CHEMILUMINESCENT DETECTION OF REACTIVE OXYGEN SPECIES

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BACKGROUND: Chemiluminescence (CL) describes the phenomenon of energy transfer from the decomposition of an energetic species (typically a 'reactive oxygen species' or 'ROS' such as peroxides) to an appropriate chromophore, elevating the chromophore to its excited state; upon relaxation and energy release back to its ground state, light is emitted at a wavelength characteristic of the chromophore.

Firefly light production, courtesy of the enzyme luciferase and its co-factor, luciferin, rely on CL; a second common example of CL is the femtomolar detection of proteins when horseradish peroxidase is incorporated into the assay.

PHYSIOLOGICAL ROS: ROS are a normal consequence of cellular metabolism and energy production. In healthy cells, ROS generation, use and disposal are tightly regulated processes in order to avoid dangerous, unwanted interactions; a cardinal sign of cellular dysregulation is the overproduction of ROS and/or the lost ability to properly dispose of them. Biologically relevant ROS include: superoxide radical; singlet oxygen; hydroxyl radical; and hydrogen peroxide. As the cellular power plant, mitochondria therefore also produce the most endogenous ROS; in the case of fNIRS, it follows that stimulation of cytochrome c oxidase (COX)- the terminal enzyme in the mitochondrial electron transport chain (ETC)- has the potential to also stimulate increased ROS production. The ability to detect this change, if any, and monitor for cellular dysregulation with regard to ROS management would be informative.

PRIOR CL RESEARCH: At a former company, we developed, patented and commercialized a system for the detection of explosives and other species (see US Patent 9005524). This device, marketed by ICx Technology/FLIR Systems as 'PaxPoint', relied on the presence of an energetic peroxide species reacting with a suitable oxalate compound, rapidly forming a highly constrained, energetic and unstable dioxetane species and then decomposing to transfer this energy to a suitable chromophore. This research experience, along with an extensive history of custom dye synthesis, provides a strong foundation into CL-based sensors and facilitates rapid application to the biological system.

SUMMARY: CL provides a sensitive and specific means of measuring the influence of fNIRS stimulation as it relates to ROS generation and regulation. Design, synthesis and optimization of the chemical components required for CL-based measurement of intracellular ROS (chromophore and oxalate-type compound) will be performed by Akita Innovations.