Introduction
Luciferin has been well studied as a reporter of cellular processes since its initial isolation in 1949. Luciferase is a highly specific enzyme due to its deep binding pocket and requirement for Mg\(^{2+}\), ATP, and Luciferin, or a Luciferin analogue preserving the thiazole and carboxylic acid moieties. In this study we sought to develop a novel set of Luciferin based sensors to probe for supramolecular assembly through bioluminescence based assays. Exploiting this previous knowledge, we designed several 6’-OH functionalized derivatives with well characterized supramolecular guest moieties to impart high binding affinity with supramolecular hosts, most specifically cucurbit[7]uril (CB[7]) and β-cyclodextrin.

Sensor Design
Scheme 1: a) D-Cysteine in aqueous, pH 8; b) DMF, 1,2 dibromoethane, cesium carbonate, 80oC; c) propargyl bromide, cesium carbonate; d) THF, quinuclidine; e) THF, trimethylamine; f) 1-azidoadamantane CuAAC; g) 1-azidoadamantane CuAAC. (Dashed arrows represent proposed synthetic steps towards functionalized luciferin)

Enzyme Inhibition Discussion
Firefly luciferin shows a steady decrease in reaction rate as CB[7] is added to the system. The initial increase observed in analogues could be caused by CB[7] binding to the Hydrophobic moiety followed by binding to the aromatic core. Luciferase has a deep active site which fully encapsulates the native substrate. The portal to the active site of luciferase has two arginine residues which would be positively charged under basic conditions. Binding of CB[7] could shield the moiety from these charges, increasing binding affinity of the analogue to luciferase, consequently increasing the rate of reaction.

References

Conclusions
In this study we have successfully designed and synthesized 6’-OH functionalized Luciferin analogues with responsibility to CB[7] by coupling them to well-characterized CB[7] binding moieties. We have also begun to characterize how these analogues interact with CB[7] and the Luciferase enzyme. Further research will focus on synthesizing control analogues, such as those proposed in the synthetic scheme, fully characterizing the binding of CB[7] and other host molecules to our novel molecules, and applying these molecules as tools to probe supramolecular assembly in situ and in vivo.

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