COMPUTATIONAL STUDY OF THE GLYCOSYLATED HRP ENZYME USED IN 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, 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 Molecular Dynamics (MD) simulations the effect of glycans on the availability of the surface groups, including the HIS-tag groups needed for the purification process, were investtigated. The results of MD 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 that present possible targets for the attachment of the linker that is necessary for PCA design. Different forms of the HRP enzyme, that are used in the HRP PCA, have been simulated.

Additionally, the influence of the mutations introduced by Martell and associates [2] was also studied by MD simulations. Key mutations that cause significant structural and dynamical changes in the HRP enzyme were identified.

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REFERENCES

[1] S.W. Michnick, P.H. Ear, E.N. Manderson, I. Remy, E. Stefan, *Nat. Rev. Drug Discov.* 2007, 6, 569–582.

[2] J.D. Martell, M. Yamagata, T.J. Deerinck, S. Phan, C.G. Kwa, M.H. Ellisman, J.R. Sanes, A.Y. Ting, *Nat Biotechnol* **2016**, 34, 774–780.

[3] S. Capone, R. Pletzenauer, D. Maresch, K. Metzger, F. Altmann, C. Herwig, O. Spadiut, *Glycobiology* **2014**, 24, 852–863.