An engineered lactate dehydrogenase with direct electron transfer to an electrode for lactate biosensing

Anastasya Boyarski,[1] Shiraz Paz[2], Noam Shlush[2] Lital Alfonta[1,2]

[1]Department of Chemistry, [2]Department of Life Sciences, Ben-Gurion University of Negev, POBox 653, Beer-Sheva, 8410501, Israel

Lactic acidosis is a process in which under endurance-based activities glycogen is consumed to produce energy and rising lactate levels. Measuring lactate levels can establish the fitness of an athlete along with exercise intensity. Lactate balance is important for acid-base homeostasis, disruption of which can also result in lactic acidosis, in addition, this irregularity can occur, due to decreased tissue oxygenation, chronic diseases, drugs, toxins and more. Therefore, to measure lactate in different bodily fluids, sensitive sensor is required. Lactate dehydrogenase from Baker's yeast (ScLDH) is a perfect candidate for such biosensor. ScLDH has two functional domains, the active-site, which uses Flavin mononucleotide (FMN) as a cofactor to convert lactate to pyruvate, and a cytochrome-b domain, which transfers electrons from the active site to an electron acceptor. In the native electron-transfer chain, electrons would have been transferred to an external cytochrome-c. In our work, we present an improved biosensor, by fusing a minimal cytochrome-c (MCD) to the native enzyme, thus enabling faster electron transfer and lower detection threshold for lactate. In our study, we have compared different engineered constructs of ScLDH: i) MCD was fused to the native enzyme to improve the electron transfer rate and to mimic the native electron transfer pathway (ScLDH-MCD); ii) the cytochrome-b domain was replaced with MCD (ScLDH-MCDΔB); iii) cytochrome-b domain was removed, and the enzyme was left only with its active-site (ScLDH-ΔB). Preliminary results, show an outstanding activity of ScLDH-MCD compared to other constructs, while different kinetic properties were compered between variants.