Minimalistic cyclopropene probes as bioorthogonal endocannabinoids to study lipid localization with increased spatiotemporal resolution

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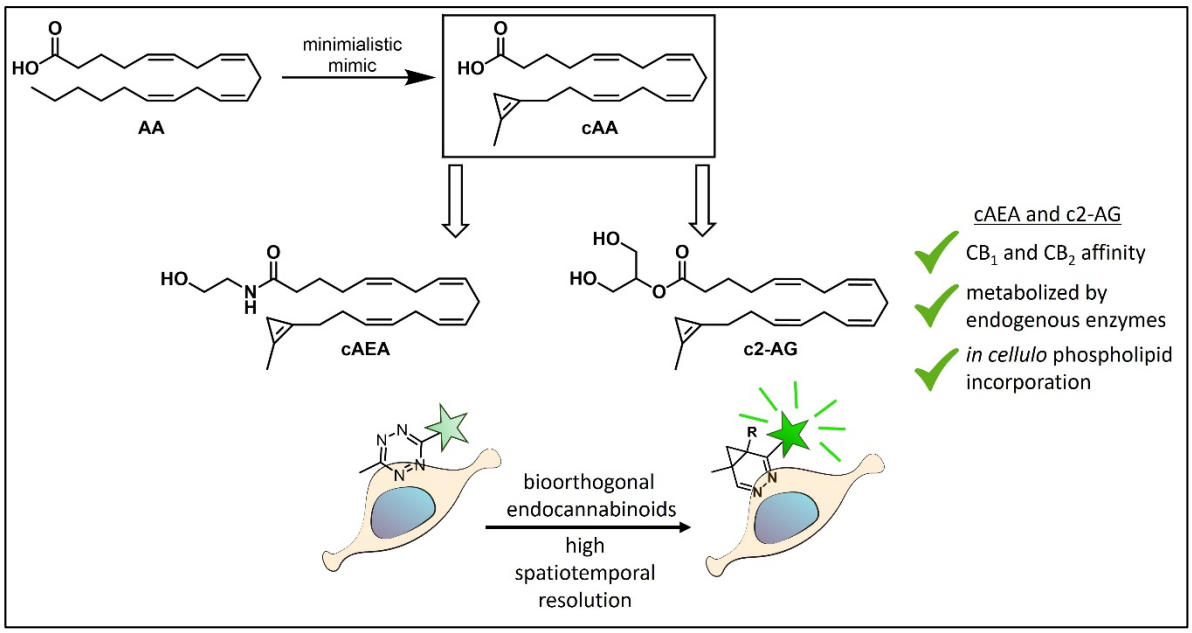
Radiolabeled analogues and alkyne probes are among the most used tools to study lipid distribution and uptake. However, these methods provide low spatiotemporal resolution and are mostly incompatible with living systems. Although these tools have adequately described the biosynthetic and metabolic pathways of lipids, they fall short to report on the dynamic localization of the species themselves.[1] Much is unclear about the way lipids traverse the hydrophilic cytosol and extracellular space, are transported between different membranes or their fate in systemic circulation. The inverse electron demand Diels-Alder reaction between cyclopropenes and tetrazines has proven a valuable method to ligate reporter moieties in a fast manner that is compatible with living systems.[2] We hypothesized that the incorporation of a 1,2-cyclopropene would constitute a minimal physiochemical change compared to the original lipid structure, therefore preserving most of the endogenous interaction profile.[3] In here, we have synthesized such as set of cyclopropene lipid probes, tailored to study the endocannabinoid lipid messengers, capable of ligating to tetrazine fluorophores for lipid localization. We show that the bioorthogonal endocannabinoids retain certain biological properties of the endogenous endocannabinoids such as activation of the CB_1 and CB_2 receptors and hydrolysis by the metabolizing enzyme MAGL. In addition, the bioorthogonal endocannabinoids react with quenched tetrazine fluorophores, are actively incorporated into other lipids by cells and can be used to measure real-time lipid uptake. Taken together, cyclopropene lipids are a promising model for endogenous lipid localization with increased spatiotemporal resolution.



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