

AspSnFR: A Genetically-Encoded Biosensor for Real-Time Monitoring of Aspartate in Live Cells

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Aspartate plays a pivotal role in nucleotide synthesis, ammonia detoxification, and redox balance through the Malate-Aspartate-Shuttle (MAS). To unravel the complex roles of aspartate metabolism, real-time and live-cell measurement tools are essential. We present AspSnFR, a green fluorescent, genetically-encoded biosensor for aspartate inside cells, developed by screening and testing libraries of biosensors in mammalian cells. AspSnFR accurately measures cytosolic aspartate levels and shows how it is synthesized from glutamine. By integrating high-content imaging of AspSnFR with drug interventions, we identify how aspartate metabolism responds under different nutrient environments. Additionally, AspSnFR helps monitor aspartate efflux from mitochondria via SLC25A12, a key transporter in the MAS. In live cells, we demonstrate that SLC25A12 rapidly links Ca²⁺ signaling with the export of mitochondrial aspartate, highlighting its essential role in connecting cellular signaling, mitochondrial function, and overall metabolism.