博士論文（要約）

Visualization analysis of extracellular ATP in the brain *in vivo* with a hybrid-type fluorescent sensor

（ハイブリッド型蛍光センサーを用いた
生体内における脳の細胞外 **ATP** の可視化解析）

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Adenosine 5’ triphosphate (ATP), which is known as the intracellular energy currency, works as an extracellular signaling molecule in various tissues and organs including the brain. In the brain, ATP is released from neurons and glia, and involved in a variety of physiological and pathological cellular processes. To clarify the mechanisms of these processes, it is important to understand the dynamics of extracellular ATP signaling in the brain. However, conventional techniques for measurement of extracellular ATP cannot provide detailed information on the spatiotemporal dynamics of extracellular ATP in the brain *in vivo*.

In this study, I describe a technique for *in vivo* imaging of extracellular ATP in the brain with high spatiotemporal resolution. This technique uses a hybrid-type fluorescent ATP sensor, in which an ATP-binding protein, *Bacillus* FoF_1-ATP synthase ε subunit, is labeled with a small-molecule fluorescent dye. Screening of the dye and the dye-labeling site within the ε subunit revealed that a fluorescent sensor in which the ε subunit is labeled with Cy3 at glutamine-105 (designated ATPOS) shows a large fluorescence response to ATP, with submicromolar affinity, pH-independence, and high selectivity for ATP over ATP metabolites and other nucleotides. For application of ATPOS to imaging of extracellular ATP in the brain, I introduced BoNT/C-Hc for binding to neuronal cell membrane and Alexa Fluor 488 for ratiometric measurement. The resulting ATPOS complex injected into the cerebral cortex of living mice works as a ratiometric fluorescent sensor for extracellular ATP. I applied the newly-developed ATP imaging technique to visualize extracellular ATP signaling in pathological neuronal excitation known as cortical spreading depression (CSD), and found a propagating wave of extracellular ATP release during CSD. I also demonstrated that wave-like propagation of extracellular ATP release repetitively takes place during brain ischemia. Furthermore, I found that the wave-like extracellular ATP signaling has capability to regulate vasoconstriction during CSD and brain ischemia. Thus, ATPOS should be useful to probe the dynamics of extracellular ATP in diverse biological processes *in vivo*, and valuable for understanding the functions of extracellular ATP signaling in the brain.