Ruud Van Deursen et al., J Proteomics Bioinform 2015, 8:10
http://dx.doi.org/10.4172/0974-276X.C1.080

OMICS International
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International Conference on
Protein Engineering
October 26-28, 2015 Chicago, USA

Expression of de novo disulfide-rich proteins with α, β-hydrolase-type folds

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VX and analogous organophosphorous (OP) compounds display their toxicity by rapidly inhibiting acetylcholinesterases (AChE) in the central nervous system. Upon binding of the OP compound to the catalytic serine, AChE and similar naturally occurring enzymes are frequently reported to undergo 'aging'. In this process the enzymatic activity is inhibited by irreversible binding of OP compounds to the histidine in the catalytic site. The membrane protein phospholipase A2 (Group VIII) is an exception and is widely resistant against aging, allowing the use of oximes as strong nucleophile in a competitive substitution reaction to restore the catalytic activity. Rosetta has been previously used to create new enzymes based on phosphotriesterases (PTE) obtained from soil bacteria. Further optimization of the catalytic efficiency is however needed. The well conserved α, β-hydrolase-type fold of the phospholipase A2 (group VIII) has been chosen as starting point of this alternate de novo design approach for the reason of the slow aging rates. Key part of this 60 residue minimal core is a flat beta sheet that is composed of 3 parallel strands with a length of 5 residues each. This sheet is surrounded by at least three alpha helices. The small size of this design asks for the introduction of disulfide bonds for stabilization. Herein we present our experimental setup to secrete these de novo designed disulfide-rich proteins using fusion constructs with different leader sequences of the Sec-, SRP- and TAT-secretion pathways.

Biography

Ruud Van Deursen has completed his PhD from University of Berne. After Postdoctoral studies at Ecole Polytechnique Federale de Lausanne in Switzerland on high-throughput screening, he has joined the Baker Laboratory at the University of Washington in Seattle to work on de novo design of nucelophilic catalysts and expression of disulfide-rich proteins. He has published 16 publications as an author and co-author in the fields of CADD and biocatalysis.

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