THE UNIVERSITY OF TEXAS MD Anderson Cancer Center

BIODESY

Determination Of Inhibitor Binding Site (Allosteric vs. Active Site) By Protein Phosphatase Conformational Signatures Detected By Second-Harmonic Generation

Young T.[1], Salafsky J.[1], Leonard P.[2], Mseeh F.[2], Czako B.[2], Jones P[2], Parker C.[2] and Geck Do M.[2]

[1]Biodesy, Inc., 384 Oyster Point Blvd. #8, South San Francisco, CA 94080 [2]Institute for Applied Cancer Science, The University of Texas MD Anderson Cancer Center

Introduction

Proteins are dynamic molecules that perform specialized functions through unique conformational changes accessible in physiological environments. An ability to specifically and selectively control protein function via conformational modulation is an important goal for development of novel therapeutics and studies of protein mechanism in biological networks and disease. This is exemplified in the SH2 domain-containing phosphatase 2 (Shp2), a bona fide proto-oncogene member of the protein tyrosine phosphatases (PTP) superfamily. Shp2 exists in equilibrium between multiple conformations, simplified herein as "open" and "closed" states. It consists of two Src Homology domain 2 (SH2) domains and a Protein Tyrosine Phosphatase (PTP) domain. Crystal structures revealed an autoinhibited state where the N-terminal SH2 domain (N-SH2) blocks access to the phosphatase active site. A gain of function mutation, E76K, results in constitutive activation by destabilizing the autoinhibited closed state. Shp2 inhibitors have been reported and they can be generally classified as PTP site binders or allosteric binders. Development of those PTP competitive inhibitors has proven challenging in part due to selectivity issues. One approach to overcome those challenges is to target non-conserved regions outside the active site via development of allosteric inhibitors. Therefore, it is desirable to have assays that can readily identify the binding mode for potential inhibitors.

Here we applied a second-harmonic generation (SHG) based technique for determining the conformational signatures of full-length wild-type Shp2 (FL-Shp2(WT)) as well as full-length Shp2(E76K) (FL-Shp2(E76K)) mutant protein. Using the Biodesy Delta System and a set of previously characterized inhibitors, we confirmed two distinct phosphatase conformations depending on the inhibitor binding mode to the allosteric or PTP catalytic site. This assay was then utilized to bin 62 test compounds into allosteric or PTP site binders.

Methods

His tagged FL-Shp2(WT) and FL-Shp2(E76K) were labeled with the succinimidyl ester of an SH-active dye. Supported lipid bilayers containing a Ni-NTA lipid were used to tether the dye-conjugated proteins to the surface. Unbound protein was washed out, and ligands were added. The SHG signal corresponding to the change in dye orientation relative to the surface normal was recorded. Percent change in SHG intensity (%Δ) at a given time point t was calculated as: $\%\Delta = ((I_t - I_{t0})/I_{t0}) \times 100$ where I_t is the SHG intensity at time t and I_{t0} is the SHG intensity at time 0 before injection.

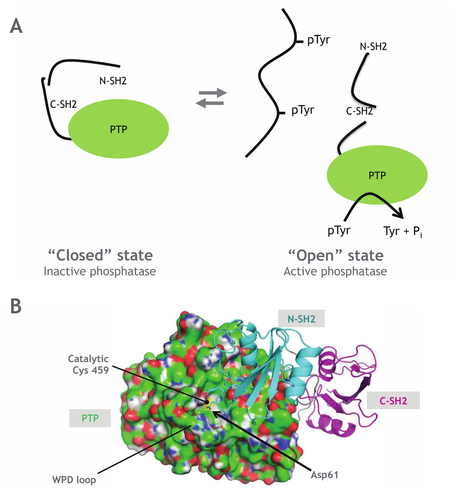

Figure 1: Shp2 Exists in Multiple Conformations. A) Stimulation by binding of phosphorylated proteins to SH2 domains relieves auto-inhibition, making the active site available. B) Asp61 from the N-SH2 domain acts like a phosphomimic forming salt bridges with Arg362 and Arg465 in the active site as well as five water-mediated hydrogen bonds. Mutations to residues in the interdomain interface between the SH2 domains and PTP domain can destabilize the closed conformation leading to increased activation of the Shp2 phosphatase. C) Gain of function mutations (e.g., E76K) promote the open form of Shp2. FL-Shp2(E76K) is as active as the isolated PTP domain.

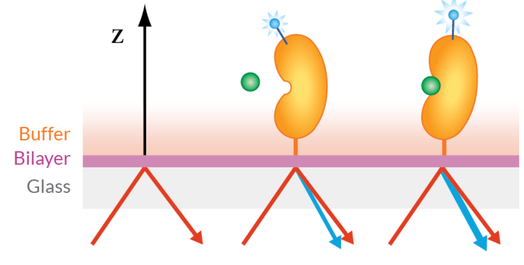

Figure 2: Schematic of Second-Harmonic Generation (SHG). SHG is an optical phenomenon that enables the measurement of sub-Angstrom changes in SH-active dye orientation. SHG intensity is exquisitely sensitive (10^6) to the angle of the dye with respect to the surface. Different changes in conformation produce differentiated SHG signatures.

Results

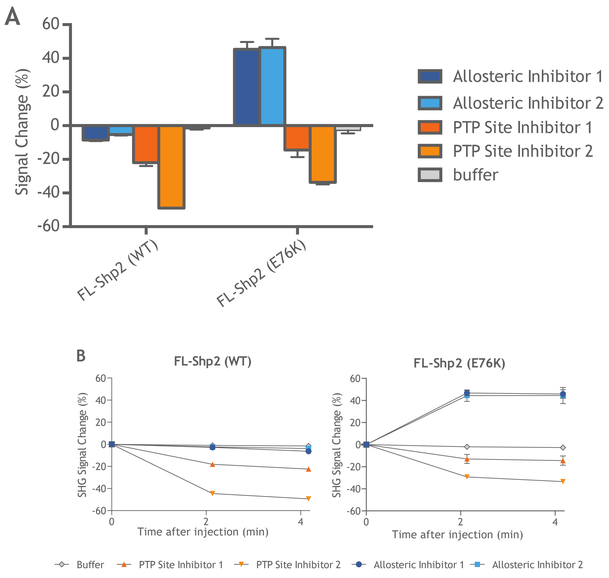

Figure 3: Shp2 Conformational Signatures. A) Allosteric inhibitors 1 and 2 show a large, positive signal change for FL-Shp2(E76K) and no/little signal change for FL-Shp2(WT), indicating distinct behavior of the auto-inhibited vs. the constitutively active proteins. The PTP site inhibitors produce a negative signal change in both proteins indicating a similar binding mode. B) Conformational change time course of the allosteric and PTP site binders on FL-Shp2(WT) and FL-Shp2(E76K).

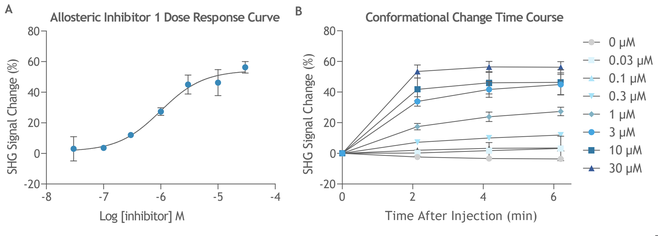

Figure 4: Dose-Response Curve for Shp2 Allosteric Inhibitor 1. The SHG conformational signature (A) and the conformational change time course (B) of FL-Shp2(E76K) is dose dependent.

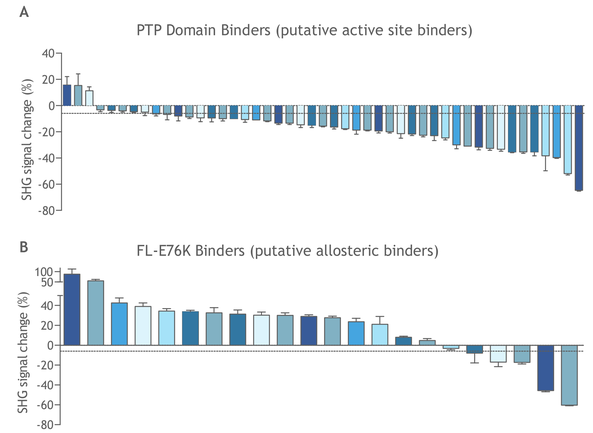

Figure 5: Binning of Allosteric and PTP Site Inhibitors by Conformational Signature of FL-Shp2(E76K). Binding mode was determined by inhibition in an enzymatic activity assay. A) Of the 42 PTP domain binders, 35 show the expected negative SHG signal change, 3 show a positive signal change, and 4 are within the noise. B) Of the 20 allosteric binders, 14 show the expected positive signal change, 4 show a negative signal change, and 1 is within the noise. Allosteric inhibitors showing a negative signal change are being explored further.

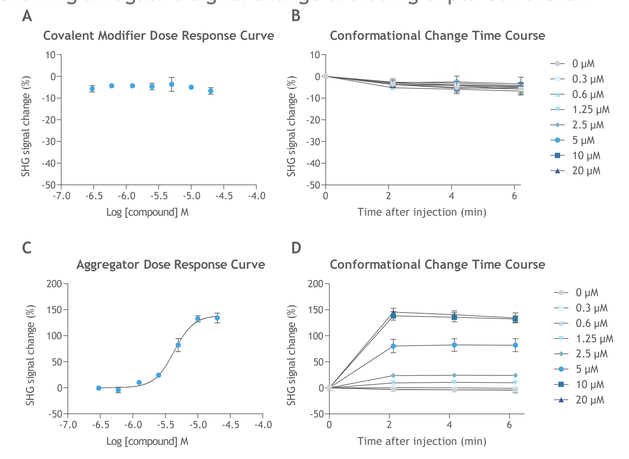

Figure 6: Bad Acting Compounds Detected by FL-Shp2(E76K) SHG Assay. A) and B) Dose-response curve and conformational change time course for a known covalent modifier. C) and D) Dose-response curve and conformational change time course for a known aggregator.

Conclusions

The design of new selective PTP inhibitors is a highly promising, yet extremely challenging task. We offer here a new approach based on SHG that could have far-reaching consequences for selective PTP inhibition and drug discovery in general. The role of conformational dynamics in drug binding is acknowledged, but the current methods for determination of binding mode are time consuming and require expertise. The SHG technique presented here sensitively detects ligand-induced conformational changes, thereby monitoring conformational changes in the protein-drug complex directly. This allows selection of compounds based not only on their ability to bind, but also, crucially, on their effect on the conformational ensemble, thus dramatically increasing the chances of identifying conformation-selective compounds. We anticipate that the unique advances delivered by the approach described here will have immediate impact in drug design for the PTP superfamily, and generally for other targets.