Towards a chemigenetic calcium ion sensor based on an engineered calmodulin and an environmentally sensitive synthetic dye fluorophore

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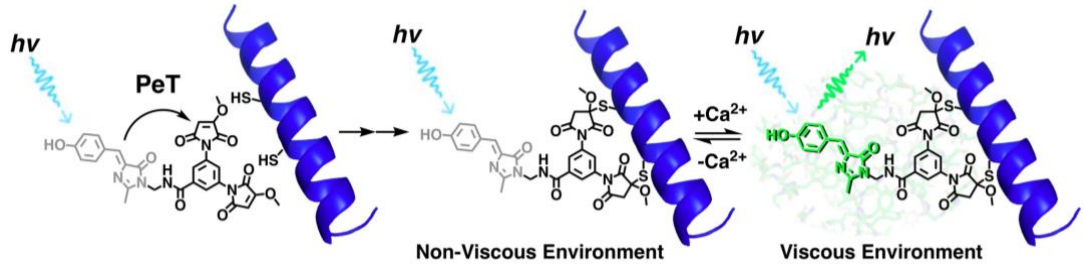
Calcium ions are a ubiquitous messenger within cells, and spatiotemporal imaging of calcium ions within cells and tissue is important to further our understanding of their role within biology. The first-generation calcium ion indicators were entirely organic and composed of a fluorophore covalently linked to a calcium chelator known as BAPTA.[2] After the discovery of the green fluorescent protein, many new types of purely genetically encoded calcium indicators were designed based on the fusion of a fluorescent protein to calmodulin, a calcium sensing domain.[3] To further expand the repertoire, "chemigenetic" calcium indicators have been recently proposed, but their structure and design are still limited.[4] In this work, we explore a novel chemigenetic calcium indicator design based on a genetically encoded calcium sensing polypeptide and a synthetic dye that undergoes a fluorogenic reaction.

For the polypeptide, we genetically fused calmodulin and M13 calcium sensing domains to an α-helix containing two cysteines. For the dye, an environmentally-sensitive green fluorescent protein (GFP) chromophore analog called hydroxybenzylidene imidazolinone (HBI) was conjugated to a dimaleimide, which will quench the dye's fluorescence by photoinduced electron transfer (PeT) until the dimaleimide has reacted with the polypeptide's dicysteine motif.[5] In the presence of calcium ions, the M13 peptide and calmodulin will change their conformation to effectively modulate the local environment surrounding the dye and therefore its brightness. Our most recent progress about the dye synthesis, in vitro characterization, and computational validation will be reported.



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