Резюмета на научните трудове след предходна хабилитация

M. Chanana, S. Jahn, **R. Georgieva**, J. F. Lutz, H. Bäumler, D. Y. Wang. Fabrication of colloidal stable, thermosensitive, and biocompatible magnetite nanoparticles and study of their reversible agglomeration in aqueous milieu. *Chemistry of Materials* 2009, 21(9), 1906-1914. **IF-5.368**

A number of catechol-terminated copolymers of di(ethylene glycol) methyl ether methacrylate (MEO2MA) and poly(ethylene glycol) methyl ether methacrylate (OEGMA) with varied MEO2MA-to-OEGMA molar ratios were synthesized via atom transfer radical polymerization triggered by dopamine derived initiators. They were grafted on magnetite nanoparticles (NPs) via ligand exchange, thus imparting the NPs with robust colloidal stability against salt and excellent biocompatibility. Of importance is that similar to the copolymers of MEO2MA and OEGMA, their coated magnetic NPs showed a lower critical solution temperature. This leads to a reversible agglomeration of the resulting composite NPs in buffer and physiological solution in response to the environment temperature. This reversible and thermosensitive agglomeration was also observed within red blood cells after loading the resulting composite NPs into the cells. The agglomeration of magnetite NPs in red blood cells endowed the NP-loaded composite cells with a better magnetic response, for example, contrast enhancement for magnetic resonance imaging.

V. Staedtke, M. Brähler, A. Müller, **R. Georgieva**, S. Bauer, N. Sternberg, A. Voigt, A. Lemke, C. Keck, J. Möschwitzer and H. Bäumler. In vitro inhibition of fungal activity by macrophages mediated sequestration and release of encapsulated amphotericin B - nanosupension in red blood cells. *Small* 2010, 6(1), 96-103. **IF-7.336**

The efficacy of antifungal treatment has been diminished by the biodistribution limitations of amphotericin B (AmB) due to its pharmacological profile, as well as the severe side effects it causes. A cellular drug-delivery system, which incorporates human erythrocytes (RBCs) loaded with an AmB nanosuspension (AmB-NS), is developed in order to improve antifungal treatment. AmB-NS encapsulation in RBCs is achieved by using hypotonic hemolysis, leading to intracellular AmB amounts of 3.81_0.47 pg RBC_1 and an entrapment efficacy of 15–18%. Upon phagocytosis of AmB-NS–RBCs, leukocytes show a slow AmB release over ten days, and no alteration in cell viability. This results in an immediate, permanent inhibition of intra- and extracellular fungal activity. AmB-NS–RBC-leukocyte-mediated delivery of AmB is efficient in amounts 1000 times lower than the toxic dose. This drug-delivery method is effective for the transport of water-insoluble substances, such as AmB, and this warrants consideration for further testing.

W. C. Mak, **R. Georgieva**, R. Reneberger, H. Bäumler. Protein particles formed by protein activation and spontaneous self-assembly. *Advanced Functional Materials* 2010, 20(23), 4139-4144 **IF-8.486**

In this article, a non-chemical crosslinking method is used to produce pure protein microparticles with an innovative approach, so-called protein activation spontaneous and self-assembly (PASS). The fabrication of protein microparticles is based on the idea of using the internal disulfide bridges within protein molecules as molecular linkers to assemble protein molecules into a microparticle form. The assembly process is triggered by an activating reagent–dithiothreitol (DTT), which only involved in the intermediate step without being incorporated into the resulting protein microparticles. Conventional protein microparticle fabrication methods usually involve emulsification process and chemical crosslink reactions using amine reactive reagents such as glutaraldehyde or EDC/NHS. The resulting protein microparticles are usually having various size distributions. Most importantly crosslinking reactions using amine reactive reagents will result in producing protein microparticles with undesired properties such as auto-fluorescence and high toxicity. In contrast to the conventional methods, our technology provides a simple and robust method to produce highly homogeneous, stable and nonfluorescence pure protein microparticles under mild conditions at physiological pH and temperature. The protein microparticles are found to be biodegradable, non-toxic to MDCK cells and with preserved biological activities. Results on the cytotoxcity study and enzyme function demonstrate the potential applications of the protein microparticles in the area of pharmaceutics and analytical chemistry.

H. Bäumler, **R. Georgieva.** Enzyme cascade in biopolymer multicompartment microparticles. *Biomacromolecules* 2010, 11(6), 1480-1487. **IF-5.325**

Spherical biopolymer particles have been fabricated, applying coprecipitation with calcium carbonate, followed by cross-linking of the macromolecules and dissolution of the inorganic support. Particles made of roughly 80% horseradish peroxidase (HRP) as well as glucose oxidase (GOX) were prepared and enzyme activities were confirmed, applying the Amplex Red assay. The enzyme particles were reusable for at least six times, with a remaining activity of 30-50% from the initial one. When multiple coprecipitation steps and one

or several crosslinking procedures were applied, multicompartment particles were obtained. Each of the resulting concentric compartments could be independently loaded with biomolecules. Three coupled enzymes, _-glucosidase (_-Glu), GOX, and HRP have been incorporated stepwise in such particles. Each of these enzymes was located in a separate compartment, in a desired sequence, and at a defined position. The distance between the enzyme containing compartments was also varied, including spacing compartments consisting of bovine serum albumin (BSA). When fluorogenic substrates for _-Glu and HPR were used, the start and the end of the coupled enzyme reaction were visualized and recorded inside of individual particles, applying confocal laser scanning microscopy. A strong influence of the spacing on the reaction kinetics of the last enzyme was observed, suggesting an impaired diffusion of the intermediate products of the chain reaction through the spacing compartments made of BSA. The influence of the spacing between compartments containing different enzymes on the reaction kinetics was demonstrated on the microscopic scale within one microparticle, which to the best of our knowledge was not achieved until now.

I. Segal, A. Zablotskaya, E. Lukevicsa, M. Maiorov, D. Zablotsky, E. Blums, A. Mishnev, **R. Georgieva**, I. Shestakova, A. Gulbe. Preparation and cytotoxic properties of goethite-based nanoparticles covered with decyldimethyl(dimethylaminoethoxy) silane methiodide. *Applied Organometallic Chemistry* 2010, 24, 193-197. **IF-2.062**

The present work describes the synthesis, physico-chemical and biological properties of the first water-soluble goethite nanoparticles covered with biologically active components: oleic acid and cytotoxic decyldimethyl (dimethylaminoethoxy) silane methiodide. The structure of initial goethite nanoparticles synthesized was proved by XRD analysis and the rough estimation of nanoparticles core size gave the value of 8 nm. The size of colloidal water-soluble nanoparticles, determined by dynamic light scattering, was within 19–35 nm. Magnetic properties and cytotoxicity (against HT-1080 and MG-22A tumor cell lines) of the nanoparticles obtained were investigated.

S. Moreno-Flores, **R. Georgieva**, Y. Xiong, K. Melzak, H. Bäumler, J. L. Toca-Herrera. Physical attachment of fluorescent protein particles to atomic force microscopy probes in aqueous media: implications for surface pH, fluorescence, and mechanical properties studies. *Microscopy Research and Technique* 2010, 73, 746-751. **IF-1.712**

Transfer of a fluorescently labeled protein particle from a surface to a microsized scanning probe has been induced by repetitive scanning in aqueous medium. The so-attached particle can in turn act as a probing tool to study particle–substrate and particle–particle interactions. Attachment of the fluorescent particle occurs at the apical region of an atomic force microscope (AFM) cantilever tip and it endures repetitive loading–unloading cycles against the sample surface. Fluorescence microscopy has been used to address the exact location of the attached particle in the cantilever and to identify the moment when the particle contacts the sample. Moreover, we have observed that fluorescence intensity at the contact point is lower when the probing particle contacts another fluorescent particle than when it contacts the nonfluorescent substrate. The change in fluorescence is attributed to local changes of pH and interparticle-quenching of fluorophores in the contact region. These findings are promising since they constitute a chemical-free way to attach bioparticles to AFM probes under physiological conditions. The atomic force microscopy combined with fluorescence microscopy provides a straight forward method to study particle/particle and particle/substrate interactions, as well as to investigate mechanical properties of biocolloids.

J. Rodrigues, C. Abramjuk, L. Vázquez, N. Gamboa, J. Domínguez, B. Nitzsche, M. Höpfner, **R. Georgieva**, H. Bäumler, C. Stephan, K. Jung, M. Lein, A. Rabien. New 4-maleamic acid and 4-maleamide peptidyl chalcones as potential multitarget drugs for human prostate cancer. *Pharmaceutical Research* 2011, 28(4), 907-919 **IF-4.093**

Purpose The objective of this study was to investigate the effect of new 4-maleamic acid and 4-maleamide peptidyl chalcone derivatives against human prostate cancer in vitro and in vivo.

Methods From a series of 21 chalcones, the effects of the three best inhibitors of PC-3 and LNCaP cell viability on growth, including cell cycle changes, adhesion, migration, and cell invasion, as well as their ability to inhibit angiogenesis, clonogenic activity, and matrix metalloproteinases MMP-2 and MMP-9, were tested. The effects in vivo were studied in PC-3 and LNCaP xenografts.

Results Three of the examined chalcones reduced cell viability in both cell lines in a strong dose- and time-dependent manner. An inhibition of the cell cycle progress was observed. These changes were accompanied with the inhibition of cell adhesion, migration, and invasion as well as with reduced neovascularization in chick embryos, tumor colony formation, and MMP-9 activity. The in vivo results demonstrated the strong activity of these structures as inhibitors of tumor development in nude mice compared to non-treated animals.

Conclusion The results suggest the multitarget efficacy of 4-maleamic acid and 4-maleamide peptidyl chalcones against human prostate cancer cells and emphasize the potential therapeutic relevance of these compounds.

N. Sternberg, K. Andreas, H. Bäumler and **R. Georgieva**. *Chapter 15:* Blood cells as carriers for magnetically targeted delivery of drugs. In: Magnetic nanoparticles: From fabrication to clinical application. Edited by N. Thanh, CRC Press, Taylor & Francis Group, London 2012, pp. 387-418 ISBN 9781439869321 / ISBN 9781439869338.

In recent years, cell-based new therapeutic strategies have been intensively explored for tissue regeneration and targeted drug delivery. In this context, cells loaded with magnetic nanoparticles (MNPs) gain increasing interest as MNP migration can easily be followed *in vivo* by magnetic resonance imaging (MRI). Moreover, an external magnetic field enables the direction and concentration of cells loaded with MNPs at the desired sites for imaging, tissue repair, drug delivery or cancer therapy by hyperthermia. Several cell types have been used to test this strategy. This chapter focuses on loading blood cells with MNPs additionally providing an overview on general strategies for entrapment of drugs and nanoparticles in cells, examples of promising results and applications.

N. Sternberg. **R. Georgieva**, K. Duft, H. Bäumler. Loaded and surface modified red blood cells for targeted drug delivery. *Journal of Microencapsulation* 2012, 29(1), 9-20 **IF-1.571**

Red blood cells (RBCs) are natural carriers which can be used for targeted drug delivery. Conditions during loading and surface modification are essential for carrier-RBC preparation for specifically targeted drug delivery. Therefore, human RBCs were loaded with albumin and magnetic nanoparticles (NPs) by different hypotonic haemolysis procedures and compared based on loading efficiency and membrane damage. Samples were analysed by flow cytometry and confocal microscopy. The optimized loading procedure resulted in 90% albumin-loaded carrier-RBCs with 54% Annexin V binding and 263 pg iron per RBC after loading with iron oxide NPs. Albumin-loaded RBCs were subsequently surface conjugated with insulin and IgG via biotin–streptavidin. Insulin-conjugated carrier-RBCs were observed to attach and to be internalized by cultured endothelial cells. Uptake was not observed for carrier-RBCs non-specifically modified with IgG. Attachment of other peptides with high specificity will open novel opportunities for targeting various cells, tissues and for crossing biological barriers.

N. Sternberg, **R. Georgieva**, A. Abdallah, A. Müller, H. Bäumler, Loaded red blood cells as natural carriers for drug delivery. In: Proceedings of the 17th International Workshop on Bioencapsulation, Gronningen, 24 – 26 September 2009, P74 p. 1-4 (http://impascience.eu/bioencapsulation/340_contribution_texts/2009-09-24_P-74)

Red blood cells (RBC) were successfully loaded with diverse model substances such as Amphotericin B (AmB) nanosuspension, quantum dots (QD) and fluorescein isothiocyanate labelled bovine serum albumin (FITC-BSA). Different loading strategies were used to achieve the appropriate surface characteristics according to the desired particular application. The surface of loaded erythrocytes was additionally modified with insulin via the biotin-avidin pair. The insulin-modified surface of the carrier RBC provoked an uptake by endothelial cells confirming the possibility of specific cell targeting by this strategy.

K. Andreas, **R. Georgieva**, M. Ladwig. S. Müller, M. Notter, M. Sittinger, J. Ringe. Highly efficient magnetic stem cell labeling with citrate-coated superparamagnetic iron oxide nanoparticles for MRI tracking. *Biomaterials* 2012 33(18), 4515-4525 **IF-7.604**

Tracking of transplanted stem cells is essential to monitor safety and efficiency of cell-based therapies. Magnetic resonance imaging (MRI) offers a very sensitive, repetitive and non-invasive in vivo detection of magnetically labeled cells but labeling with commercial superparamagnetic iron oxide nanoparticles (SPIONs) is still problematic because of low labeling efficiencies and the need of potentially toxic transfection agents. In this study, new experimental citrate-coated SPIONs and commercial Endorem and Resovist SPIONs were investigated comparatively in terms of in vitro labeling efficiency, effects on stem cell functionality and in vivo MRI visualization. Efficient labeling of human mesenchymal stem cells (MSCs) without transfection agents was only achieved with Citrate SPIONs. Magnetic labeling of human MSCs did not affect cell proliferation, presentation of typical cell surface marker antigens and differentiation into the adipogenic and osteogenic lineages. However, chondrogenic differentiation and chemotaxis were significantly impaired with increasing SPION incorporation. Transplanted SPION labeled MSCs were visualized in vivo after intramuscular injection in rats by 7T-MRI and were retrieved ex vivo by Prussian Blue and immunohistochemical stainings. Though a careful titration of SPION incorporation, cellular function and MRI

visualization is essential, Citrate SPIONs are very efficient intracellular magnetic labels for in vivo stem cell tracking by MRI.

M. Delcea, N. Sternberg, A. M. Yashchenok, **R. Georgieva**, H. Bäumler, H. Möhwald, A. G. Skirtach. Nanoplasmonics for Dual-Molecule Release through Nanopores in the Membrane of Red Blood Cells. *ACS Nano* 2012, 6(5), 4169-4180 **IF-12.062**

A nanoplasmonics-based opto-nanoporation method of creating nanopores upon laser illumination is applied for inducing diffusion and triggered release of small and large molecules from red blood cells (RBCs). The method is implemented using absorbing gold nanoparticle (Au-NP) aggregates on the membrane of loaded RBCs, which, upon near-IR laser light absorption, induce release of encapsulated molecules from selected cells. The binding of Au-NPs to RBCs is characterized by Raman spectroscopy. The process of release is driven by heating localized at nanoparticles, which impacts the permeability of the membrane by affecting the lipid bilayer and/or trans-membrane proteins. Localized heating and temperature rise around Au-NP aggregates is simulated and discussed. Research reported in this work is relevant for generating nanopores for biomolecule trafficking through polymeric and lipid membranes as well as cell membranes, while dual- and multi-molecule release is relevant for theragnostics and a wide range of therapies.

K. Gawlitza, C. Wu, **R. Georgieva**, D. Wang, M. B. Ansorge-Schumacher, R. von Klitzing. Immobilization of lipase B within micronsized poly-NIsopropylacrylamide hydrogel particles by solvent exchange. *Physical Chemistry Chemical Physics* 2012, 14(27), 9594-9600 **IF-3.829**

The aim of the present work is the use of a water soluble enzyme in an organic solvent, still with a pronounced catalytic activity. Therefore, lipase B from Candida antarctica (CalB) is immobilized within micron-sized thermosensitive p-NIPAM hydrogel particles using a solvent exchange from polar to organic solvents. The absorbed amount of CalB is investigated at different immobilization temperatures. Confocal laser scanning microscopy (CLSM) shows that CalB is homogeneously distributed within the polymer network. An enhanced specific activity of CalB in n-hexane is achieved after immobilization within the p-NIPAM microgels. In order to get information on the supply of the substrate depending on the temperature, the activity is determined at different reaction temperatures. Additionally, the system is stable in the organic solvent, namely n-hexane, and shows a good reusability.

K. Gawlitza, C. Wu, **R. Georgieva**, M. B. Ansorge-Schumacher, R. von Klitzing. Temperature controlled activity of Lipase B from *Candida Antarctica* after immobilization within p-NIPAM microgel particles. *Zeitschrift für Physikalische Chemie* 2012, 226(7-8), 749-759 **IF-1.128**

The immobilization of lipase B from *Candida antarctica* (CalB) within micronsized poly-*N*Isopropylacrylamide (p-NIPAM) microgel particles with a crosslinker content of 5% is reported. The immobilization of the enzyme was reached by an exchange from polar to organic solvents. After determining the embedded amount of CalB within the polymer network, an enhanced specific activity in *n*-hexane was obtained. Due to the thermoresponsibility of the polymer particles, the activity reaction was done at 25 ◦C and 50 ◦C. The results presented show that the reversible collapse of the microgel leads to a decreased activity with increasing temperature. Hence, p-NIPAM microgels display a good opportunity to tailor the activity of CalB. An interesting side effect is that CalB presents a suitable probe to estimate the mesh size of the polymer network, since it penetrates in the unlabeled form but not after labeling with FITC.

Y. Xiong, A. Steffen, K. Andreas, S. Müller, N. Sternberg, **R. Georgieva**, H. Bäumler. Hemoglobin-Based Oxygen Carrier Microparticles – Synthesis, Properties, and In Vitro and In Vivo Investigations. *Biomacromolecules* 2012, 13(10), 3292-3300 **IF-5.371**

Bovine hemoglobin microparticles (Hb-MPs) as suitableoxygen carriers are fabricated easily by three key steps: coprecipitation of Hb and $CaCO_3$ to make Hb-$CaCO_3$-microparticles (Hb-$CaCO_3$-MPs), cross-linking by glutaraldehyde (GA) to polymerize the Hb and dissolution of $CaCO_3$ template to obtain pure Hb-MPs. The Hb entrapment efficiency ranged from 8 to 50% corresponding to a hemoglobin quantity per Hb-MP of at least one-third of that in one erythrocyte. The Hb-MPs are spherical, with an average diameter of 3.2 μm and high oxygen affinity. The methemoglobin level was increased after preparation, but can be reduced to less than 7% with ascorbic acid. Phagocytosis assays showed low immunogenicity of Hb-MPs if the particles were cross-linked with low concentration of GA and treated with sodium borohydride. Magnetite loaded Hb-MPs circulated up to 4 days after intravenous application.

Y. Xiong, Z.Z. Liu, **R. Georgieva**, K. Smuda, A. Steffen, M. Sendeski, A. Voigt, A. Patzak, H. Bäumler. Non-Vasoconstrictive Hemoglobin Particles as Oxygen Carriers. *ACS Nano* 2013, 7(9), 7454-7461 **IF-12.033**

Artificial oxygen carriers, favorably hemoglobin-based oxygen carriers (HBOCs), are being investigated intensively during the last 30 years with the aim to develop a universal blood substitute. However, serious side effects mainly caused by vasoconstriction triggered by nitric oxide (NO) scavenging due to penetration of nanosized HBOCs through the endothelial gaps of the capillary walls and/or oxygen oversupply in the precapillary arterioles due to their low oxygen affinity led to failure of clinical trials and FDA disapproval. To avoid these effects, HBOCs with a size between 100 and 1000 nm and high oxygen affinity are needed. Here we present for the first time unique hemoglobin particles (HbPs) of around 700 nm with high oxygen affinity and low immunogenicity using a novel, highly effective, and simple technique. The fabrication procedure provides particles with a narrow size distribution and nearly uniform morphology. The content of hemoglobin (Hb) in the particles corresponded to 80% of the Hb content in native erythrocytes. Furthermore, we demonstrate a successful perfusion of isolated mouse glomeruli with concentrated HbP suspensions in vitro. A normal, nonvasoconstrictive behavior of the afferent arterioles is observed, suggesting no oxygen oversupply and limited NO scavenging by these particles, making them a highly promising blood substitute.

K. Gawlitza, **R. Georgieva**, N. Tavraz, J. Keller, R. von Klitzing. Immobilization of Water Soluble HRP within Poly-N-Isopropylacrylamide Microgel Particles for Use in Organic Media. *Langmuir* 2013, 29(51), 16002-16009 **IF-4.384**

In the present work, the immobilization of enzymes within poly-Nisopropylacrylamide (p-NIPAM) microgels using the method of solvent exchange is applied to the enzyme horseradish peroxidase (HRP). When the solvent is changed from water to isopropanol, HRP is embedded within the polymer structure. After the determination of the immobilized amount of enzyme, an enhanced specific activity of the biocatalyst in isopropanol can be observed. Karl Fischer titration is used to determine the amount of water within the microgel particles before and after solvent exchange, leading to the conclusion that an “aqueous cage” remains within the polymer structure. This represents the driving force for the immobilization due to the high affinity of HRP for water. Beside, confocal laser scanning microscopy (CLSM) images show that HRP is located within the microgel network after immobilization. This gives the best conditions for HRP to be protected against chemical and mechanical stress. We were able to transfer a water-soluble enzyme to an organic phase by reaching a high catalytic activity. Hence, the method of solvent exchange displays a general method for immobilizing enzymes within p-NIPAM microgels for use in organic solvents. With this strategy, enzymes that are not soluble in organic solvents such as HRP can be used in such polar organic solvents.

M. Koziol, T. Sievers, K. Smuda, Y. Xiong, A. Müller, F. Wojcik, A. Steffen, M. Dathe, **R. Georgieva**, H Bäumler. Kinetics and Efficiency of a Methyl-Carboxylated 5 Fluorouracil Bovine Serum Albumin Adduct for Targeted Delivery. *Macromolecular Bioscience* 2014, 14(3), 428-439 **IF-3.851**

5-Fluorouracil (5-FU) is a clinically well-established anti-cancer drug effectively applied in chemotherapy, mainly for the treatment of breast and colorectal cancer. Substantial disadvantages are adverse effects, arising from serious damage of healthy tissues, and shortcoming pharmacokinetics due to its low molecular weight. A promising approach for improvement of such drugs is their coupling to suitable carriers. Here, a 5-FU adduct, 5-fluorouracil acetate (FUAc) is synthesized and covalently coupled to bovine serum albumin (BSA) as model carrier molecule. On average, 12 molecules FUAc are bound to one BSA. Circular dichriosm (CD)-spectra of BSA and FUAc-BSA are identical, suggesting no significant conformational differences. FUAc-BSA is tested on T-47D and MDA-MB-231 breast cancer cells. Proliferation inhibition of membrane albumin binding protein (mABP)-expressing T-47D cells by FUAc-BSA is similar to that of 5-FU and only moderate for MDA-MB-231 cells that lack such expression. Therefore, a crucial role of mABP expression in effective cell growth inhibition by FUAc-BSA is assumed.

H. Bäumler, Y. Xiong, Z.Z. Liu, A. Patzak, **R. Georgieva**. Novel Hemoglobin Particles – Promising New Generation Hemoglobin Based Oxygen Carriers (HBOCs). *Artificial Organs* 2014, 38(8), 708-714 **IF-2.050**

During the last 30 years, artificial oxygen carriers have been investigated intensively with the aim to develop universal blood substitutes. Favorably, hemoglobin-based oxygen carriers (HBOCs) are expected to meet the sophisticated requirements. However, the HBOCs tested until now show serious side effects, which resulted in failure of clinical trials and Food and Drug Administration disapproval. The main problem consists in vasoconstriction triggered by nitric oxide (NO) scavenging or/and oxygen oversupply in the pre-capillary

arterioles. HBOCs with a size between 100 nm and 1 μm and high oxygen affinity are needed. Here we present a highly effective and simple fabrication procedure, which can provide hemoglobin particles (HbPs) with a narrow size distribution of around 700 nm, nearly uniform morphology, high oxygen affinity, and low immunogenicity. Isolated mouse glomeruli are successfully perfused with concentrated HbP suspensions without any observable vasoconstriction of the afferent arterioles. The results suggest no oxygen oversupply and limited NO scavenging by these particles, featuring them as a highly promising blood substitute.

B. Tacheva, A. Zheleva, **R. Georgieva**, W. Tong, C. Gao, M. Karabaliev. Interactions of BSA-nanoparticles with some electroactive drugs. Trakia Journal of Sciences 2014, 12 (Suppl.1), pp. 84-88.

The interaction of three different drugs with Bovine serum albumin nanoparticles (BSA-NPs) is investigated in the work. Two phenothiazine drugs, chlorpromazine and thioridazine, and a spin-labeled nitosourea drug (SLCNUgly) are used to investigate the loading efficiency of BSA-NPs. The presented results indicate penetration of the drugs in the BSA-NPs, according to their hydrophobicity.

A. Eleta, J. Etxebarria, N. Reichardt, **R. Georgieva**, H. Bäumler, J. L. Toca-Herrera. On the molecular interaction between albumin and ibuprofen: an AFM and QCM-D study. *Colloids and Surfaces B: Biointerfaces* 2015, 134, 355-362 **IF-3.902**

The adsorption of proteins on surfaces often results in a change of their structural behavior and consequently, a loss of bioactivity. One experimental method to study interactions on a molecular level is single molecular force spectroscopy that permits to measure forces down to the pico-newton range.In this work, the binding force between human serum albumin (HSA) covalently immobilized on glutaraldehyde modified gold substrates, and ibuprofen sodium salt was studied by means of sin-gle molecular force spectroscopy. First of all, a protocol was established to functionalize atomic force microscopy (AFM) tips with ibuprofen. The immobilization protocol was additionally tested by quartz crystal microbalance with dissipation (QCM-D) and contact angle measurements. AFM was used to characterize the adsorption of HSA on gold substrates, which lead to a packed monolayer of thickness slightly lower than the reported value in solution. Finally, single molecule spectroscopy results were used to characterize the binding force between albumin and ibuprofen and calculate the distance of the transition state (0.6 nm) and the dissociation rate constant (0.055 s−1). The results might indicate that part of the adsorbed protein still preserves its functionality upon adsorption.

B. Tacheva, B. Parvanova, N. Sandev, I. Zarkov, M. Karabaliev, H. Bäumler, **R. Georgieva**, Polyelectrolyte microcapsules with potential for cellular delivery of drugs. Science & Technologies 2015, 5(1): 411-416.

Polyelectrolyte microcapsules (PEMC) have a great potential for targeted delivery of drugs. PEMCs prepared on fixes erythrocytes were loaded with the water insoluble antifungal drug Amphotericin B (AmB) by solvent exchange. The surface of loaded capsules was modified with polyethylene glycol to prevent from immune responses. The interaction of loaded and surface modified capsules with leucocytes was investigated in whole blood samples applying a standard phagocytosis test with flowcytometry and CLSM. The most effective prevention from phagocytosis was found for PEMC modified by PEG-5000. Our results confirmed the great potential of the PEMCs as a carrier-system for water insoluble drugs protecting them from inactivating effects and minimizing immune responses. Further surface functionalization by coupling antibodies will provide drug carrier PEMCs as an effective tool for targeting specific cells and tissues.

B. Tacheva, **R. Georgieva**, M. Karabaliev. Interactions of the spin-labeled chloroethylnitrosourea SLCNUgly with electrode-supported lipid films. *Electrochimica Acta* 2016, 192, 439-437 **IF-4.798**

The spin-labeled chloroethylnitrosourea containig glycine SLCNUgly is an analogue of the clinically used nitrosourea drug lomustine (1-(2-chloroethyl)-3-cyclohexyl-1-nitrosourea, CCNU), showing promising properties and features in vitro as well as in vivo. In this work the interaction of SLCNUgly with a lipid model membrane is investigated. The presented results indicate penetration of the drug in the membranes without causing defects of the lipid structure and reveal the potential of both SLCNUgly and electrode-supported lipid films as models for investigating nitrosourea drugs-membrane interactions.

A. N. Severyukhina, N. Petrova, K. Smuda, G. Terentyuk, B. Klebtsov, **R. Georgieva**, H. Bäumler and D. A. Gorin. Photosensitizer-Loaded Electrospun Chitosan-Based Scaffolds for Photodynamic Therapy and Tissue Engineering. *Colloids and Surfaces B:Biointerfaces* 2016, 144, 57-64 **IF-3.887**

Novel chitosan-based nanofibrous composite materials containing different amounts of the photo-sensitizer Photosens were obtained by electrospinning and were characterized by scanning electron microscopy and by confocal laser scanning microscopy. The release of Photosens from the materials was investigated in water and in phosphate-buffered saline. A noncancerous (MC3T3-E1 murine osteoblasts)and a cancerous [T-47D (mammary gland)] cell line were cultivated on Photosens-containing scaffolds, and cell growth and metabolic activity were examined by confocal laser scanning microscopy and by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide assay, respectively. The viability of both cell lines on Photosens-containing fibers decreased in a spatial manner upon laser irradiation of an appropriate wavelength and power density. Interestingly, the noncancerous MC3T3-E1 cells grown on Photosens −containing scaffolds were less affected by the irradiation. We conclude that the Photosens-containing electrospun chitosan nanofibers described here are of potential interest for biomedical applications, particularly topical photodynamic therapy and tissue engineering.

H. Bäumler, L. Hamberger, P. Zaslansky, U. Kalus, **R. Georgieva**, A. Pruss. Non-destructive determination of alograft bone-implant stiffness by analytic centrifugation. *Experimental Mechanics* 2016, 56(9), 1653-1660 **IF-2.091**

An analytical centrifugation technique (LUMiFuge) for mechanical testing is introduced. Measurements on calibrated springs with LUMiFuge and BOSE LM1- ElectroForce TestBench delivered similar values for E-moduli and spring constants. Native cylindrical scaffolds from cancellous femoral bone were successfully tested using this new technique. The E-moduli obtained for scaffolds with trabecular orientation parallel to the cylindrical axis (longitudinal trabeculae) were significantly higher than that of scaffolds with trabeculae perpendicular to the cylindrical axis (lateral trabeculae). The mean value of elastic modulus increased in dependence on the number of stress rounds from 37.3 ± 19.1 to 63.0 ± 24.2 MPa for samples with longitudinal trabeculae and from16.0 ± 8.1 to 33.3 ± 13.8MPa for samples with lateral trabeculae, respectively. We assume that due to the mechanical stress a not completely reversible structural displacement of trabeculae occurs, which results in more compact trabecular arrangement and increase of elastic modulus. The new analytical centrifugation technique offers advantages for characterization of mechanical properties of small bone samples. The method is relatively simple, allows simultaneous testing of several samples, non-destructive testing by application of low loads and can be used to follow the influence of repetitive stress on deformation and recovery of the bone.

A. N. Severyukhina, N. V. Petrova, A. M. Yashchenok, D.N. Bratashov, K. Smuda, I. A. Mamonova, N. A. Yurasov, D. M. Puchinyan, **R. Georgieva**, H. Bäumler, A. Lapanje and D. A.Gorin. Light induced antibacterial activity of electrospun chitosan-based material containing photosensitizer. *Materials Science & Engineering C* 2017, 70(1): 311-316 **IF-4.164 (2016)**

Increasing antimicrobial resistance requires the development of novel materials and approaches for treatment of various infections. Utilization of photodynamic therapy represents an advanced alternative to antibiotics and metal-based agents. Here, we report the fabrication of electrospun material that possesses benefits of both topical antimicrobial and photodynamic therapies. This material combines chitosan, as a biocompatible polymer, and a second generation photosensitizer. The incorporation of photosensitizer doesn't affect the material morphology and its nearly uniform distribution in fibers structure was observed by confocal Raman microscopy. Owing to photosensitizer the prepared material exhibits the light-induced and spatially limited antimicrobial activity that was demonstrated against Staphylococcus aureus, an important etiological infectious agent. Such material can be potentially used in antibacterial therapy of chronic wounds, infections of diabetic ulcers, and burns, as well as rapidly spreading and intractable soft-tissue infections caused by resistant bacteria.

L. Zhao, W. Kaewprayoon, H. Zhou, **R. Georgieva**, H. Bäumler. RBC aggregation in dextran solutions can be measured by flow cytometry, *Clinical Hemorheology and Microcirculation* 2017, 65(1): 93-101 **IF-1.679 (2016)**

The impact of macromolecules on RBC aggregation continues to be of interest, nevertheless present measurements still have limitations and need improvement.We applied flowcytometry to measure RBC aggregation in dextran T500 (Dx500) solution. The samples were fixed in the aggregated state by glutaraldehyde. Fixed RBC exhibit auto fluorescence, which can be detected by flow cytometry. Single cells,

doublets, triplets and larger aggregates can be distinguished quantitatively and quickly due to the correlation between auto fluorescence intensity and number of RBC per measured event. With the increase in concentration of Dx500, percentages of all aggregates and bigger aggregates increased significantly at concentration of 2%, 4% and 6%, while decreased when the concentration reached 8% and 10%. The percentage of bigger aggregates in concentration of4%was higher than that in2%and 6%. The data of flowcytometry was confirmed by microscopic observation and are in good agreement with the literature. The method provides additional advantages to the conventional measurement of RBC aggregation. It gets the distribution of single cells and aggregates as derived from the microscopic observation with hematocrit of physiological level. It uses sample volume as 1/5∼1/10 as needed in sedimentation and photometric methods.

I. T. Ivanov, B. K. Paarvanova, V. A. Ivanov, K. Smuda, H. Bäumler, **R. Georgieva**. Effects of heat and freeze on isolated erythrocyte submembrane skeletons. *General Physiology and Biophysics* 2017, 36(1): 155-165 **IF-1.170 (2016)**

In this study we heated insoluble residues, obtained after Triton-X-100 (0.1 v/v%) extraction of erythrocyte ghost membranes (EGMs). Specific heat capacity, electric capacitance and resistance, and optical transmittance (280 nm) sustained sharp changes at 49°C (TA) and 66°C (TC), the known denaturation temperatures of spectrin and band 3, respectively. The change at TA was selectively inhibited by diamide (1 mM) and taurine mustard (1 mM) while its inducing temperature was selectively decreased by formamide in full concert with the assumed involvement of spectrin denaturation. In the residues of EGMs, pretreated with 4,4‘-diiso-thiocyanato stilbene-2,2‘-disulfonic acid (DIDS), the change at TC was shifted from 66 to 78°C which indicated the involvement of band 3 denaturation. The freeze and rapid thaw of EGM residues resulted in a strong reduction of cooperativity of band 3 denaturation while the slow thaw completely eliminated the peak of this denaturation. These effects of freeze-thaw were prevented in residues obtained from DIDS-treated EGMs. The freeze-thaw of residues slightly affected spectrin denaturation at 49°C although an additional denaturation appeared at 55°C. The results indicate preserved molecular structure and dynamics of the membrane skeleton in Triton-X-100 extracts of EGMs. The freeze-thaw inflicted strong damage on band 3 and spectrin-actin skeleton of EGM extracts which is relevant to cryobiology, cryosurgery and cryopreservation of cells.

B. Paarvanova, B. Tacheva, **R. Georgieva**, M. Karabaliev, I. T. Ivanov. Interactions of nitrosourea SLENU with supported lipid films and erythrocyte membranes. *Science & Technologies* 2017, 7(1): 166-171.

Dielectic spectroscopy of heated suspensions, containing either human erythrocytes or resealed erythrocyte ghost membranes, assays the state of sub-membrane spectrin-actin skeleton and its attachment to the lipid bilayer (Ivanov and Paarvanova, 2016). Using this method across a set of frequencies we measured the changes in the complex electric impedance and capacitance of tested suspensions at the spectrin denaturation temperature, 49.5 °C. In the impedance (-ΔZim vs ΔZre) plot these changes depicted two semicircles expressing two dielectric relaxations, while the capacitance plot (ΔCim vs ΔCre) expressed a single semicircle, corresponding to the second relaxation. DNAse I is an enzyme that disintegrates actin polymers. Relatedly, the impedance plot of erythrocyte ghost membranes, resealed with DNAse I, demonstrated strong reduction of the radius of first semicircle compared to the impedance plot of control membranes. In this study we compared the impedance plot obtained with erythrocytes of a tested patient and the plot of isolated erythrocyte membranes which contained DNAse I. The two plots were identical indicating that the patient could have a kind of erythrocyte membranopathy related to reduced polymerization of actin or impaired spectrrin-actin association.

B. Tacheva, V. Gadjeva, **R. Georgieva**, B. Parvanova, I. T. Ivanov, M. Karabaliev. Interactions of nitrosourea SLENU with supported lipid films and erythrocyte membranes. *Science & Technologies* 2017, 7(1): 219-226.

Nitrosourea 1-ethyl-1-nitroso-3-[4-2,2,6,6-tetramethylpiperidine-1-oxyl (SLENU) is a spin labeled analogue of the clinically used non-labeled antitumor drug lomustine (CCNU). The objective of this study is to characterize the interactions of SLENU with supported lipid films and erythrocyte membranes. Thin lipid films prepared on the surface of a glassy carbon electrode are used as a model membrane system for studying the interaction between SLENU and the lipid fraction of biomembranes. The effects of SLENU on the structure of the lipid film are investigated by cyclic voltammetry (CV) and electrochemical impedance spectroscopy (EIS). It is shown that due to the amphiphilic properties of SLENU it penetrates the lipid layers. The possible formation of defects in the lipid films was studied by the aid of hydrophilic ions - the electroactive couple ferri-ferrocyanide. The SLENU penetration in the lipid phase provokes a little decrease in the film thickness. Up to concentration of 1 mM SLENU there is no change of the redox currents of ferriferrocyanide suggesting

absence of defects in the lipid structure. Acidic hemolysis of erythrocytes is also not influenced by the presence of SLENU, suggesting the lack of destabilizing effect of SLENU on erythrocyte membranes.

Б. Тачева, Б. Първанова, И. Т. Иванов, **Р. Георгиева**, М. Карабалиев. Обмен на лекарствени средства между еритроцитни мембрани и протеинови наночастици. *Science & Technologies* 2017, 7(1): 172-178

Влиянието на хлорпромазин и тиоридазин върху еритроцитни мембрани е изследвано чрез метода хемолиза в хипотонична среда. И двете вещества имат двуфазов ефект на въздействие върху мембраните - стабилизиране при ниски концентрации и дестабилизиране след достигане до съответна критична концентрация. Критичната концентрация на тиоридазина е по-малка от тази на хлорпромазина, което е в съответствие със съотношението на коефицентите им на разпределение. При наличие на наночастици от говежди серумен албумин (BSA) в разтвора се наблюдава нарастване в критичните концентрации, дължащо се на инкорпориране на част от лекарствените вещества в наночастиците.

Б. Тачева, Б. Първанова, **Р. Георгиева**, М. Карабалиев. Метод за електрохимично изследване на вграждането на амфифилни лекарствени вещества в липозоми. *Science & Technologies* 2017, 7(1): 201-210

Вграждането на амфифилното електроактивно лекарствено вещество хлорпромазин (CPZ) е изследвано in situ с помощта на метода на цикличната волтамперометрия (CV). Посредством CV се определя концентрацията на електроактивни вещества в изследвания разтвор, обменящи електрони с работния измервателен електрод в процесите на окисление и редукция. При добавяне на липозоми в разтвора голяма част от наличния хлорпромазин се вгражда в липидния им бислой, което се регистрира по намаляването на сигнала от окислението на останалия невграден в липозомите хлорпромазин. Допълнително с помощта на електрохимична импедансна спектроскопия (EIS) е показано, че наличието на липозоми не променя повърхността на електрода и полученият резултат се дължи именно на взаимодействието на лекарственото вещество с липозомите. Проведени са и паралелни експерименти с хидрофилното вещество фери/фероцианид, което не ваимодейства с липозомите и при което съответно няма промяна на CV-сигнала.

Б. Тачева, Б. Първанова, **Р. Георгиева**, А. Желева, М. Карабалиев. Сравнително изследване на взаимодействието на лекарствени вещества с липозоми и с BSA-наночастици. *Science & Technologies* 2017, 7(1): 211-218

Определени са коефицентите на разпределение между липозоми и водна среда на хлорпромазин, тиоридазин и спин-белязаната нитрозоурея SLCNUgly. Използван е методът на втората производна на абсорбционния спектър. Резултатите са сравнени с резултати за вграждането на същите вещества в протеинови наночастици от говежди серумен албумин (BSA). Въз основа на сравнението е направено предположението, че вграждането на веществата в BSA-наночастиците се дължи предимно на хидрофобни взаимодействия.

H. Y. Li, Y. Xiong, W. Tong, **R. Georgieva**, H. Bäumler, C.Y. Gao. Photo-decomposable submicrometer albumin particles cross-linked by *ortho*-nitrobenzyl derivatives. *Macromolecular Chemistry and Physics* 2017, 218(24), art. Nr. 1700413 (p.1-6), DOI: 10.1002/macp.201700413 **IF-2.500 (2016)**

Stimuli-responsive particles are widely used as carriers for on-demand delivery of drugs, genes, proteins, etc., due to their response to environmental stimuli. In this study, photoresponsive albumin sub-micrometer particles are fabricated by using *ortho*-nitrobenzyl derivative 4-bromomethyl-3-nitrobenzoic acid (BNBA) as a cross-linker. Bovine serum albumin (BSA)-doped MnCO3 particles are used as the sacrificial template, in which the BSA molecules are cross-linked by BNBA under the activation of 4-(4,6-dimethoxy-1,3,5-triazin-2-yl)-4-methylmorpholinium chloride. After removal of MnCO3 particles by ethylene diamine tetraacetic acid, photoresponsive BSA–BNBA sub-micrometer particles are obtained. Since the C-N bond formed via reaction of benzyl bromide and amine is photocleavable, the particles can be decomposed upon UV irradiation at 365 nm under acidic environment. The loaded macromolecules can be released upon UV irradiation, exhibiting great potential applications of the particles in the field of controlled release.

Y. Xiong, **R. Georgieva**, A. Steffen, K. Smuda, H. Bäumler. Structure and properties of hybrid biopolymer particles fabricated by co-precipitation cross-linking dissolution procedure. *Journal of Colloid and Interface Science* 2018, 514, 156-164, https://doi.org/10.1016/j.jcis.2017.12.030 **IF-4.233 (2016)**

The Co-precipitation Crosslinking Dissolution technique (CCD-technique) allows a few-steps fabrication of particles composed of different biopolymers and bioactive agents under mild conditions. Morphology and properties of the fabricated biopolymer particles depend on the fabrication conditions, the nature of the biopolymers and additives, but also on the choice of the inorganic templates for co-precipitation. Here, we investigate the influence of an acidic biopolymer, hyaluronic acid (HA), on the formation of particles from bovine hemoglobin and bovine serum albumin applying co-precipitation with CaCO3 and MnCO3. CaCO3 templated biopolymer particles are almost spherical with particle size from 2 to 20 μm and protein entrapment efficiency from 13 to 77%. Presence of HA causes significant structural changes of the particles and decreasing protein entrapment efficiency. In contrast, MnCO3 templated particles exhibit uniform peanut shape and submicron size with remarkably high protein entrapment efficiency of nearly 100%. Addition of HA has no influence on the protein entrapment efficiency or on morphology and size of the particles. These effects can be attributed to the strong interaction of Mn2+ with proteins and much weaker interaction with HA. Therefore, entrapment efficiency, size and structure of biopolymer particles can be optimized by varying the mineral templates and additives.