

## MEETING ABSTRACTS

# PROKARYOTIC EXPRESSION OF HUMAN BUTYRYLCHOLINESTERASE AS A TOOL FOR CATALYTIC BIOSCAVENGER DEVELOPMENT

Xavier Brazzolotto<sup>1</sup>, Alexandre Iger<sup>1</sup>, Virginia Guillon<sup>1</sup>, Gianluca Santoni<sup>2</sup>, Florian Nachon<sup>1</sup>

<sup>1</sup> Institut de Recherche Biomédicale des Armées, Département de Toxicologie et Risques Chimiques, Brétigny-sur-Orge, France

<sup>2</sup> European Synchrotron Radiation Facility, Grenoble, France

Currently, the best bioscavenger candidate against nerve agent intoxication is human butyrylcholinesterase (BChE). However, the effective dose cost, estimated to about 200 milligrams of pure enzyme, remains challenging despite the production and purification progresses realized these last years. A strategy for reducing dosage and cost would be to turn this scavenging protein into a nerve agent hydrolyzing enzyme, a catalytic bioscavenger. Up to now, screening of large mutant libraries has been hindered by the restricted eukaryotic expression of active BChE. Here we present the successful prokaryotic expression of an active human BChE variant designed with *PROSS*, a sequence- and structure-based algorithm for the soluble prokaryotic expression of difficult proteins. The protein is easily purified with two simple chromatographic steps. Despite 47 point mutations, the enzyme presents similar enzymatic parameters than the wild-type enzyme and its active site gorge structure is identical to that of the native enzyme produced in eukaryotic systems as determined by X-ray crystallography. These data validate the prokaryotic expression of human BChE which will greatly facilitate the screening of variants with nerve agent hydrolytic properties. We have initiated animal studies to assess the protein potency (immunogenicity, pharmacokinetic and bioscavenger efficiency) and will study the production of the tetramer form. On the other hand, we are currently developing high-throughput protocols for the prokaryotic expression, purification and screening of nerve agent hydrolysis.