

Direct, Real-time Detection of Protein Conformation: Revealing Therapeutic Opportunities Using Second Harmonic Generation (SHG) Detection

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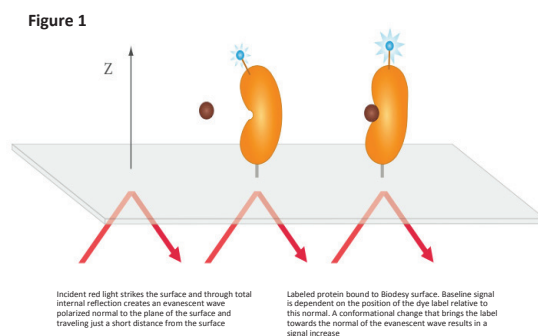
Poster M143

Abstract

Changes in conformation underlie the fundamental function of all proteins. Innovative therapeutics such as Gleevec function by holding its target protein in a non-active conformation that is unique across the kinome. Direct detection of protein conformational change offers a significant opportunity for the design of structurally-specific therapeutics. We have adapted Second Harmonic Generation (SHG), an optical technique to a discovery format to detect conformational change in a series of protein standards which represent a range of movement (rigid to floppy.) We will describe our use of SHG to screen and identify inhibitors of spermine-induced conformational change in monomeric alpha-synuclein, a key protein implicated in the etiology of Parkinson's disease. In addition, we will introduce SHG as a means of uniquely identifying conformation-specific changes in kinases.

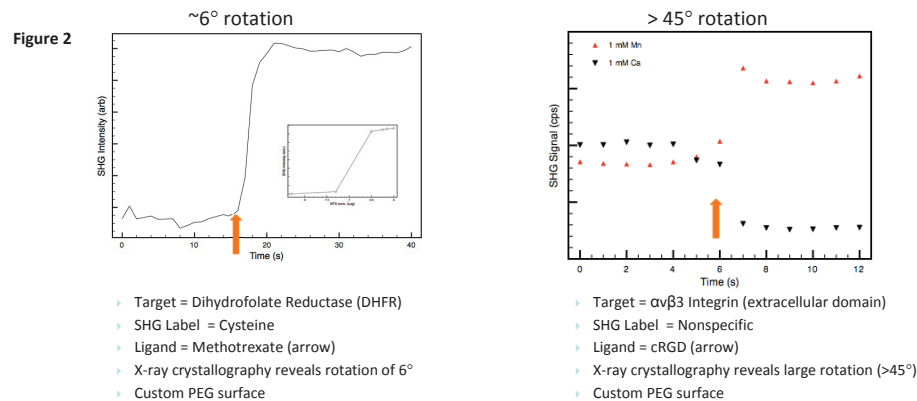
Introduction

Biodesy has developed a platform for directly detecting conformational changes in proteins sensitively and rapidly using a technique called 'Second-harmonic generation' or SHG^{1,2}. A schematic of the SHG process is shown in Fig. 1. A protein is labeled with a second-harmonic-active dye (typically also fluorescent) of ~500 Da, either to amines or cysteines. The labeled protein is placed on a proprietary surface and incident light from an ultrafast laser is shone on the surface, shown in red. A small portion of the incident red light is converted to the second harmonic: two photons of red light are combined by the dye to generate one photon of blue light. The blue light (the second harmonic) is the baseline signal and is highly sensitive to the orientation of the label relative to the surface plane. Rotation of the average orientation of the label, which occurs upon ligand binding to the protein due to conformational change, also changes the intensity of the blue light. The signal response occurs instantaneously as conformational change occurs.



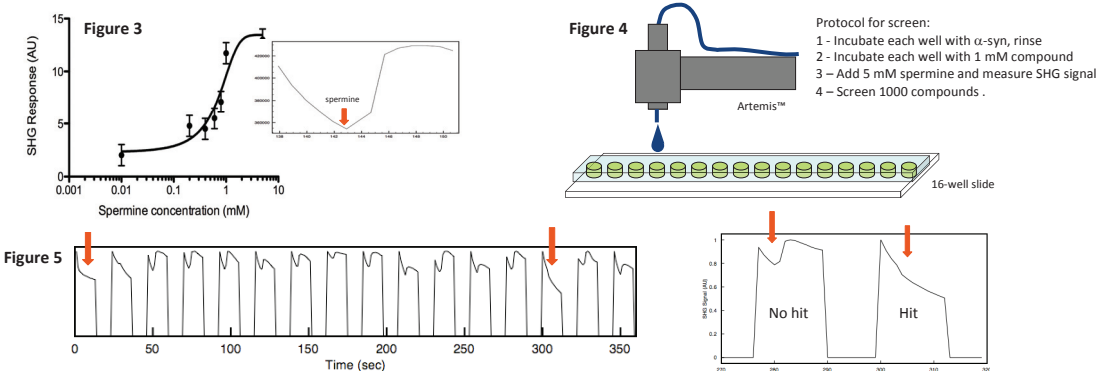
SHG detects both small and large conformational changes

Methotrexate (MTX) induces a significant SHG signal change upon exposure to Dihydrofolate reductase (DHFR), which undergoes a small, 6° rotation between lobes³ (Fig. 2 left). The effect of MTX on conformational change is concentration-dependent (inset). cRGD induces signal changes upon binding to αvβ3 integrin (Fig. 2 right). The signal changes depend on whether the protein is co-incubated with Ca²⁺ or Mn²⁺. cRGD in the presence of the divalent cations induce different signal changes in direction and magnitude due to different conformations induced by these ligands, as confirmed by electron microscopy⁴.



Spermine induces conformational change in α-synuclein (α-syn)

We have applied our platform to develop an assay for spermine-induced conformational change of monomeric α-synuclein, a protein implicated in the pathophysiology of Parkinson's disease (PD)⁵. We screened a fragment library for compounds that stabilize the 'closed' conformation of the protein (5,6). A mutant of α-syn (A90C) was labeled with an SHG-active dye, bound to a surface and exposed to spermine, which induces an upward shift in SHG signal (Fig. 3 inset). Spermine was titrated to determine its EC₅₀ (0.58 ± 0.09 mM (n=3)) compared with spermine's known KD = 0.63 mM⁵ (Fig. 3).



Screen for fragment inhibition

Using Biodesy's Artemis™ platform (Fig. 4), the Maybridge Ro3 fragment library (1000 compounds) was screened for inhibitory activity. An example of data (16 wells from a slide) is shown in Fig. 5 left. Putative hits (orange arrows; expanded traces shown in Fig. 5 right) were identified by an ability to completely prevent spermine-induced conformational change. Hits were retested a minimum of six times to verify activity.

SHG identifies multiple hits

Five fragments were identified in the SHG screen to completely inhibit spermine's effect. The compounds range from 120 – 230 MW, are all water soluble up to 1 mM and do not appear to be amphipathic from physical-chemical considerations. They completely inhibited spermine's effect in the presence of 0.001% Triton X-100, confirming their mechanism of action does not involve aggregation. Their IC₅₀'s were determined to be between 100 μM and 1 mM by SHG titration experiments. Two of the four compounds possess known blood-brain barrier (BBB) permeability. These were tested in a BiFc α-syn dimerization assay in H4 neuronal cells⁶. Both compounds inhibited dimerization compared to controls with compounds from the Ro3 library that did not register as hits in the SHG assay (Table 1 - compounds 3 and 4 are controls).

SHG detection occurs in real-time, so the effect of each compound on α-syn conformation by itself was also measured. One of the five compounds produced a response very similar in direction and magnitude to that produced by spermine (Fig. 6). This compound is the only diamine in the library and structurally similar to spermine, a tetramine. We believe it is acting like spermine to produce the 'open' conformation of the protein, preventing any further change when spermine is added. The other four fragment hits either have no effect on α-syn conformation or produce a response that is different from spermine's. NMR studies currently underway confirm that at least one compound binds to the C-terminus of monomeric α-syn, where spermine also binds. These structure-specific inhibitors may represent attractive therapeutics for the treatment of PD.

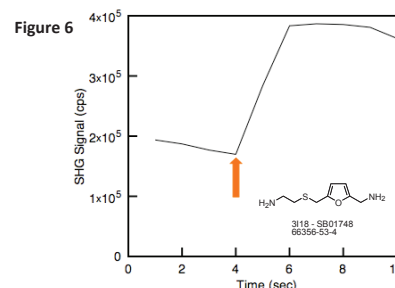


Table 1

	Compound 1	Compound 2	Compound 3	Compound 4
SHG Inhibition	Complete	Complete	None	None
BiFc Inhibition (% control)	0.69 ± 0.23	0.74 ± 0.21	0.96 ± 0.20	0.95 ± 0.34
Hits	✓	✓	-	-

References:

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