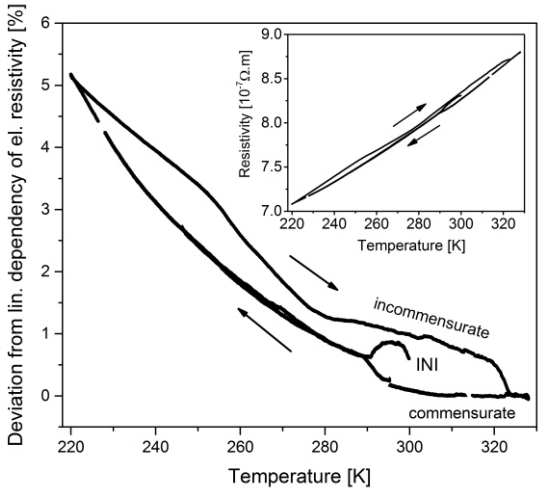

Figure 2. Deviation from linear dependency of electrical resistivity with temperature within 10M modulated martensite of the $Ni_{50}Mn_{27}Ga_{22}Fe_1$ alloy. Measured resistivity curve within the studied region of the 10M martensite phase is presented in the inset [4].

tains yet another *commensurate* metastable state followed by the intermartensitic transformation to 14M modulated martensite [4]. Observed changes in modulation exhibit a thermal hysteresis, Fig. 1. Interestingly, these minor changes in modulation can be corelated with the changes of physical properties, such as electrical resistivity, Fig. 2, and elastic moduli.

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Session III

SL10

PHOTOCHEMICAL DEGRADATION OF SELECTED PHARMACEUTICALS UNDER LIGHT CONDITIONS RELEVANT TO NATURAL WATERS AND STUDY OF TOXICITY OF PHOTOPRODUCTS

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Xenobiotics in the environment include a wide variety of compounds, e.g. pesticides, drugs, textile dyes, personal care products, stabilisers, and many others. Among xenobiotics, pharmaceuticals have recently acquired increasing attention [1, 2]. Pharmacological products enter natural waters mainly via wastewater either from manufacturing facilities or from municipal wastewater (excretion of unmetabolised drugs, disposal of unused drugs). The contamination of natural aquatic systems results in adverse negative effects on aquatic organisms.

In surface waters, physical, chemical, and biological processes contribute to the transformations of polluting substances. Photoinitiated processes may represent important degradation pathways in surface waters for compounds resistant to both biological degradation and chemical reactions such as hydrolysis [3]. Photochemical degradation may lead to a decrease in contaminant concentration, and, in some cases, generate photoproducts with even higher harmful effects than that of the parent compound [4].

Ecotoxicology represents a framework enabling to test a given compound and to reveal or at least estimate its potential harmful effect. This study is focused on aquatic organisms. In autotrophs, algae *Chlorella sp*. and *Desmodesmus sp*. are often used due to their simple laboratory maintenance [5, 6]. The flagship of heterotrophs toxicity testing in surface water is the planktonic microcrustacean *Daphnia magna* [7]. It has several characteristics that in toxicological tests, especially those targeted at acute toxicity estimation – it can be relatively easily maintained in the laboratory and, when under suitable conditions, *D. magna* reproduces parthenogenetically. A common model of vertebrates in ecotoxicology is the zebrafish *Danio rerio*. Although the extrapolation of the obtained results to higher vertebrates is not straightforward and should be done with care, the response of fish to xenobiotics is a significant indicator of how a particular compound (or products of its photodegradation) affect fish assemblages in surface waters.

In this study, toxicity of atorvastatin, a widely prescribed hypolipidemic drug, and the mixture of its photoproducts were investigated. The photoproduct mixture was produced by irradiation of the solution of atorvastatin (c = 50 mg/l) by the radiation in the range between 300 – 350 nm (to imitate the short-wavelength solar radiation that reaches the Earth´s surface) for 15 minutes. Then, two toxicity assays based on OECD 202 [8] and 236 [9] guidelines were performed.

In the case of acute toxicity test on the model organism *Daphnia magna* (OECD 202), 2 juveniles not older than 24 h were introduced into 5 ml of pure media, other juveniles in pairs in the media with atorvastatin in the concentration range from 1 to 10 000 µg/l; photoproducts solutions were tested at concentration range of remaining atorvastatin in the irradiated solution up to 1000 µg/l. During this test (48 hrs) constant temperature was held at 18.7 ± 0.2 °C; photoperiod was 16 hrs light and 6 hrs dark and the juvenils were not fed. Atorvastatin did not cause any mortality, photoproducts caused 20 % mortality at the highest concentration used. The LC50 value could not be evaluated from this experiment. The data show that lethal concentration for 50 % of daphnids is higher than the highest used concentration. Photoproducts seem to be more toxic than atorvastatin itself since in addition to the observed mortality each daphind showed odd swimming at the highest concentration of photoproducts.

Toxicity assay based on OECD 236 guideline was done on embryos of *Danio rerio*. One fertilized egg was introduced into 2 ml of ISO water (control) or into 2 ml of atorvastatin or 2 ml of photoproducts (concentration ranges as in the tests with *D.magna)*. Tested embryos were kept in the incubator Climacell EVO line, for 96 hours at the temperature 25 – 26 °C and the photoperiod 14 hrs light/ 10 hrs darkness. The evolution of the embryos was monitored visually every 24 hours. Four key parameters indicating the lethality were sought for: coagulated embryos, lack of somite formation, non – detachment of the tail and lack of heartbeat. The value of LC50 for atorvastatin was determined by software Prism 6, its value is 1976 µg/l. Regarding photoproducts, 40 % mortality was observed at the photoproduct mixture with remaining 500 µg/l of atorvastatin. In the higher concentration there was also retardation in the development and the drug had adverse effect on blood formation – lack of cell flow, transparent cells without pigmentation.

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SL11

DECORIN BINDING PROTEINS FROM EUROPEAN *BORRELIA* – DO STRUCTURAL DIFFERENCES INFLUENCE LIGAND BINDING?

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Adhesion of spirochetes from *Borrelia burgdorferi* sensu lato complex is the crucial step in early phase of Lyme disease infection. Decorin binding proteins (Dbp) are glycosaminoglycan (GAG) binding adhesins exposed on the surface of *borrelia* spirochetes. Dbps are expressed in two homologous forms A and B, both of them were characterized as main factors of *borrelia* virulence [1]. Based on the previous described differences in binding mechanisms of Dbp-GAG interaction [2], we focused on the relations between structural differences and GAG binding. We aim to describe the structural differences in detail among Dbps from european *Borelia* species and their particular interactions with different GAGs using solution nuclear magnetic resonance (NMR) spectroscopy at atomic resolution. Almost complete backbone and sidechain assignments of DbpA from *B. Afzelii* and *B. Bavariensis* have been achieved. Predictions of secondary structure propensity for both variants, calculated from assigned chemical shifts, were compared with available NMR structures of North American *borrelia* species. Backbone dynamics was described by T1 and T2 spin relaxations and ^{1}H ^{15}N heteronuclear NOE (Nuclear Overhauser effect) experi-

ments. We performed initial protein-GAG interaction studies of both variants of DbpA with different GAGs by NMR titrations including protein dynamics measurement by heteronuclear NOE experiments, hydrogen-deuterium exchange mass spectrometry (HDX-MS) and surface plasmon resonance (SPR) trial measurement. NMR-based prediction of secondary structure propensity and protein backbone dynamics combined with initial protein-ligand interaction experiments, which indicates interspecific differences in GAG binding, provided insight into structural characteristics of DbpA and will set the starting point for future extensive research of specific differences in structure and dynamics of Dbps and how it influence the interaction mechanism with GAG ligands.

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SL12

LIPOLYTIC SYSTEM IN THE HARD TICK *IXODES RICINUS*

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Ticks are obligatory blood-feeding ectoparasites capable of transmitting a wide variety of pathogens comprising bacteria, viruses, and protozoa to humans and animals. After mosquitoes, ticks are the second most dangerous vectors of arthropod-borne diseases. Hard tick *Ixodes ricinus* is a typical representative of the 3-host tick and its life cycle comprises three life stages. Each of the parasitic stages, except for adult males, feeds on a host. In contrast to mosquitoes, hard ticks *I. ricinus* feed much longer. Nymphal feeding takes typically from 3 to 4 days, adult females feed twice longer approximately 6 – 9 days. Adult ticks are capable to imbibe and digest huge amounts of host blood exceeding hundred times their unfed weight (Sonenshine, 1991).

Tick midgut lumen serves as the main organ for storage of the engorged blood. Most of the hematophagous ectoparasites (such as insect blood-feeders) digest host blood extracellularly in the midgut lumen. By contrast, digestion in ticks is a slow process occurring intracellularly in the midgut epithelium cells. Host blood is the sole source of energy and nutrients for overall tick development and reproduction. During feeding of each developmental stage, dynamic changes of tick midgut epithelium reflect the changes in the physiological processes occurring in this tissue (Sonenshine, 1991). Furthermore, the midgut serves as the primary interface between the tick and tick-borne pathogens that determines tick vector competence (O'Neal et al., 2020).

Limited information and functional studies about vector insect lipid metabolism are available. Lipids, in the form of sterols or free fatty acids, are the main and essential components of the insect dietary requirements (Canavoso et al., 2001; Toprak et al., 2020). However, insects are not able to synthesize sterols by themselves (Clark and Block, 1959; Jing and Behmer, 2020). During oxidation of the fatty acids (FAs), twice more energy (approximately 9 kcal/g) is released than during the complete oxidation of carbohydrates (approximately 4 kcal/g) (Toprak et al., 2020). The main source of lipids for these invertebrates is host blood. Lipid digestion occurs mainly in the midgut lumen, where lipases (EC 3.1.1.3; the major lipid digestive enzymes) catalyze the hydrolysis of the ester bond in triacylglycerols (TAGs) as well as in di- and monoacylglycerols (DAGs, MAGs) to the final products - glycerol and free fatty acids (FAs) (Derewenda, 1994; Toprak et al., 2020).

In *I. ricinus*, poor understanding of lipid metabolism exists. As mentioned above, digestion of proteins in tick midgut occurs intracellularly in the midgut epithelium cells. Nothing is known about digestion of lipids in ticks and no digestive lipases have been yet functionally characterized in any tick species. Some esterases and lipases involved in the incorporation of the nutrients to the oocytes were identified during embryogenesis in the eggs of the camel tick *Hyalomma dromedarii* (Fahmy et al., 2004). Earlier, Koh et al. (1991) described the utilization of the nutritional reserves stored in the form of lipid droplets in the midgut epithelium cells. These lipids droplets are used for the growth of tissues during feeding in the nymphal stage of hard tick *Haemaphysalis longicornis* (Koh et al., 1991). More recently, it was demonstrated in another tick species *Dermacentor variabilis*, that lipids are also exploited in the course of the long off-host starvation period, during which the large lipid droplets are released and used as the endogenous energy recourses (Rosendale et al., 2018). In the midgut transcriptome of *I. ricinu*s, lipases from several classes have been identified (Perner et al., 2016). Phospholipase A2, acid sphingomyelinase, and lipase (pancreatic-like type) were identified as the most up-regulated lipases in the midgut on the 3rd day of adult

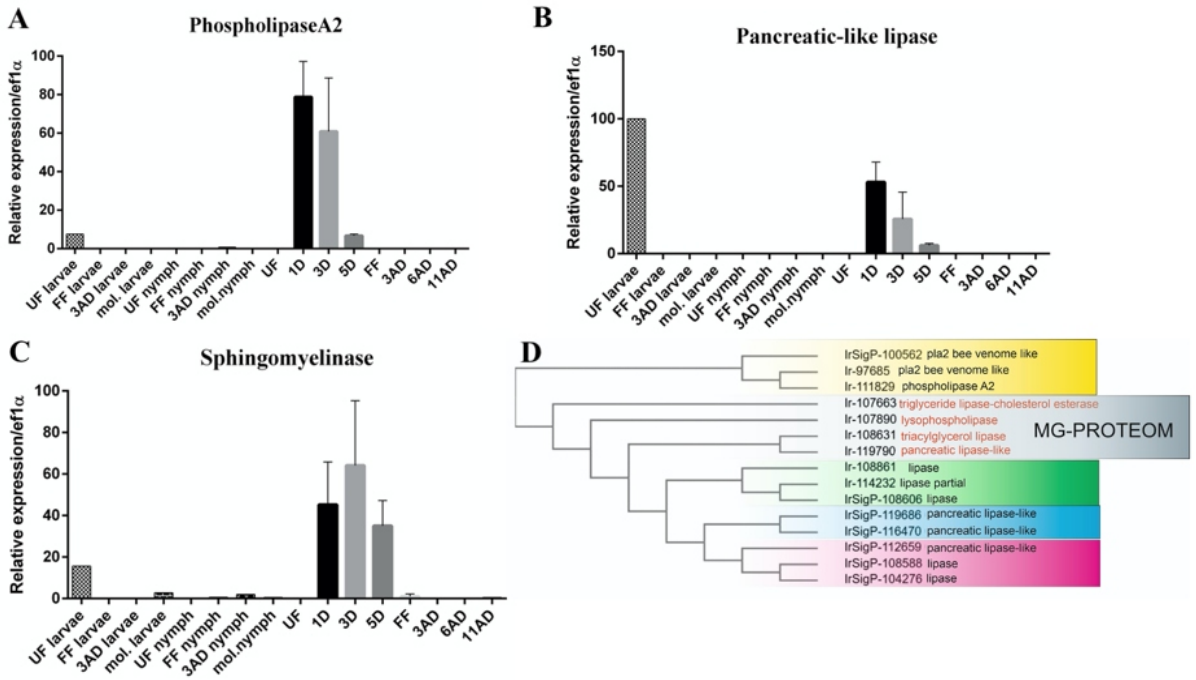

Figure 1: (A-C) - mRNA expression of individual lipases in the midgut during developmental and feeding stages. Quantitative real-time PCR (qRT-PCR) profiling of the phospholipase A2 (PlA2), pancreatic-like lipase, and sphingomyelinase. Bars indicate the standard deviation. UF: unfed; 1D,3D, 5D: one, three and five days of feeding; FF: fully fed females; 3AD, 6AD, 11AD: three, six and eleven days after detachment; mol.: molting. **(D) – Phylogenetic three of lipases identified in the midgut transcriptome (Perner et al., 2016a) and in the midgut proteome.** Lipases identified in the midgut proteome are highlighted grey. Phylogenetic tree was performed using ClustalOmega server (https://www.ebi.ac.uk/Tools/msa/clustalo/).

female feeding (Perner et al., 2016). Detailed analysis of these midgut-specific lipases using quantitative real time PCR (qRT-PCR) across tick development revealed that these lipases are mainly expressed during the early stage of adult female feeding (Fig. 1A-C). The RNAi mediated-knockdown (RNAi KD) of individual lipases in adult females did not show any phenotype differences between RNAi KD and control groups (data not shown). Furthermore, in the midgut proteome analysis, either form unfed females or from females fed for five days, only rare lipases were identified (Fig. 1D).

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SL13

STRUCTURAL STUDIES OF Si3 ENDOLYSIN MUTANTS

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For more than half a century, antibiotics have been used as the most common tool in the fight against infectious diseases. However, due to the misuse and overuse of these substances, an increasing number of bacteria are emerging that are completely resistant to all currently known antibiotics (multidrug resistant). The emergence of these types of bacteria renders most, if not all, available antibiotics ineffective. This situation is a global health crisis that threatens effective prevention and control of ever-increasing infectious diseases. This crisis is also exacerbated by increasingly sophisticated development new antibiotics.

A way out of this crisis may be the use of novel substances in the fight against infectious diseases. One of the potential candidates are endolysins. These enzymes, originally derived from bacteriophages, are peptidoglycan hydrolases that destroy bacterial cell wall. They have high specificity, much higher capability and efficiency, and also have not been found to develop resistance [1, 2]. An extremely fast and efficient lysis of peptidoglycan leads to a

Table 1. Selected data collection statistics

Parameters	**lysECD7-SMAP**	**lysAAA**	**LysHE**
Resolution (Å)	1.70	1.43	2.00
Average unit cell (*a, b, c* Å; ( )	48.66, 58.03, 196.63 90.00	66.307, 76.548, 65.633 90.00	48.183, 66.381, 119.26 90.00
Space group	$P2_12_12_1$	$C222_1$	$P22_12_1$
Number of molecules in asymmetric unit	4	1	2
R	0.216	0.166	0.183
R_{free}	0.260	0.204	0.221

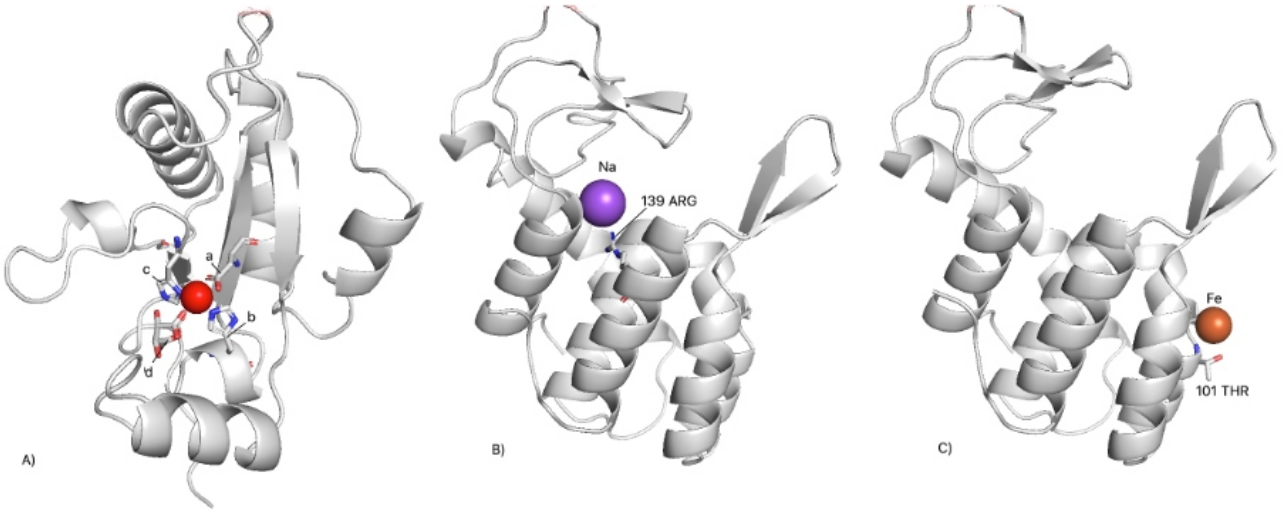

Figure 1. Secondary structures of Si3 mutants. A) the lysECD7-SMAP mutant molecule has a Zn atom (red ball) that is stabilized by amino acids 62 HIS (a), 69 ASP (b) and 117 HIS (c). Also shown is a glycerol molecule (d) located in the region of the Zn atom; B) lysAAA mutant with Na ion (purple ball); C) lysHE mutant, the structure contains the Fe ion (orange ball).

sudden drop in turgor pressure and osmotic lysis, causing bacterial cell death [3]. The most important aspect for us is how molecular engineering can be used to change lytic spectrum of endolysins [4].

The aim of our work was to obtain crystals of three Si3 endolysin mutants (lysAAA, lysHE and lysECD7-SMAP), followed by the study of their structure.

In the course of the work carried out, we obtained crystals of high diffraction quality for all three mutants presented above, which allowed us to study their structure in sufficient detail. X-ray diffraction data wore collected at BESSY II, Helmholtz Zentrum Berlin and processed used XDS-app and CCP4 software. The main statistical parameters are given in Table 1.

As can be seen from Figure 1, the structure of the lysECD7-SMAP mutant is fundamentally different from the structures of the lysAAA and lysHE mutants, which, in turn, are similar to each other. It is worth noting separately the presence of different metal atoms in the structure of each molecule; however, only in the lysECD7-SMAP mutant is the Zn atom stabilized by amino acid residues. ECD7-SMAP mutant has a 19-amino-acid C-terminal tail which changes overall conformation that may impact its enzymatic activity. It is, therefore, our main focus of further structural studies.

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SL14

MOLECULAR, BIOCHEMICAL AND STRUCTURAL CHARACTERIZATION OF SECRETED FERRITIN II FROM *IXODES RICINUS*

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Ferritin is a ubiquitous protein with crucial role in tick biology. Ticks digest large amounts of host blood and are exposed to an enormous amount of free iron, which has to be treated properly to avoid its toxicity. Two types of ferritin were discovered in the tick *Ixodes ricinus* – tagged as ferritin I and ferritin II [1]. Ferritin I is a globular protein composing 24 subunits (25kDa each) and forming a hollow-sphere complex [2, 3]. Ferritin I functions as an intracellular scavenger of potentially toxic free iron and is capable to sequester up to 4 500 iron atoms [2]. The function of ferritin II is not entirely clear, but it probably plays a role in the transport of non-heme iron between the tick gut and the peripheral tissues. Silencing of ferritin II using RNA interference had a detrimental effect on tick development and reproduction [1]. The vaccination of mammalian hosts with recombinant ferritin II revealed its promising potential as an efficient anti-tick vaccine [4].

This study focus on the molecular, biochemical and structural characterization of ferritin II from *Ixodes ricinus.* We have cloned ferritin II into two *E. coli* expression vectors (pET-SUMO and pASK-37+), optimized its production in various expression cells and conditions (e.g., temperature, times and concentrations of inducer). Further, we will focus on improving the protein solubility and its purification in order to get sufficient amounts of pure recombinant ferritin II for following structural and biochemical studies.

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