**COMPUTATIONAL STUDY OF THE HYPERGLYCOSYLATED HRP ENZYME USED IN THE PATHOGEN DETECTION**

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The main objective of the MARILIA project is to develop a novel pathogen detection concept for water samples. Powerful tools for detecting protein-protein interactions (PPI), created between a pathogen and a protein detector, can be developed by using protein-fragment complementation assays (PCA) [1]. A new type of PCA has been recently developed by Martell and associates [2] based on the horseradish peroxidase (HRP). The HRP enzyme PCA has numerous advantages over other similar PCA, such as: functioning in extracellular environments, generating spatially restricted fluorescent signal, etc. However, hyperglycosylation can present a significant hurdle when expressing recombinant HRP in yeast cells [3].

Using the Molecular Dynamics (MD) simulations we have studied the effect of glycans on the availability of the surface groups, including the HIS-tag groups needed for the purification process. The results of the simulations show strong influence of the glycan on the overall stability of the HRP enzyme, but also indicate some critical drawbacks in availability of the surface groups. Different forms of the HRP enzyme, that are used in the HRP PCA, have also been simulated.

Additionally, the influence of the mutations introduced by Martell and associates was also studied by MD simulations. We have identified key mutations that cause significant structural changes in the HRP enzyme.

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