Genetically encoded biosensors for cellular metabolism

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Historically, L-lactate (lactic acid) has been viewed as a waste by-products of glucose metabolism. However, growing evidence suggests that L-lactate is better considered a biological fuel currency and signaling molecule, that can be shuttled over scales and environments ranging from subcellular, to intercellular, to interorgan. For example, tumor cells with enhanced aerobic glycolysis produce and release L-lactate, by which L-lactate accumulation in tumor microenvironment hampers T cell activation and its survival. To comprehensively explore the emerging roles of L-lactate, methods for monitoring of its concentration in live tissues are of broad utility and high impact. However, there remains a technological gap in high-performance biosensors capable of monitoring L-lactate metabolism in living organisms with high spatiotemporal resolution.

Herein, we present that directed protein evolution and extensive biosensor expression optimization can enable the engineering of fluorescent protein-based biosensors[1], designated the LACCO series, for a versatile metabolite L-lactate with high sensitivity, brightness, specificity, and spatiotemporal resolution in living cultured cells and *in vivo*[2–5]. We used these state-of-the-art biosensors to achieve a visualization of the L-lactate dynamics across cellular compartments, revealing an unprecedented L-lactate shuttle in a spatiotemporal manner. We also have resolved their structures using X-ray crystallography and cryo-electron microscopy (cryo-EM), and provided insights into their molecular mechanism of lactate-dependent fluorescence response. The latest LACCO series represents a major technological leap towards interrogations of the emerging roles of L-lactate in living cells and tissues.

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