Second harmonic generation (SHG) characterization of membrane protein conformational change – A novel technology for on-target binding and mechanism of actions studies

David Shaya, Ben Moree, Tracy Young

Biodesy Inc. 170 Harbor Way #100, South San Francisco, CA 94080

Second-harmonic generation (SHG) has recently emerged as a biophysical tool for conformational sensing of bi-molecular interactions between target biomolecules such as membrane protein and a wide range of ligands.

The technique relies on rendering a target protein SH-active by labeling with a dye-probe either via lysine or cysteine residues. The labeled biomolecules are tethered to a surface. The efficiency of SHG-light generation depends very sensitively on the angular orientation of SH-active dyes with respect to the surface to which the labeled biomolecules are tethered. Ligand-induced conformational changes of the labeled biomolecule that result in net dye movement are observed by a change of the SHG signal intensity. Thus differences in SHG signal intensities allow for sensing on-target binding and/or discrimination of ligands by mechanism of action.

Here, we describe SHG characterization of membrane protein conformational changes upon binding of tool compounds. We demonstrate how SHG enables the discovery of new chemical matter through screening in a 384 or 1536 well format. In addition, we demonstrate how SHG can distinguish different mechanisms of action through ligand binding (e.g. agonist vs. antagonist). We are looking for collaborations and early adaptors to help us refine this technology for analysis of membrane protein conformation change and binding characterization.

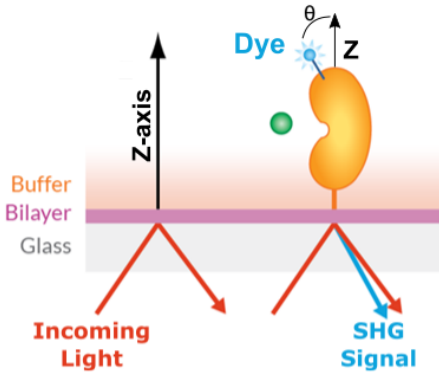

Figure legend: The target protein is denoted as a yellow kidney bean. SHG signal sensitivity/efficiency depends on the angle θ between the SH-active dye probe and the surface normal Z.

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