolated from healthy carriers of variant A allele of prothrombin 20210G>A polymorphism exhibited a higher level of activity and a higher degree of stiffness at the stage.

15. Laczkó-Dobos H., **Todinova S.J**., Sözer Ö., Komenda J., Kis M., Sallai A., Dobrikova A.G., Ughy B., Debreczeny M., Gombos Z., Apostolova E.L., Domonkos, I.. Identification of thylakoid membrane thermal transitions in Synechocystis sp. PCC6803 photosynthetic mutants. Photosynthesis Research, **2011**, 107(3), 237-46. DOI:10.1007/s11120-011-9627-3, 237-246. SJR:1.01, **ISI IF:3.091 (WebS), Q1**, не оглавява ранглистата

We used differential scanning calorimetry (DSC) as a technique capable of identifying photosynthetic complexes on the basis of their calorimetric transitions. Annotation of thermal transitions was carried out with thylakoid membranes isolated from various photosynthetic mutants of Synechocystis sp. PCC6803. The thylakoid membranes exhibited seven major DSC bands between 40 and 85°C. The heat sorption curves were analyzed both by mathematical deconvolution of the overall endotherms and by a subsequent annealing procedure. The successive annealing procedure proved to be a more reliable technique than mathematical deconvolution in assigning thermal transitions. The main DSC band, around 47°C, resulting from the high enthalpy change that corresponds to non-interacting complex of PSII, was assigned using the PSI-less/apcE(-) mutant cells. Another band around 68-70°C relates to the denaturation of PSII surrounded by other proteins of the photosynthetic complexes in wild-type and PSI-less/apcE(-) cells. A further major transition found at 82-84°C corresponds to the PSI core complex of wild type and PSII-deficient BE cells. Other transition bands between 50-67 and 65-75°C are believed to relate to ATP synthase and cytochrome b(6)f, respectively. These thermal transitions were obtained with thylakoids isolated from PSI(-)/PSII(-) mutant cells. Some minor bands determined at 59 and 83-84°C correspond to an unknown complex and NADH dehydrogenase, respectively. These annotations were done by PSI-less/apcE(-) and PSI(-)/PSII(-) mutants.

16. Petrova N., **Todinova S.,** Laczko-Dobos H., Zakar T., Vajravel S., Taneva S.G., Gombos Z., Krumova S. Structural integrity of Synechocystis sp. PCC 6803 phycobilisomes evaluated by means of differential scanning calorimetry. Photosynth Res, **2018,** 137(1), 95–104. DOI:https://doi.org/10.1007/s11120-018-0481-4, 95-104. ISI **IF:3.057 (WebS), Q1,** не оглавява ранглистата

Phycobilisomes (PBSs) are supramolecular pigment–protein complexes that serve as light-harvesting antennae in cyanobacteria. They are built up by phycobiliproteins assembled into allophycocyanin core cylinders (ensuring the physical interaction with the photosystems) and phycocyanin rods (protruding from the cores and having light-harvesting function), the whole PBSs structure being maintained by linker proteins. PBSs play major role in light-harvesting optimization in cyanobacteria; therefore, the characterization of their structural integrity in intact cells is of great importance. The present study utilizes differential scanning calorimetry and spectroscopy techniques to explore for the first time, the thermodynamic stability of PBSs in intact Synechocystis sp. PCC 6803 cells and to probe its alteration as a result of mutations or under different growth conditions. As a first step, we characterize the thermodynamic behavior of intact and dismantled PBSs isolated from wild-type cells (having fully assembled PBSs) and from CK mutant cells (that lack phycocyanin rods and contain only allophycocyanin cores), and identified the thermal transitions of phycocyanin and allophycocyanin units in vitro. Next, we demonstrate that in intact cells PBSs exhibit sharp, high amplitude thermal transition at about 63 °C that strongly depends on the structural integrity of the PBSs supercomplex. Our findings implicate that calorimetry could offer a valuable approach for the assessment of the influence of variety of factors affecting the stability and structural organization of phycobilisomes in intact cyanobacterial cells.

17. Petrova N., **Todinova S.,** Paunov M., Kovacs L., Taneva S., Krumova S. Thylakoid membrane unstacking increases LHCII thermal stability and lipid phase fluidity. Journal of Bioenergetics and Biomembranes, **2018,** 50(6), 425-435. DOI:https://doi.org/10.1007/s10863-018-9783-7. **JCR- IF:2.548 (WebS), Q2**

Thylakoids are highly protein-enriched membranes that harbor a number of multicomponent photosynthetic complexes. Similarly to other biological membranes the protein constituents are heterogeneously distributed laterally in the plane of the membrane, however the specific segregation into

stacked (grana patches) and unstacked (stroma lamellae) membrane layers is a unique feature of the thylakoid. Both the lateral and the vertical arrangements of the integral membrane proteins within the three-dimensional thylakoid ultrastructure are thought to have important physiological function. In this work we explore the role of membrane stacking for the thermal stability of the photosynthetic complexes in thylakoid membranes. By means of circular dichroism and differential scanning calorimetry we demonstrate that the thermal stability of the monomeric and trimeric forms of the major light harvesting complex of photosystem II (LHCII) increases upon unstacking. This effect was suggested to be due to the detachment of LHCII from photosystem II and consequent attachment to photosystem I subunits and/or the fluidization of the lipid matrix upon unstacking. The changes in the physical properties of the protein and lipid membrane components upon unstacking result in strongly reduced photosystem II excitation energy utilization.

II. Publications under Indicator **Г7**

Abstracts of selected publications of Assoc. Prof. Svetla Todinova for participation in the concourse for the academic position "Professor"