# РЕЗЮМЕТА на публикациите на английски език

Референциите на публикациите и техните резюмета са подредени съгласно списъка с публикациите на кандидата.

**1. R Tsekovska, E Gatev, R Mironova, S Kerezieva, S Ilieva, T Ilieva, B Vasileva, T Niwa, D Popova, V Vasilev. Serum levels of $N^{\varepsilon}$-(carboxymethyl)-lysine in chronic kidney disease and diabetes. *Biomedicines* (2025) 13(7), 1672.**


## ABSTRACT

Background: $N^{\varepsilon}$-(carboxymethyl)-lysine (CML) is formed in the human body by non-enzymatically driven reactions including glycation, oxidation, and lipoxidation. CML is aubiquitous product of normal physiology, but its levels are increased under disease conditions like chronic kidney disease (CKD) and diabetes mellitus (DM). Free CML is eliminated from the human body mainly through kidney excretion, and its accumulation in the kidney tissue is linked to CKD pathogenesis. Aim: The main goal of this study was to evaluate the relative contribution of CKD and Type 2 DM (T2DM) to the accumulation of CML in patients' sera. Methods: The study included 22 patients with CKD without DM, 55 with CKD and comorbid T2DM, and 21 with T2DM without CKD. Serum CML levels were measured by ELISA. The Kruskal-Wallis test was used to detect differences among groups. Spearman correlation analysis was performed, and the one-tailed Dunn test was considered to indicate statistical significance at $p < 0.05$. Results: The median serum CML levels (CKD, 658.4 ± 434.3 ng/mL; CKD + T2DM, 431.3 ± 327.9 ng/mL; T2DM, 273.9 ± 134.2 ng/mL) differed significantly ($p < 0.05$) among the three patient groups. A positive correlation was observed between serum CML and microalbuminuria ($p = 0.004$; r = 0.58), proteinuria ($p = 0.002$; r = 0.6), and age ($p = 0.007$; r = 0.52) only in the CKD patients. In all T2DM patients, independent of CKD status, serum CML correlated negatively ($p < 0.05$) with postprandial glucose and duration of diabetes, while its correlation with fasting glucose and HbA1c was negative only in the T2DM cohort without CKD. Conclusions: In patients with CKD, higher levels of CML were observed compared to those with T2DM. Serum CML correlated positively with proteinuria, albuminuria, and patient age in non-diabetic CKD patients, and negatively with blood glucose, HbA1c, and DM duration of T2DM in patients without CKD.


**2. B Vasileva, E Gatev, A Aleksandrov, S Kerezieva, S Ilieva, D Popova, V Vasilev, T Niwa, R Mironova, B Deliyska, R Tsekovska. Serum fructosamine as a marker for glycemic control in patients with diabetic nephropathy. *In Proceedings of the Bulgarian Academy of Sciences* (2024) 77(3): 442-450.**


## ABSTRACT

Diabetes mellitus (DM) is a metabolic disease of chronic insulin deficiency or resistance. The progression of DM is associated with long-term damage to macro- and micro-vascular body systems and causes serious health complications. Up to 40% of DM patients develop chronic kidney disease (CKD), mainly as a consequence of diabetic nephropathy (DN). Hence, strict glycemic control is recommended to slow down CKD progression. Hyperglycemia causes complications in diabetic patients through the glycation of various proteins. The interaction of glucose with the free $NH_2$ groups of proteins results in the formation of the early glycation

product fructosamine (FA). FA has a short half-life (1 to 2 weeks) and hence it has the potential of an early marker for glycemic control. Although long used in the clinical practice, the FA diagnostic and prognostic significance remains questionable. In this study, we tested the FA potential to serve as a glycemic marker in DM patients with DN. We found that DM patients have significantly higher serum FA levels than non-diabetics. Further, the serum FA level in diabetics correlates positively with the blood glucose concentration. Finally, the mean serum FA level was higher in DM patients with DN, compared to those without DN. These results establish FA as a promising marker for glycemic control in DN patients.

**3. E Boteva, K Doychev, K Kirilov, Y Handzhiyski, R Tsekovska, E Gatev, R Mironova. Deglycation activity of the Escherichia coli glycolytic enzyme phosphoglucose isomerase. *International Journal of Biological Macromolecules* (2024) 257(1), 128541.**

**ABSTRACT**

Glycation is a spontaneous chemical reaction, which affects the structure and function of proteins under normal physiological conditions. Therefore, organisms have evolved diverse mechanisms to combat glycation. In this study, we show that the *Escherichia coli* glycolytic enzyme phosphoglucose isomerase (Pgi) exhibits deglycation activity. We found that *E. coli* Pgi catalyzes the breakdown of glucose 6-phosphate (G6P)-derived Amadori products (APs) in chicken lysozyme. The affinity of Pgi to the glycated lysozyme ($K_m$, 1.1 mM) was ten times lower than the affinity to its native substrate, fructose 6-phosphate ($K_m$, 0.1 mM). However, the high kinetic constants of the enzyme with the glycated lysozyme ($k_{cat}$, 396 $s^{-1}$ and $k_{cat}/K_m$, 3.6 × 105 $M^{-1}$ $s^{-1}$) indicated that the Pgi amadoriase activity may have physiological implications. Indeed, when using total *E. coli* protein (20 mg/mL) as a substrate in the deglycation reaction, we observed a release of G6P from the bacterial protein at a Pgi specific activity of 33 μmol/min/mg. Further, we detected 11.4 % lower APs concentration in protein extracts from Pgi-proficient *vs*. deficient cells ($p$ = 0.0006) under conditions where the G6P concentration in Pgi-proficient cells was four times higher than in Pgi-deficient cells ($p$ = 0.0001). Altogether, these data point to physiological relevance of the Pgi deglycation activity.

**4. N Jeliazkova, E Longhin, N Yamani, E Rundén-Pran, E Moschini, T Serchi, I Vrček, M Burgum, S Doak, M Cimpan, I Rios-Mondragon, E Cimpan, C Battistelli, C Bossa, R Tsekovska, D Drobne, S Novak, N Repar, A Ammar, P Nymark, V Battista, A Sosnowska, T Puzyn, N Kochev, L Iliev, V Jeliazkov, K Reilly, I Lynch, M Bakker, C Delpivo, A Jiménez, AS Fonseca, N Manier, ML Fernandez-Cruz, S Rashid, E Willighagen, MD Apostolova, M Dusinska. A template wizard for the cocreation of machine-readable data-reporting to harmonize the evaluation of (nano)materials. *Nature Protocols* (2024) 16 May, 1-43.**

**ABSTRACT**

Making research data findable, accessible, interoperable and reusable (FAIR) is typically hampered by a lack of skills in technical aspects of data management by data generators and a lack of resources. We developed a Template Wizard for researchers to easily create templates suitable for consistently capturing data and metadata from their experiments. The templates are easy to use and enable the compilation of machine-readable metadata to accompany data generation and align them to existing community standards and databases, such as

eNanoMapper, streamlining the adoption of the FAIR principles. These templates are citable objects and are available as online tools. The Template Wizard is designed to be user friendly and facilitates using and reusing existing templates for new projects or project extensions. The wizard is accompanied by an online template validator, which allows self-evaluation of the template (to ensure mapping to the data schema and machine readability of the captured data) and transformation by an open-source parser into machine-readable formats, compliant with the FAIR principles. The templates are based on extensive collective experience in nanosafety data collection and include over 60 harmonized data entry templates for physicochemical characterization and hazard assessment (cell viability, genotoxicity, environmental organism dose-response tests, omics), as well as exposure and release studies. The templates are generalizable across fields and have already been extended and adapted for microplastics and advanced materials research. The harmonized templates improve the reliability of interlaboratory comparisons, data reuse and meta-analyses and can facilitate the safety evaluation and regulation process for (nano) materials.

**5. E Boteva, K Doychev, R Tsekovska, Y Handzhyski, I Ivanov, R Mironova. Protein amadoriase activity of the *Escherichia coli* K-12 glycolytic enzyme phosphoglucose isomerase. *In Proceedings of the Bulgarian Academy of Sciences* (2023), 76(1): 56-64.**

**ABSTRACT**

The Maillard reaction (glycation) is a spontaneous non-enzymatic reaction between primary amines and carbonyl compounds, which affects proteins and DNA of both pro- and eukaryotes. In recent studies, we have shown that the glycolytic enzyme of *Escherichia coli* phosphoglucose isomerase (PGI) catalyzes in vitro the deglycation of DNA modified with glucose 6-phosphate (G6P)-derived Amadori products (APs). APs are early products of the Maillard reaction, which are formed not only on DNA but also on other amines including proteins. The aim of the current study was to test the *E. coli* PGI for protein deglycation (amadoriase) activity. To this end, we used chicken lysozyme glycated with G6P as a model protein. Treatment of the glycated lysozyme with protein extract from an *E. coli* PGI proficient but not deficient strain resulted in the release of G6P, which was indicative of PGI protein amadoriase activity. G6P-derived APs represent fructose 6-phosphate (F6P) residues bound to free amino groups of the model protein and because of that we compared the kinetic constants of the *E. coli* PGI for the glycated lysozyme and for free F6P. PGI demonstrated nearly two times higher affinity to the glycated lysozyme ($K'_m$ = 0.06 mM) than to free F6P ($K'_m$ = 0.1 mM). However, the apparent catalytic constant of the enzyme with the glycated lysozyme ($K'_{cat}$ = 93 $s^{-1}$) was eight times lower than with F6P ($K'_{cat}$ = 736 $s^{-1}$). Future studies are expected to shed light on the physiological relevance of the PGI protein amadoriase activity we report here.

**6. R Tsekovska, K Kirilov, A Sredovska–Bozhinov, E Gatev, I Ivanov, T Niwa, R Mironova, Y Handzhyiski. Antiglycation properties of resveratrol and glucosamine. *Journal of Chemical Technology and Metallurgy* (2023), 58(2): 270-274.**

**ABSTRACT**

The non-enzymatic glycosylation (glycation) is a natural process occurring in living organisms, which is associated with aging and diabetic complications. This non-enzymatic reaction affects not only long-lived proteins, such as eye crystalline, but also DNA, RNA and aminolipids. The

long-standing search for substances that can inhibit this process is still on-going. In this study we examined the effect of two natural compounds, resveratrol and glucosamine, on the formation of early and fluorescent advanced glycation end products (AGEs). We found that resveratrol inhibited formation of fluorescent AGEs in bovine serum albumin (BSA) glycated with D-ribose, with inhibition percentage of 80 %, while glucosamine inhibited the formation of the same products in the same test system only by 4 % compared to a control sample of glycated BSA. Both test compounds, resveratrol and glucosamine, did not inhibit the formation of early glycation products in the glycated BSA. Our results showed that resveratrol has strong antiglycation activity in contrast to glucosamine demonstrating very low antiglycation potential.

**7. R Tsekovska, K Kirilov, E Gatev, R Mironova, Y Handzhyiski. Investigation of purine compounds for anti-glycation activity *in vitro*. *Journal of Chemical Technology and Metallurgy* (2022), 57 (1): 19-24.**

**ABSTRACT**

Non-enzymatic glycosylation (glycation) is a common process for all living organisms. It is associated with the accumulation of glycation products in proteins, amino lipids and DNA, potentially leading to disruption of their biological functions. Therefore different substances have been interrogated for any anti-glycation activity. Some of these substances, like aminoguanidine, were found to be potent glycation inhibitors in vitro/ex vivo and in animal models. However, due to side effects of aminoguanidine, phase II clinical trials of this drug have been canceled. In this study, we investigated the impact of six purine compounds (theobromine, theophylline, caffeine, xanthine, hypoxanthine and uric acid) on the formation of fluorescent glyation products, as well as on the covalent aggregation of bovine serum albumin (BSA) glycated with D-ribose in vitro. Maximum inhibitory activity (ca. 50% inhibition) was achieved with theophylline and xanthine, followed by theobromine and hypoxanthine. Caffeine had neutral effect on glycation, whereas uric acid exhibited clear pro-glycation activity. It enhanced the formation of fluorescent adducts in BSA up to 125 % of baseline control and enhanced the covalent aggregation of that protein.

**8. S Engibarov, R Tsekovska, K Kirilov, E Gatev, I Ivanov, R Mironova, Y Handzhyiski. Deoxyribonuclease activity of allenic acids. *Journal of Chemical Technology and Metallurgy* (2022), 57(2): 291-297.**

**ABSTRACT**

Non-enzymatic cleavage of nucleic acids (DNA or RNA) is essential for developing novel antibiotics and chemotherapeutics, as well as new gene manipulation techniques. In this study, we investigated for deoxyribonuclease activity of the allenic acids 2-methyl-4-phenylhexa-2,3-dienoic acid (racemic mixture, AK-1), S-2-methyl-4-phenylbuta-2,3-dienoic acid (AK-2) and R-2-methyl-4-phenylbuta-2,3-dienoic acid (AK-3) using supercoiled plasmid pUC18 DNA as a substrate. All three allenic acids demonstrated single strand deoxyribonuclease activity in the order AK2 ≥ AK3 > AK1. Increasing concentrations of potassium-phosphate buffer (pH 7.5) in the reaction mixture completely inhibited the relaxation of the supercoiled pUC18 DNA, thus implying that the phosphodiester bond in DNA is the vulnerable site of attack by the allenic acids. When plasmid pUC18 DNA nicked with allenic acids was treated with T4 DNA ligase, it failed to regain its covalently closed circular form. This indicates that the allenic acids act as

either hydrolytic agents producing 3'-$PO_4$ and 5'-OH termini, or oxidizers, damaging DNA by free radical mechanism.

**9. AS Jiménez, R Puelles, M Pérez-Fernández, L Barruetabeña, N Raun Jacobsen, B Suarez-Merino, C Micheletti, N Manier, B Salieri, R Hischier, R Tsekovska, Y Handzhiyski, J Bouillard, Y Oudart, KS Galea, S Kelly, N Shandilya, H Goede, J Gomez-Cordon, KA Jensen, Martie van Tongeren, MD Apostolova, I Rodriguez-Llopis. Safe(r) by design guidelines for the nanotechnology industry. *NanoImpac*t (2022), 25: 100385.**

**ABSTRACT**

Expectations for safer and sustainable chemicals and products are growing to comply with the United Nations and European strategies for sustainability. The application of Safe(r) by Design (SbD) in nanotechnology implies an iterative process where functionality, human health and safety, environmental and economic impact and cost are assessed and balanced as early as possible in the innovation process and updated at each step. The EU H2020 NanoReg2 project was the first European project to implement SbD in six companies handling and/or manufacturing nanomaterials (NMs) and nano-enabled products (NEP).

The results from this experience have been used to develop these guidelines on the practical application of SbD. The SbD approach foresees the identification, estimation, and reduction of human and environmental risks as early as possible in the development of a NM or NEP, and it is based on three pillars: (i) safer NMs and NEP; (ii) safer use and end of life and (iii) safer industrial production. The presented guidelines include a set of information and tools that will help deciding at each step of the innovation process whether to continue, apply SbD measures or carry out further tests to reduce uncertainty. It does not intend to be a prescriptive protocol where all suggested steps have to be followed to achieve a SbD NM/NEP or process. Rather, the guidelines are designed to identify risks at an early state and information to be considered to identify those risks. Each company adapts the approach to its specific needs and circumstances as company decisions influence the way forward.

**10. R Tsekovska, R Mironova, I Ivanov. Protein glycosylation in bacteria. *C R Acad Bulg Sci* (2021), 74(4): 467-487.**

**ABSTRACT**

Glycosylation can be generally defined as attachment of carbohydrate residues to biomolecules such as proteins, nucleic acids, etc. There are two main types of glycosylation in living systems – enzymatic and non-enzymatic. The latter type is called also glycation or the Maillard reaction. In the current review, the term "glycosylation" is used to designate the enzymatic glycosylation and "glycation" to denote the non-enzymatic process. The two types of glycosylation differ by the involvement (or not) of enzyme catalysts. The main difference between them, however, relates to their physiological significance. The enzymatic catalysis itself implies a genetically encoded process, which is important for the structure, stability and function of the biomolecules. In contrast, glycation is a spontaneous, yet unavoidable chemical reaction, compromising the structure and function of the target molecules. It has long been believed that both processes, glycosylation and glycation, do occur in eukaryotes only. The reason for this is that prokaryotes are devoid of organelles that carry out glycosylation, and their short life span does not allow glycation to occur. However, the discovery of glycosylated proteins in Archaea and Bacteria in

1976 as well as the discovery of non-enzymatic glycosylation in Escherichia coli in our laboratory in 2001, have refuted these beliefs. This review is dedicated to the enzymatic type of protein glycosylation in bacteria.

**11. A Giusti, R Atluri, R Tsekovska, A Gajewicz, MD Apostolova, CL Battistelli, EAJ Bleeker, C Bossa, J Bouillard, M Dusinska, P Gómez-Fernández, R Grafström, M Gromelski, Y Handzhiyski, N Raun Jacobsen, P Jantunen, K Alstrup J, A Mech, J Maria Navas, P Nymark, AG Oomen, T Puzyn, K Rasmussen, C Riebeling, I Rodriguez-Lopis, S Sabella, JR Sintes, B Suarez-Merino, S Tanasescu, H Wallin, A Haase. Nanomaterial grouping: Existing approaches and future recommendations. *NanoImpact* (2019), 16: 100182.**

**ABSTRACT**

The physico-chemical properties of manufactured nanomaterials (NMs) can be fine-tuned to obtain different functionalities addressing the needs of specific industrial applications. The physico-chemical properties of NMs also drive their biological interactions. Accordingly, each NM requires an adequate physico-chemical characterization and potentially an extensive and time-consuming (eco)toxicological assessment, depending on regulatory requirements. Grouping and read-across approaches, which have already been established for chemicals in general, are based on similarity between substances and can be used to fill data gaps without performing additional testing. Available data on "source" chemicals are thus used to predict the fate, toxicokinetics and/or (eco)toxicity of structurally similar "target" chemical(s). For NMs similar approaches are only beginning to emerge and several challenges remain, including the identification of the most relevant physico-chemical properties for supporting the claim of similarity. In general, NMs require additional parameters for a proper physico-chemical description. Furthermore, some parameters change during a NM's life cycle, suggesting that also the toxicological profile may change.

This paper compares existing concepts for NM grouping, considering their underlying basic principles and criteria as well as their applicability for regulatory and other purposes. Perspectives and recommendations based on experiences obtained during the EU Horizon 2020 project NanoReg2 are presented. These include, for instance, the importance of harmonized data storage systems, the application of harmonized scoring systems for comparing biological responses, and the use of high-throughput and other screening approaches. We also include references to other ongoing EU projects addressing some of these challenges.

**12. A Mech, K Rasmussen, P Jantunen, L Aicher, M Alessandrelli, U Bernauer, EAJ Bleeker, J Bouillard, P Di Prospero Fanghella, R Draisci, M Dusinska, G Encheva, G Flament, A Haase, Y Handzhiyski, F Herzberg, J Huwyler, NR Jacobsen, V Jeliazkov, N Jeliazkova, P Nymark, R Grafström, AG Oomen, ML Polci, C Riebeling, J Sandström, B Shivachev, S Stateva, S Tanasescu, R Tsekovska, H Wallin, MF Wilks, S Zellmer, MD Apostolova. Insights into possibilities for grouping and read-across for nanomaterials in EU chemicals legislation. *Nanotoxicology* (2019), 13(1): 119-141.**

**ABSTRACT**

This paper presents a comprehensive review of European Union (EU) legislation addressing the safety of chemical substances, and possibilities within each piece of legislation for applying grouping and read-across approaches for the assessment of nanomaterials (NMs). Hence, this review considers both the overarching regulation of chemical substances under REACH (Regulation (EC) No 1907/2006 on registration, evaluation, authorization, and restriction of chemicals) and CLP (Regulation (EC) No 1272/2008 on classification, labeling and packaging of substances and mixtures) and the sector-specific pieces of legislation for cosmetic, plant protection and biocidal products, and legislation addressing food, novel food, and food contact materials. The relevant supporting documents (e.g. guidance documents) regarding each piece of legislation were identified and reviewed, considering the relevant technical and scientific literature. Prospective regulatory needs for implementing grouping in the assessment of NMs were identified, and the question whether each particular piece of legislation permits the use of grouping and read-across to address information gaps was answered.

**13. R Tsekovska, M Boyanova, R Mironova, I Ivanov. Impact of glycation inhibitors on the biologic activity of recombinant human interferon-gamma. *Biotechnology& Biotechnological Equipment* (2012), 26(1): 170-174.**

**ABSTRACT**

Glycation of recombinant human interferon-gamma (rhIFN-γ) causes conformational alterations of the molecule and results in reduction of its biologic activity. The aim of this study was to find adequate approaches for prevention of glycation in order to obtain a stable recombinant protein with sustained biologic activity. To this end we investigated the effect of seven chemical compounds (acetylsalicylic acid, vitamin B1, aminoguanidine, arginine, pyridoxine, pyridoxal 5'-phosphate and pyridoxamine) on the biologic activity of rhIFN-γ produced in *Escherichia coli*. The obtained results showed that rhIFN-γ isolated from bacterial cells grown in the presence of 0.1 mM acetylsalicylic acid was the least affected by glycation. It demonstrated high stability in solution and biologic activity of about $2x10^6$ IU/mg over three months of storage at -20°C.

**14. R Tsekovska, M Boyanova, R Mironova, I Ivanov. Effect of arginine on glycation and stability of recombinant human interferon-gamma. *Biotechnology&Biotechnological Equipment* (2009), 23(1): 1063-1067.**

**ABSTRACT**

Recombinant human interferon-gamma (rhIFN-γ) produced in *Escherichia coli* (*E. coli*) undergoes structural and functional alterations as a result of two different but parallel processes – aggregation and non-enzymatic glycosylation (glycation). Finding approaches for their inhibition is of great importance for the quality of the rhIFN-γ. In this study we used arginine for this purpose. We found that arginine added to the *E. coli* culture medium, inhibits formation of fluorescent glycation adducts and imidazolone in the total bacterial protein but does not interfere with the early glycation stages. In addition, refolding and storage of rhIFN-γ in the presence of arginine led to delayed accumulation of $N^{\varepsilon}$-(carboxymethyl)lysine and structural stabilization of the recombinant protein.

**15. G Stoynev, R Dimitrova, L Srebreva, R Mironova, I Ivanov. Glycation in Escherichia coli: Detection of glycating compounds using histone H1 as a substrate. *C R Acad Bulg Sci* (2004), 57(9): 77-82.**

**ABSTRACT**
Glycation is a condensation reaction between reducing sugars and primary amino groups in proteins. It proceeds in many stages leading to formation of reversible Schiff bases, Amadori products and finally to irreversible products called advanced glycation end products (AGEs). Glycation is extensively studied in relation to diabetes and aging. Because of the common belief that it is a slow process under physiological conditions affecting long-lived proteins, until recently glycation has not been studied in prokaryotes. Our recent data, however, have shown that glycation takes place also in bacteria. The aim of this study is to check whether bacterial cytosol contains glycating compounds. Using histone H1 as a substrate for glycation, we show that incubation of histone H1 (placed in dialysis bags) in bacterial lysates leads to accumulation of both early and late (AGEs) glycation products. This means that the glycating compounds of *Escherichia coli* are low molecular mass substances dissolved in bacterial cytoplasm.

**16. R Mironova, T Niwa, R Dimitrova, M Boyanova, I Ivanov. Glycation and post-translational processing of human interferon-gamma expressed in Escherichia coli. *J Biol Chem* (2003), 278(51): 51068-51074.**

**ABSTRACT**
Until recently, nonenzymatic glycosylation (glycation) was thought to affect the proteins of long living eukaryotes only. However, in a recent study (Mironova, R., Niwa, T., Hayashi, H., Dimitrova, R., and Ivanov, I. (2001) *Mol. Microbiol.* 39, 1061–1068), we have shown that glycation takes place in *Escherichia coli* as well. In the present study, we demonstrate that the post-translational processing (proteolysis and covalent dimerization) observed with cysteineless recombinant human interferon-γ (rhIFN-γ) is tightly associated with its *in vivo* glycation. Our results show that, at the time of isolation, rhIFN-γ contained early (but not advanced) glycation products. Using reverse phase high performance liquid chromatography in conjunction with fluorescence measurements, enzyme-linked immunosorbent assay, and mass spectrometry, we found that advanced glycation end products arose in rhIFN-γ during storage. The latter were identified mainly in the Arg/Lys-rich C terminus of the protein, which was also the main target of proteolysis. Mass spectral analysis and N-terminal sequencing revealed four major ($Arg^{140} \downarrow Arg^{141}$, $Phe^{137} \downarrow Arg^{138}$, $Met^{135} \downarrow Leu^{136}$, and $Lys^{131} \downarrow Arg^{132}$) and two minor ($Lys^{109} \downarrow Ala^{110}$ and $Arg^{90} \downarrow Asp^{91}$) cleavage sites in this region. Tryptic peptide mapping indicated that the covalent dimers of rhIFN-γ originating during storage were formed mainly by lateral cross-linking of the monomer subunits. Antiviral assay showed that proteolysis lowered the antiviral activity of rhIFN-γ, whereas covalent dimerization completely abolished it.

**17. Y Handzhiyski, Tsekovska, K Kirilov, T Niwa, I Ivanov, R Mironova. Enhancing effect of L-lysine on glycation of histone H1 and bovine serum albumin *in vitro*. Chapter 3 in: A Closer Look at Glycation: A Potential Hotspot for Age-related Complications and Diseases, Nova Science Publishers, Inc, NY, USA (2021), 79-98, ISBN: 978-1-53619-176-9.**

**ABSTRACT**

Glycation is a non-enzymatic post-translational reaction between primary amino groups in proteins and reducing sugars resulting in the formation of advanced glycation end products (AGEs). This reaction contributes to many complications in diabetic patients and to other pathophysiological processes in humans. Therefore, a number of low molecular weight amino and carbonyl compounds have been tested thus far for inhibitory effect on glycation. Data obtained with L-lysine are controversial and it is not yet clear whether L-lysine is an inhibitor or enhancer of glycation. Glycation is a multistage reaction and therefore L-lysine may exert various effects on the different stages of the glycation reaction. With this in mind, we focused particularly on the effect of L-lysine on the advanced stage of glycation. We found that L-lysine enhances the formation of fluorescent AGEs in rat histone H1 and bovine serum albumin glycated with glucose 6-phosphate and D-ribose, respectively. Histone H1 also underwent accelerated covalent aggregation and proteolysis in the presence of L-lysine. Resveratrol was further found to suppress the enhancing effect of L-lysine on AGEs-formation in BSA, which implies that this effect is oxidationdependent. In conclusion, L-lysine appears inappropriate for inhibition of glycation *in vivo*, where over-dosages might accelerate glycation associated pathophysiological processes.

**18. E Popova, L Zagorchev, R Tsekovska, R Mironova, M Odjakova. Protective role of salinity against the accumulation of advanced glycation end products in embryogenic suspension cultures of Dactylis glomerata L. Chapter 5 in: Advanced Glycation End-Products: Sources and Effects, Nova Science Publishers, Inc, NY, USA (2020), 105-126, ISBN:978-1-53617-555-4**

**ABSTRACT**

The non-enzymatic glycosylation (glycation) of proteins results in the formation of advanced glycation end products (AGEs) that irreversibly modify proteins and impair their functions. Various abiotic stresses in plants cause an overproduction of reactive oxygen species, which are known to accelerate glycation. Despite this fact, protein glycation in plants under stress conditions has not been examined yet. In this study, we prepared poly-L-lysine modified with AGEs and used it as an antigen to generate a single-chain fragment variable monoclonal antibody (scFv mAb) by the phage display technology. The scFv mAb demonstrated strong and specific antigen binding and was used to study protein glycation in *Dactylis glomerata* L. embryogenic suspension cultures, subjected to moderate (0.085 M NaCl) or severe (0.17 M NaCl) salt stress. We found a negative correlation between the AGEs levels in the intra- and extracellular proteins of *D. glomerata* L. and the concentration of NaCl in the suspension cultures, which points to the protective role of salinity against glycation.

**19. R Tsekovska, Y Handzhiyski, E Boteva, K Kirilov, T Niwa, I Ivanov, R Mironova. Advanced glycation end products in *Escherichia coli*: A sign of aging. Chapter 5 in: Advances in Medicine and Biology, Nova Science Publishers, Inc. NY, USA (2017), 118:51-81, ISBN: 978-1-53611-010-4.**

**ABSTRACT**

Recent studies have challenged the paradigm that bacteria do not age and are immortal. Stewart et al. (PLoS Biology, 2005, 3(2), e45) provided an intriguing evidence that during division *Escherichia coli* K-12 exhibits functional asymmetry, leaving behind a mother cell with delayed

growth and survival rate and a younger daughter cell, empowered to successfully perpetuate the species over time. In view of this new finding, bacteria, and in particular *E. coli*, emerged as a promising model for exploration of basic mechanisms of aging. It is not yet clear to what extent pro- and eukaryotic cells age similarly but at least some features of aging, especially at the molecular level, should be common. Spontaneous chemical reactions including hydrolysis, oxidation and glycation (the Maillard reaction) are well known to deteriorate macromolecular structure and function. The Maillard reaction, yielding the so-called Advanced Glycation End Products (AGEs) on proteins, DNA and aminolipids, has been long associated with diseases (diabetes, Alzheimer's and Parkinson's diseases) and aging in humans. We have demonstrated that despite the short life span of *E. coli* of tens of minutes to hours, its chromosomal DNA accumulates AGEs under normal growth conditions (Mironova et al., Mol. Microbiol., 2005, 55(6), p1801). Such progressive modification of a key biological molecule provides an independent line of evidence for *E. coli* aging. This article reviews data on the Maillard reaction as a cause of stochastic damage in *E. coli*, and subsequently, of *E. coli* aging.

**20. R Tsekovska, A Sredovska-Bozhinov, T Niwa, I Ivanov, R Mironova. Maillard reaction and immunogenicity of protein therapeutics. *World Journal of Immunology*, Baishideng Publishing Group, (2016), 6(1): 19-38.**

**ABSTRACT**

The recombinant DNA technology enabled the production of a variety of human therapeutic proteins. Accumulated clinical experience, however, indicates that the formation of antibodies against such proteins is a general phenomenon rather than an exception. The immunogenicity of therapeutic proteins results in inefficient therapy and in the development of undesired, sometimes life-threatening, side reactions. The human proteins, designed for clinical application, usually have the same amino acid sequence as their native prototypes and it is not yet fully clear what the reasons for their immunogenicity are. In previous studies we have demonstrated for the first time that interferon-b (IFN-b) pharmaceuticals, used for treatment of patients with multiple sclerosis, do contain advanced glycation end products (AGEs) that contribute to IFN-b immunogenicity. AGEs are the final products of a chemical reaction known as the Maillard reaction or glycation, which implication in protein drugs' immunogenicity has been overlooked so far. Therefore, the aim of the present article is to provide a comprehensive overview on the Maillard reaction with emphasis on experimental data and theoretical consideration telling us why the Maillard reaction warrants special attention in the context of the well documented protein drugs' immunogenicity.

**21. A Sredovska-Bozhinov, R Tsekovska, Y Handzhiyski, R Mironova. Interferon-beta for treatment of relapsing-remitting multiple sclerosis: Problems and Perspectives. *International Journal of Recent Research in Arts and Sciences* (2015), 4: 407-416.**

**ABSTRACT**

Therapeutic proteins like human interferon-β (IFNβ) can induce immune response in treated patients. The development of an immune intolerance is a slow process, in which the frequency of occurrence and the amount of the antibodies vary widely - from low levels in most cases to very high in others. In many cases the antibodies do not have clinical consequences, but often together with the loss of drug activity they can neutralize the activity of the endogenous drug counterparts and provoke immune responses in treated patients such as flu-like symptoms, allergies,

anaphylaxis and serum sickness. In this review article we focus on the problems associated with the immunogenicity of human IFNβ used for treatment of relapsing-remitting multiple sclerosis and outline the perspectives for the production of less immunogenic therapeutic proteins.

**22. R Dimitrova, R Mironova, I Ivanov. Formation of 3-deoxyglucosone in Escherichia coli. In: Proceedings (Human Medicine) of the Scientific Conference with International Participation, St. Zagora, June 3-4, (2004), 4:96-100.**

**ABSTRACT**

During the process of nonenzymatic glycosylation (glycation), highly reactive α-dicarbonyl compounds, known as α-oxoaldehydes, are formed. These compounds participate in the formation of advanced glycation end products (AGEs). Among them are 3-deoxyglucosone, glyoxal, and methylglyoxal, which have so far been described only in eukaryotes. In our previous studies, we demonstrated that the glycation process in bacteria depends on their metabolic activity. In the present study, we examined the dependence of this process on the type of culture medium (rich or minimal) in order to show that the bacterial cytoplasm contains substances with glycation potential. This, in turn, led us to investigate the bacterial cytosol directly for the presence of glycation agents, in particular 3-deoxyglucosone.