

NIH COMMON FUND HIGH-RISK HIGH-REWARD RESEARCH SYMPOSIUM

DECEMBER 15 – 17, 2014

SPEAKER ABSTRACTS

From sperm to stroke: the science of tethering enzymes with applications from nanoscale energy production to handheld diagnostics for neural injury

Awardee: Alexander Travis

Award: Pioneer Award

Awardee Institution: Cornell University

The ability to attach functional enzymes to nanostructures could enable a number of future medical applications, ranging from devices traveling through our bloodstreams to deliver drugs at a specific tissue, to handheld devices diagnosing disease within minutes from a few drops of blood. However, multiple challenges exist. Being bound to a surface can interfere with enzyme activity by limiting diffusion of substrates, blocking substrate binding sites, or interfering with conformational changes. Mammalian sperm have overcome these challenges by having specialized glycolytic enzymes, designed to function when tethered to a cytoskeletal structure in the flagellum. Through this “solid-state” design, sperm produce energy locally where needed. This inspired us to pursue a biomimetic strategy of oriented immobilization, tethering enzymes by modifying or replacing their biological targeting domains.

Using this approach, we demonstrated several coupled glycolytic reactions, including the first demonstration of co-tethered, sequential steps of any biological pathway. We’ve shown that when attached using oriented immobilization, enzymes representing several enzyme classes had much higher specific activities than if attached by random adsorption or by chemically-specific but non-oriented immobilization (e.g. carboxyl-amine binding). These improvements have enabled us to achieve our goal of tethering all eleven enzymes of glycolysis to nanoparticles in series and generating the end product of lactate from glucose. This is the first time any complete biological pathway has been reconstituted on a scaffold, and is a significant advancement in tethered reactions over the previous high of three enzymes. Although overall glucose utilization was low, the efficiency of flux through the tethered pathway was higher than when the enzymes were in solution. These data provide proof of principle that tethered glycolytic enzymes could serve as an energy-generating platform technology. Toward this goal, we are working to achieve net ATP production.

Our tethered enzymes enable us to investigate fundamental relationships such as the effects of nanoparticle size and composition on enzyme function and multilayer formation. We are also pursuing immediate applications for tethered enzymes including developing point-of-care diagnostic devices for time-sensitive pathologies such as stroke. This application capitalizes on several advantages of tethered enzymes, including speed, sensitivity, ability to be incorporated into microfluidic devices, and portability. Moving beyond our Pioneer Award, we now show in rat models of stroke and samples from human patients that our tethered enzymes can detect physiological and pathological levels of blood-borne biomarkers within 5-10 minutes, in comparison to hours typically needed for existing technologies (e.g. ELISA).