Abstracts of Assoc. Prof. Sashka Krumova's publications for participation in the competition for the academic position of Professor

1. Todinova S. J., **Krumova S. B.,** Radoeva R., Gartcheva L., Taneva S. G., Calorimetric markers of Bence Jones and nonsecretory multiple myeloma serum proteome. Analytical Chemistry (2014) 86, 24, 12355-12361, DOI:10.1021/ac503677d. ISI IF:5.636, Q1/Q1

The present work provides a thermodynamic description of blood serum from patients diagnosed with Bence Jones myeloma (BJMM) and nonsecretory myeloma (NSMM) by means of differential scanning calorimetry (DSC), serum protein electrophoresis and free light chain assay. Specific alterations in the thermodynamic behaviour of both BJMM and NSMM proteome have been revealed. Based on the transition temperature of the main transition in the calorimetric profiles and the shape similarity criterion we defined BJMM and NSMM sets/subsets of thermograms with very similar thermodynamic features. We show that some of the BJMM and NSMM subsets correlate with previously defined secretory myeloma subsets (Todinova et al. Anal. Chem. 2011, 83, 7992). The established analogies strongly suggest that common molecular markers contribute to the calorimetric profiles of the different, secretory and nonsecretory, myeloma types; our data show robust evidence that these are ligands stabilizing the major serum proteins. We demonstrate that the DSC approach might be highly beneficent especially for NSMM patients since the characteristic modifications in the DSC profiles might serve as calorimetric markers when no monoclonal proteins can be detected in the blood stream and the diagnosis heavily relies on invasive methods.

2. Danailova A., Dzhonova D., Todinova S., Gartcheva L., Taneva S., **Krumova S.** Serum NAD(P)H fluorescence vs. serum proteome calorimetry for IgM multiple myeloma discrimination. J. BioSci. Biotechnol. (2015), SE/ONLINE: 49-53

Multiple myeloma (MM) is hematological disease with increasing incidence. Due to the development of novel tools for diagnosis and monitoring of the effect of treatment there is a significant improvement in patients' survival but there is still no cure and the nature of the disease remains elusive. Our previous works have demonstrated the potential of differential scanning calorimetry (DSC) to classify the different MM types into specific calorimetric groups and to distinguish them from healthy controls. Here we elaborate on another biophysical technique based on the fluorescent properties of NAD(P)H claimed to be useful for metabolism dysfunction detection in cancer sera. A group of patients diagnosed with MM with secretion of immunoglobulin M (IgM) paraprotein that are well characterized on basic clinical criteria was studied. Our data reveal that about half of the studied population could be distinguished on the basis of NAD(P)H fluorescence and demonstrate that the ability of NAD(P)H fluorescence approach to detect the malignant state is far lower than that of DSC.

3. **Krumova S. B.,** Todinova S. J., Danailova A., Petkova V., Dimitrova K., Gartcheva L., Taneva S. G., Calorimetric features of IgM gammopathies. Implication for patient's diagnosis and monitoring. Thermochimica Acta (2015) 615, 23-29, DOI:10.1016/j.tca.2015.07.002. ISI IF:2.184, Q1/Q2

The serum proteome of patients featuring immunoglobulin M (IgM) gammopathy and diagnosed as having IgM multiple myeloma or Waldenström's macroglobulinemia was

characterized by differential scanning calorimetry and HevyLite^{TM} (HLC) assay. The thermodynamic properties of the thermograms and their deviation from the typical healthy thermogram were found to correlate with the monoclonal protein concentration and the level of the involved (monoclonal) IgM heavy/light chains. Patients monitoring during treatment showed that the variations in the shape of the DSC profiles corresponded to fluctuations of the IgM heavy/light chain levels verifying the two parameters as non-invasive markers for disease progression.

4. Todinova S., **Krumova S.,** Andreeva T., Dimitrova K., Gartcheva L., Taneva S. G., 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 (2016) 2, 3, 416-426, DOI:doi: 10.17980/2016.416.

Differential scanning calorimetry (DSC) gains speed and success in the last decade in the characterization of blood plasma and serum. Numerous publications reveal the potential of this technique to identify calorimetric markers specific for variety of diseases and their staging. In our previous works we have clearly demonstrated that DSC can serve to classify multiple myeloma cases in a number of calorimetric groups whose thermodynamic parameters are strongly affected by the level and isotype of the secreted monoclonal immunoglobulins or free light chains (FLC). In this report we present a case of multiple myeloma with secretion of monoclonal κ FLC (stage III according to ISS classification). High FLC level (about 20% from the total protein content) was found in the patient's serum that remained persistent for the monitoring period of 1 year. The calorimetric profile of the serum revealed the occurrence of an unusual calorimetric transition at 46-47 °C, unique among nearly 500 multiple myeloma patients studied by us so far. This transition was assigned to unstable monoclonal free light chains that also led to the formation of amorphous aggregates (imaged by atomic force microscopy) in the patient's serum. Additional studies of patients with similar calorimetric features are needed in order to relate the emergence of the 47 °C transition and protein aggregation to the disease activity status of multiple myeloma or to other pathology.

5. Danailova A., Todinova S. J., Dimitrova K., Petkova V., Guenova M., Mihaylov G., Gartcheva L., **Krumova S.,** Taneva S. G., Effect of autologous stem-cells transplantation of patients with multiple myeloma on the calorimetric markers of the serum proteome. Correlation with the immunological markers. Thermochimica Acta (2017) 655, 351-357, DOI:http://dx.doi.org/10.1016/j.tca.2017.08.001. ISI IF:2.189, Q2/Q2

The thermodynamic stability of biofluids is currently extensively studied by means of differential scanning calorimetry (DSC), a biophysical technique that measures thermally induced conformational transitions of biomolecules in solution. In this work we utilize the calorimetric approach to monitor myeloma patients after autologous stem cell transplantation. The thermodynamic parameters determined from the calorimetric profiles of blood sera collected before and at different periods of time after the transplantation were compared with the variation in the levels of the secreted paraproteins, monoclonal free light chains and involved heavy/light chains searching for correlations between the calorimetric markers and the immunological indicators of prognosis prediction and monitoring response. We established that the change in the paraprotein level and thus the patient's clinical status is

clearly reflected in the serum thermogram. Our study proves the potential of microcalorimetry as a non-invasive tool for patients′ monitoring after autologous stem cell transplantation.

6. **Krumova S.,** Todinova S., Mavrov D., Marinov P., Atanassova V., Atanassov K., Taneva S. G., Intercriteria analysis of calorimetric data of blood serum proteome. Biochimica et Biophysica Acta - General Subjects (2017) 1861, 409-417, DOI:dx.doi.org/10.1016/j.bbagen.2016.10.012. ISI IF:3.679, Q1/Q1

BACKGROUND: Biological microcalorimetry has entered into a phase where its potential for disease diagnostics is readily recognized. A wide variety of oncological and immunological disorders have been characterized by differential scanning calorimetry (DSC) and characteristic thermodynamic profiles were reported. Now the challenge before DSC is not the experimental data collection but the development of analysis protocols for reliable data stratification/classification and discrimination of disease specific features (calorimetric markers).

METHODS: In this work we apply InterCriteria Analysis (ICA) approach combined with Pearson's and Spearman's correlation analysis to a large dataset of calorimetric and biochemical parameters derived for the serum proteome of patients diagnosed with multiple myeloma (MM).

RESULTS: We have identified intercriteria dependences that are general for the various types of MM and thus can be regarded as a characteristic of this largely heterogeneous disease: strong contribution of the monoclonal (M) protein concentration to the excess heat capacity of the immunoglobulins-assigned thermal transition; shift of the albumin assigned calorimetric transition to allocation where it overlaps with the globulins assigned transition and strong shift of the globulins assigned transition temperature attributable to M proteins conformational changes.

CONCLUSIONS: Our data justify the applicability of ICA for deciphering of the complex thermodynamic behavior of the MM blood serum proteome.

GENERAL SIGNIFICANCE: The applied approach is suitable for more general application in the analysis of biocalorimetric data since it can help identify the biological relevance of the distinguished thermodynamic features observed for variety of diseases.

7. Todinova S., **Krumova S.,** Danailova A., Petkova V., Guenova M., Mihaylov G., Gartcheva L., Taneva S. G., Calorimetric markers for monitoring of multiple myeloma and Waldenstrom's macroglobulinemia patients. European Biophysics Journal with Biophysics Letters (2018) 47, 5, 549-559, DOI:10.1007/s00249-018-1277-3, ISI IF:1.472, Q2/Q3

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.

8. 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. https://doi.org/10.1016/j.tca.2018.07.005, ISI IF:2.189, Q2/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.

9. **Krumova S. B.,** Rukova B., Todinova S. J., Gartcheva L., Milanova V., Toncheva D., Taneva S. G., Calorimetric monitoring of the serum proteome in schizophrenia patients. Thermochimica Acta (2013) 572, 59-64, https://doi.org/10.1016/j.tca.2013.09.015, IF:2.105, Q2/Q2

Schizophrenia (Sz) is a multifactorial mental disorder with high frequency. Due to its chronic and relapsing nature there is a strong need for biomarkers for early psychosis detection and objective evaluation of drug (usually antipsychotics) treatment effect. Here differential scanning calorimetry (DSC) is applied to thermodynamically characterize the blood serum proteome of paranoid schizophrenia patients on routine antipsychotic treatment in comparison to healthy controls. DSC revealed significant modifications in the thermodynamic behavior of blood sera from Sz patients, the overall thermal profile being changed in all Sz cases under study. The calorimetric profiles were classified in four distinct groups, reflecting different thermal stabilization of the high-abundance portion of the serum proteome. The observed positive (thermograms becoming closer to the healthy profile) or negative (thermograms deviating stronger from the healthy profile) proteome thermal stability switches and the Sz thermograms persistence in patients' follow-up corresponded well with the effect of drug treatment.

10. Dobrikova A., Vladkova R., Rashkov G., Todinova S. J., **Krumova S. B.,** Apostolova E., Effects of exogenous 24-epibrassinolide on the photosynthetic membranes under non-stress conditions. Plant Physiology and Biochemistry (2014) 80, 75 - 82. doi: 10.1016/j.plaphy.2014.03.022, IF:2.756, Q1/Q1

In the present work the effects of exogenous 24-epibrassinolide (EBR) on functional and structural characteristics of the thylakoid membranes under non-stress conditions were evaluated 48 h after spraying of pea plants with different concentrations of EBR (0.01, 0.1 and 1.0 mg.L^{-1}). The results show that the application of 0.1 mg.L^{-1} EBR has the most pronounced effect on the studied characteristics of the photosynthetic membranes. The observed changes in 540 nm light scattering and in the calorimetric transitions suggest alterations in the structural organization of the thylakoid membranes after EBR treatment, which in turn influence the kinetics of oxygen evolution, accelerate the electron transport rate, increase the effective quantum yield of photosystem II and the photochemical quenching. The EBRinduced changes in the photosynthetic membranes are most probably involved in the stress tolerance of plants.

11. **Krumova S. B.,** Varkonyi Zs., Lambrev P. H., Kovacs L., Todinova S. J., Busheva M., Taneva S. G., Garab, G., Heat- and light-induced detachment of the light-harvesting antenna complexes of photosystem I in isolated stroma thylakoid membranes. Journal of Photochemistry and Photobiology B (2014), 137, 4 - 12. https://doi.Org/10.1016/j.jphotobiol.2014.04.029, IF:2.96, Q1/Q2

The multisubunit pigment–protein complex of photosystem I (PSI) consists of a core and peripheral light-harvesting antenna (LHCI). PSI is thought to be a rather rigid system and very little is known about its structural and functional flexibility. Recent data, however, suggest LHCI detachment from the PSI supercomplex upon heat and light treatments. Furthermore, it was suggested that the splitting off of LHCI acts as a safety valve for PSI core upon photoinhibition (Alboresi et al., 2009). In this work we analyzed the heat- and light-induced reorganizations in isolated PSI vesicles (stroma membrane vesicles enriched in PSI). Using differential scanning calorimetry we revealed a stepwise disassembly of PSI supercomplex above 50 °C. Circular dichroism, sucrose gradient centrifugation and 77 K fluorescence experiments identified the sequence of events of PSI destabilization: 3 min heating at 60 °C or 40 min white light illumination at 25 °C resulted in pronounced Lhca1/4 detachment from the PSI supercomplex, which is then followed by the degradation of Lhca2/3. The similarity of the main structural effects due to heat and light treatments supports the notion that thermo-optic mechanism, structural changes induced by ultrafast local thermal transients, which has earlier been shown to be responsible for structural changes in the antenna system of photosystem II, can also regulate the assembly and functioning of PSI antenna.

12. Andreeva T., **Krumova S. B.,** Minkov I. L., Busheva M., Lalchev Z., Taneva S. G., Protonation-induced changes in the macroorganization of LHCII monolayers. Colloids and Surfaces A: Physicochemical and Engineering Aspects (2014) 460, 196 - 203. https://doi.org/10.1016/j.colsurfa.2013.12.044, IF:2.752, Q2/Q2

The major light-harvesting complex of photosystem II (LHCII) is an important regulatory

protein in photosynthetic membranes. In vivo LHCII forms stable trimers and is found either associated to photosystem II or in LHCII-only containing domains. It was suggested that in native thylakoid membrane LHCII changes its conformation and macroorganization upon switching from light-harvesting to photoprotective state. Herein we have analyzed LHCII Langmuir monolayers at different subphase salt compositions and in two different states − partly deprotonated (LHCII), at low basic pH 7.8, and highly protonated (p-LHCII), at pH 5.2, mimicking the functional light-harvesting and light-protective states of the protein, respectively. We have found strong difference in the supramolecular organization of the protein in these two functional states, the protonated monolayer exhibiting higher order of organization and significantly higher stability compared to the partly deprotonated one. Both LHCII and p-LHCII monolayers are composed of trimers self-assembling in aggregates with different packing density − loosely packed compiling homogeneous well-ordered monolayer areas and tightly packed organized in heterogeneous disordered phase. These two types of macroorganization are found in different proportions in protonated and partly deprotonated LHCII monolayers, the p-LHCII monolayer being much more heterogeneous than LHCII one.

13. Stoichev S., **Krumova S. B.,** Andreeva T., Busto J. V., Todinova S., Balashev K., Busheva M., Goni F. M., Taneva S. G., Low pH modulates the macroorganization and thermal stability of PSII supercomplexes in grana membranes. Biophysical Journal (2015) 108, 4, 844 - 853. doi: 10.1016/j.bpj.2014.12.042, IF:3.632, Q1/Q1

Protonation of the lumen-exposed residues of some photosynthetic complexes in the grana membranes occurs under conditions of high light intensity and triggers a major photoprotection mechanism known as energy dependent nonphotochemical quenching. We have studied the role of protonation in the structural reorganization and thermal stability of isolated grana membranes. The macroorganization of granal membrane fragments in protonated and partly deprotonated state has been mapped by means of atomic force microscopy. The protonation of the photosynthetic complexes has been found to induce largescale structural remodeling of grana membranes—formation of extensive domains of the major light-harvesting complex of photosystem II and clustering of trimmed photosystem II supercomplexes, thinning of the membrane, and reduction of its size. These events are accompanied by pronounced thermal destabilization of the photosynthetic complexes, as evidenced by circular dichroism spectroscopy and differential scanning calorimetry. Our data reveal a detailed nanoscopic picture of the initial steps of nonphotochemical quenching.

14. Andreeva T., Castano S., **Krumova S.,** Lecomte S., Taneva S., Effect of protonation on the secondary structure and orientation of plant light harvesting complex II studied by PM-IRRAS, Langmuir (2015), 31, 42, 11583 - 11590. doi: 10.1021/acs.langmuir.5b02653, IF:3.993, Q1/Q1

The major light-harvesting pigment−protein complex of photosystem II, LHCII, has a crucial role in the distribution of the light energy between the two photosystems, the efficient light capturing and protection of the reaction centers and antennae from overexcitation. In this work direct structural information on the effect of LHCII protonation, which mimics the switch from light-harvesting to photoprotective state of the protein, was revealed by polarization-modulated infrared reflection−absorption spectroscopy (PMIRRAS). PMIRRAS on LHCII monolayers verified that the native helical structure of the protein is preserved in both partly deprotonated (pH 7.8, LHCII) and protonated (pH 5.2, p-LHCII) states. At low surface pressure, 10 mN/m, the orientation of the α-helices in these two LHCII states is

different - tilted (θ ≈ 40°) in LHCII and nearly vertical (θ ≈ 90°) in p-LHCII monolayers; the partly deprotonated complex is more hydrophilic than the protonated one and exhibits stronger intertrimer interactions. At higher surface pressure, 30 mN/m, which is typical for biological membranes, the protonation affects neither the secondary structure nor the orientation of the transmembrane α-helices (tilted ~45° relative to the membrane surface in both LHCII states) but weakens the intermolecular interactions within and/or between the trimers.

15. **Krumova S.,** Todinova S., Tileva M., Bouzhir-Sima L., Vos M.H., Liebl U., Taneva S G., Thermal stability and binding energetics of thymidylate synthase ThyX, International Journal of Biological Macromolecules (2016) 91, 560-567. DOI: 10.1016/j.ijbiomac.2016.05.083, IF:3.671, Q2/Q1

The bacterial thymidylate synthase ThyX is a multisubstrate flavoenzyme that takes part in the de novo synthesis of thymidylate in a variety of microorganisms. Herein we study the effect of FAD and dUMP binding on the thermal stability of wild type (WT) ThyX from the mesophilic Paramecium bursaria chlorellavirus-1 (PBCV-1) and from the thermophilic bacterium Thermotoga maritima (TmThyX), and from two variants of TmThyX, Y91F and S88W, using differential scanning calorimetry. The energetics underlying these processes was characterized by isothermal titration calorimetry. The PBCV-1 protein is significantly less stable against the thermal challenge than the TmThyX WT. FAD exerted stabilizing effect greater for PBCV-1 than for TmThyX and for both mutants, whereas binding of dUMP to FAD-loaded proteins stabilized further only TmThyX. Different thermodynamic signatures describe the FAD binding to the WT ThyX proteins. While TmThyX binds FAD with a low μM binding affinity in a process characterized by a favorable entropy change, the assembly of PBCV-1 with FAD is governed by a large enthalpy change opposed by an unfavorable entropy change resulting in a relatively strong nM binding. An enthalpy-driven formation of a high affinity ternary ThyX/FAD/dUMP complex was observed only for TmThyX.

16. Danova K., Motyka V., Todorova M., Trendafilova A., **Krumova S.,** Dobrev P., Andreeva T., Oreshkova T., Taneva S., Evstatieva L., Effect of cytokinin and auxin treatments on morphogenesis, terpenoid biosynthesis, photosystem structural organization, and endogenous isoprenoid cytokinin profile in Artemisia alba Turra in vitro. Journal of Plant Growth Regulation (2017) 37, 2, 403-418, doi: 10.1007/s00344-017-9738-y, IF:2.073, Q1/Q2

Developmental pattern modification in essential oil bearing *Artemisia alba* Turra was obtained by exogenous plant growth regulator (PGRs) treatments in vitro. Enhanced rooting (in PGR-free and auxin-treated plants) led to elevation of the monoterpenoid/sesquiterpenoid ratio in the essential oils of aerials. On the contrary, root inhibition and intensive callusogenesis [combined cytokinin (CK) and auxin treatments] reduced this ratio more than twice, significantly enhancing sesquiterpenoid production. Both morphogenic types displayed sesquiterpenoid domination in the underground tissues, which however differed qualitatively from the sesquiterpenoids of the aerials, excluding the hypothesis of their shoot-to-root translocation and implying the possible role of another signaling factor, affecting terpenoid biosynthesis. Inhibited rooting also resulted in a significant drop of endogenous isoprenoid CK bioactive-free bases and ribosides as well as CK *N*-glycoconjugates and in decreased *trans*-zeatin (*trans*Z):*cis*-zeatin (*cis*Z) ratio in the

aerials. Marked impairment of the structural organization of the photosynthetic apparatus and chloroplast architecture were also observed in samples with suppressed rooting. It is well known that in the plant cell monoterpenoid and *trans*Z-type CKs biogenesis are spatially bound to plastids, while sesquiterpenoid and *cis*Z production are compartmented in the cytosol. In the present work, interplay between the biosynthesis of terpenoids and CK bioactive free bases and ribosides in *A. alba* in vitro via possible moderation of chloroplast structure has been hypothesized.

17. Todinova S., Stoyanova E., **Krumova S.,** Iliev I., Taneva S.G., Calorimetric signatures of human cancer cells and their nuclei, Thermochimica Acta (2016) 623, 95-101, https://doi.org/10.1016/j.tca.2015.11.002, IF: 2.236, Q2/Q2

The human cancer cell lines HeLa, JEG-3, Hep G2, SSC-9, PC-3, HT-29, MCF7 and their isolated nuclei were characterized by differential scanning calorimetry. The calorimetric profiles differed from normal human fibroblast (BJ) cells in the two well distinguished temperature ranges—the high-temperature range (H_T, due to DNA-containing structures) and the low-temperature range (L_T, assigned to the nuclear matrixand cellular proteins). The enthalpy of the L_T range, and, respectively the ratio of the enthalpies of the L_T-vs. H_T-range, $\Delta H_L/\Delta H_H$, is strongly reduced for all cancer cells compared to normal fibroblasts. On the contrary, for most of the cancer nuclei this ratio is higher compared to normal nuclei. The H_T-29 human colorectal cancer cells/nuclei differed most drastically from normal human fibroblast cells/nuclei. Our data also reveal that the treatment of HT-29 cancer cells with cytostatic drugs affect snot only the DNA replication but also the cellular proteome.

18. Dinarelli S, Longo G., **Krumova S.,** Todinova S., Danailova A., Taneva S., Lenzi E., Mussi V., Girasole M., Insights into the morphological pattern of erythrocytes' aging: Coupling quantitative AFM data to microcalorimetry and Raman spectroscopy. Journal of Molecular Recognition (2018) 31, 11, e2732, DOI:10.1002/jmr.2732, ISI IF:1.868, Q3/Q3

Erythrocytes (RBCs) constitute a very interesting class of cells both for their physiological function and for a variety of peculiarities. Due to their exceptionally strong relationship with the environment, the morphology and nanoscale characteristics of these cells can reveal their biochemical status and structural integrity. Among the possible subjects of investigations, the RBCs' ageing is of the utmost importance. This is a fundamental phenomenon that, in physiological conditions, triggers the cell turnover and ensures the blood homeostasis. With these premises, in recent years, we have presented an atomic force microscopy–based methodology to characterize the patterns of RBC ageing from the morphological point of view. In the present work, we used an ageing protocol more similar to the physiological conditions and we used differential scanning calorimetry and atomic force microscopy to probe the cross correlation between important structural and functional proteins. We also assessed the role played by fundamental structural and membrane proteins in the development of the most relevant morphological intermediates observed along the ageing. Furthermore, we coupled the morphological ageing patterns to the (bio)chemical alterations detected by Raman spectroscopy. This allowed identifying the chronology of the ageing morphologies and the metabolic pathways most involved in their development. As a whole, the present study provides the base to correlate specific molecular alterations to the development of structural anomalies, and these latter to the functional status of blood cells.

19. 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, ISI IF:2.914, Q2/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 threedimensional 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.

20. 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. Photosynthesis Research (2018) 137, 1, 95-104, DOI:https://doi.org/10.1007/s11120-018-0481-4. ISI IF:3.091, Q1/Q1

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.

21. Petrova N., Koleva P., Velikova V., Tsonev T., Andreeva T., Taneva S., **Krumova S.,** Danova K., Relations between photosynthetic performance and polyphenolics productivity of Artemisia alba Turra in in vitro tissue cultures. International Journal of Bioautomation (2018) 1, 73-82, DOI:10.7546/ijba.2018.22.1.73-82. SJR:0.23, Q3

Establishing optimal growth conditions for secondary metabolites production in vitro is vital for the biotechnological development of medicinal plants. In the present work we investigate the relations between the supplementation of plant growth regulators (benzyl adenine and indole-3-butyric acid) to in vitro shoot cultures of the medicinal plant Artemisia alba Turra, the productivity of antioxidant polyphenolic compounds and the structural and functional characteristics of the photosynthetic apparatus. We assayed the structural characteristics of isolated thylakoid membranes from the aerial parts by means of circular dichroism spectroscopy and atomic force microscopy, and the photosynthetic performance by pulse amplitude fluorescence modulated imaging.

Although a complex relationship between benzyl adenine and indole-3-butyric acid supplementation, the polyphenolic levels and the architecture and functionality of the photosynthetic thylakoid membranes was revealed, a clear correlation was established between the concentration of the produced polyphenolic compounds and the quantum yield of photosystem II. Our data demonstrate that there is an optimal combination of the applied plant growth regulators that triggers efficient photosynthesis and high phenolics production.