of scFv, specific to hIL7, from combinatorial antibody libraries. The fusion protein, consisting of hIL7 for scFv binding and bacterial alkaline phosphatase with enhanced catalytic activity (BAPmut) for immune complex detection, was developed. The DNA sequences encoding human IL-7 and BAPmut were subcloned into pET24a(+) plasmid vector under control of T7 promoter. E. coli BL21(DE3) cells were transformed with pET24-IL7-BAPmut expression vector and protein synthesis was induced with auto-induction protocol. Target protein was accumulated in the form of bacterial inclusion bodies. IMAX was used for purification of solubilized proteins with subsequent optimization of renaturation method. Bifunctional activity of IL-7-BAPmut fusion protein after renaturation was confirmed in ELISA and Western blot. It was shown that application of hIL7-BAPmut allows at least three times shortening the time of the screening of immune combinatory libraries of variable genes of IgG and does not require using of specific primary and secondary antibodies.

P-41-074
Analysis of butyrylcholinesterase interactions with old inhibitors and new reactivators

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Inhibition of the enzyme butyrylcholinesterase (BChE) in human tissues by binding of compounds to its active site serine is important for the detoxification and scavenging of xenobiotics such as organophosphates (OP). Although BChE is generally considered as having no physiological function, a growing body of evidence indicates that BChE plays a central role in the development of the symptomatology of Alzheimer's disease and related dementias. The most likely function for BChE is as a backup for acetylcholinesterase (AChE) and protection of synaptic AChE from man-made and naturally occurring poisons. Newly considered strategies in medical protection against nerve agents focus on the use of exogenously administered BChE. The overall idea is to administer such an enzyme in combination with a specific oxime, to scavenge an OP before it can reach and inhibit native AChE, thus helping organism detoxification from the excess OP. Starting with a directed library of pyridinium aldoximes, we identified efficient reactivators of sarin, cyclosarin, VX, and tabun-BChE conjugates and kinetically characterized their interactions in detail. Moreover, for several oximes BChE reactivation potency was showed to be promising when compared to the standard oximes used in medical practice. However, an absence of universality of reactivators underlies comprehensiveness of the reactivation mechanism and importance of the stabilization of the oxime group in vicinity of the phosphorus conjugated at the catalytic serine. Its convenient position for the nucleophilic attack is the major criteria for efficient reactivation of OP-BChE conjugates. Notwithstanding, we identified several efficient reactivators of phosphorylated BChE that, due to a cumulative capacity to reactivate both AChE and BChE, possess the potential for bioscavenging of OP. Acknowledgments: This work was supported by the Croatian Science Foundation (IP-2018-01-7683).

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Insights on the mechanism of action of class IIa bacteriocins

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Bacteriocins are ribosomally synthesized peptides with antimicrobial activity against pathogenic Gram positive bacteria. These peptides have high potency and low toxicity, and are usually specific for one or a few target microorganisms. Sakacin-A is a class IIa bacteriocin, produced by *Lactobacillus sakei*, with a specific anti- *Listeria* activity. For this reason, it has the potential to be employed to reduce the risk of *L. monocytogenes* poisoning of ready-to-eat food products. Class IIa bacteriocins exert their anti-*Listeria* activity by binding to a transmembrane receptor on the target cells, but the exact underlying mechanism has yet to be fully elucidated. The binding properties of the N- and the C-terminal domains of sakacin-A were tested against different microorganisms, by using confocal laser scanning microscopy and peptides conjugated with 5(6)-carboxyfluorescein, to investigate the molecular mechanism responsible for the specific recognition of *Listeria*. In addition, the full length peptide was inserted into liposomes of appropriate composition – where the C-terminal domain is hidden into the lipid bilayer, whereas the N-terminal half is exposed to the solvent – to perform pulldown experiments on bacterial lysates. Analysis of the liposome-bound proteins confirms a specific interaction between sakacin-A and transmembrane receptors on *Listeria* cells. Our data point to a general role of the N-terminal domain in the binding to different Gram positive bacteria, although this region is not "*per se*" sufficient for the specific recognition of *Listeria*. In conclusion, a better understanding of the mechanisms underlying the specificity and anti-microbial activity of sakacin-A will pave the way for a more efficient exploitation of class IIa bacteriocins as food preservative or as an alternative to non-protein antibiotics.