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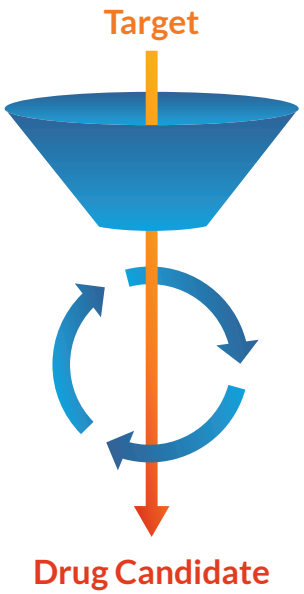
Screening with conformation: the Biodesy Delta

Abstract

Protein structure defines function. Modern efforts to develop selective and potent therapies seek to improve human health by perturbing protein conformation. Thus, measuring ligand-induced conformational change earlier in the screening process holds promise to finding better drugs faster.

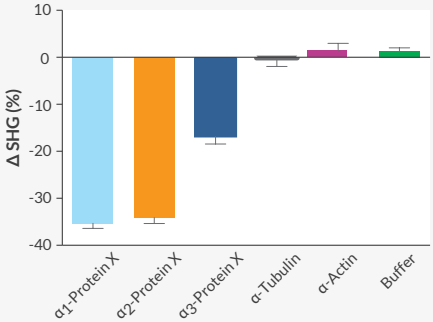
The Biodesy® Delta measures thousands of conformational changes a day in real time, thereby providing structural insight early in the screening pipeline. Delta performs primary screens of fragment or targeted libraries, orthogonal screens of HTS hits, and can also be used to test analogs during hit-to-lead and lead optimization. The information obtained allows analyte clustering on the basis of their potency, mechanism (allosteric vs orthosteric) and function (agonist vs antagonist).

He we present the typical drug discovery workflow involved when using the Delta as a primary screening tool.

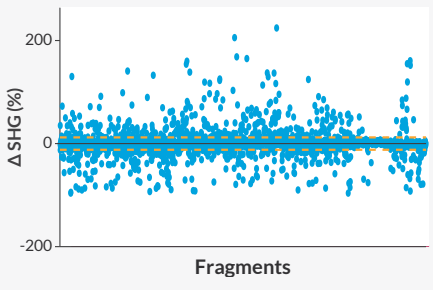


Highlights

Develop conformational response assays



Identify and confirm hits



Classify and prioritize hits

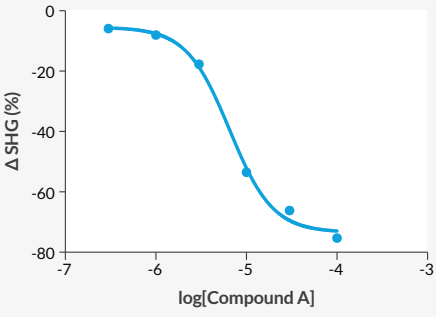
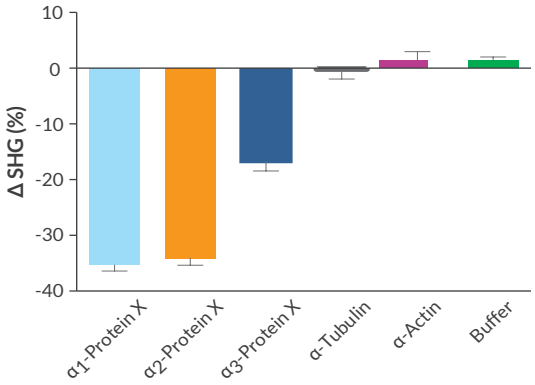


Figure 1: Developing a conformational response assay for Protein X



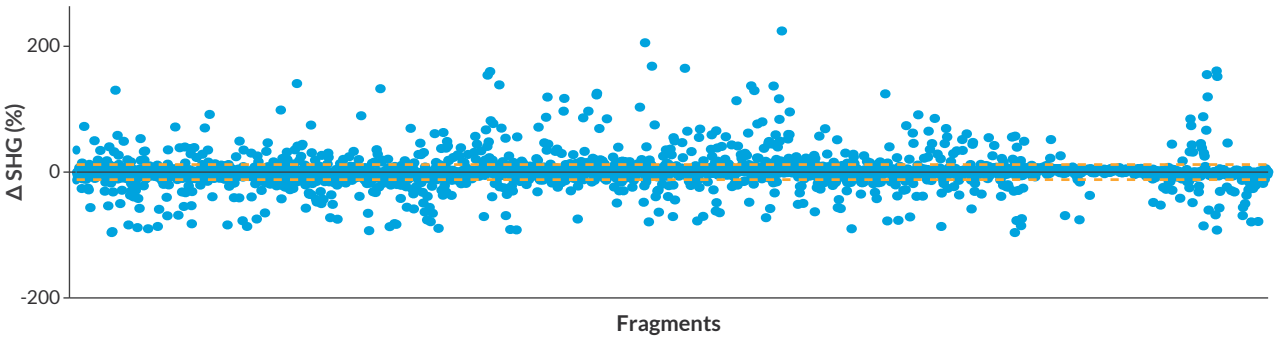
Protein X was labeled with Biodesy's orientation-sensitive, second harmonic generation (SHG) dye and tethered to a lipid bilayer at the bottom of a 384-well plates (see page 4). SHG intensity was measured before (baseline, SHG_B) and after (final, SHG_F) injection of the indicated analyte. Conformational change induced by each analyte is calculated as follows:

$$\Delta \text{ SHG (\%)} = 100\% \text{ X } (SHG_F - SHG_B)/SHG_B$$

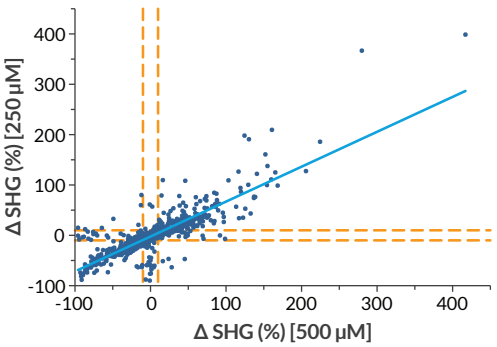
In this assay, three positive control antibodies induced negative signal changes, while buffer and antibodies to tubulin or actin produced no response.

Figure 2: Identifying and confirming hits

A



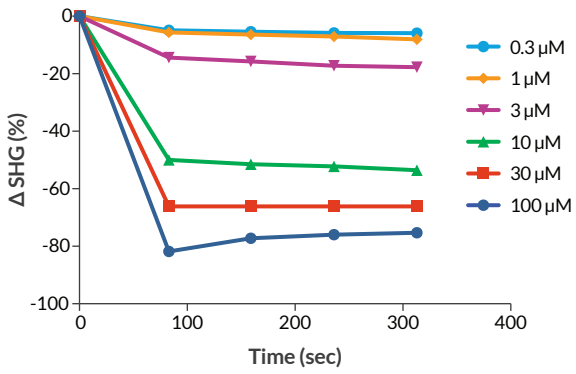
B



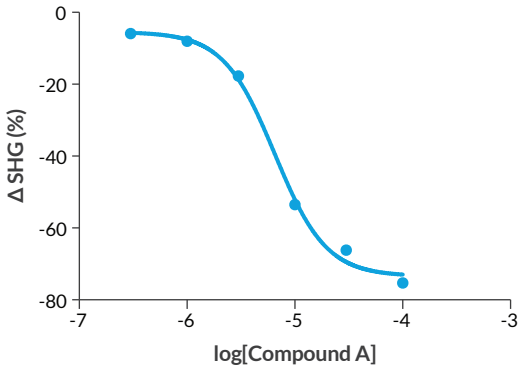
Screening for hits that modulate Protein X conformation was performed using a 2,714 fragment library (A). Hits were identified using a 3σ cutoff indicated by dashed orange lines. Hit confirmation was performed in duplicate (B). Note that some hits induce a positive signal change while others induce a negative signal change, indicating a diversity of conformational landscapes revealed by the Delta.

Figure 3: Classifying and prioritizing hits

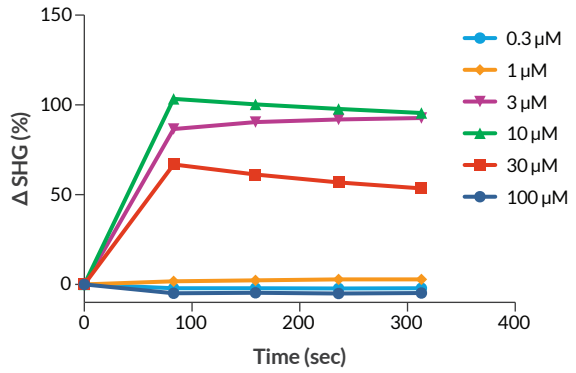
A



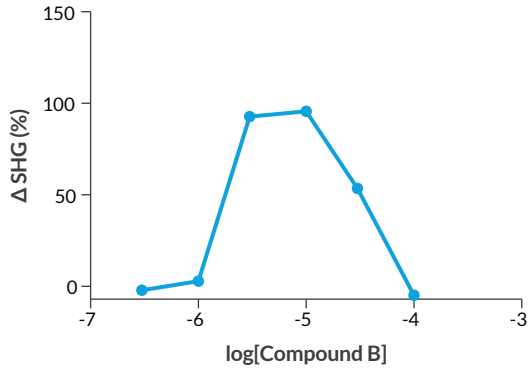
B



C



D



E

Stage	Description	Hits	Hit Rate
Screen	Hits	490	18.0%
Confirmation	Confirmed Hits	150	5.5%
Clustering	(-) ΔSHG; EC_{50}>100 µM	50	1.8%
	(-) ΔSHG; EC_{50}<100 µM	7	0.3%
	(+) ΔSHG; EC_{50}>100 µM	57	2.1%
	(+) ΔSHG; EC_{50}<100 µM	26	1.0%
	Nuisance	10	0.6%

Concentration response and time course data is routinely collected during hit follow-up to expose false positives and to cluster hits by conformational signature. The signature is comprised of direction and magnitude of conformational change, EC_{50} calculated from concentration response curves (CRC), and kinetics as determined from time course traces.

True hits exhibit time course traces that plateau (A) and sigmoidal CRC (B), in contrast to data for nuisance compounds (C, D). Here we organized confirmed hits into five clusters based on their signatures: compounds that induced either a negative or positive conformational change with high (EC_{50} < 100 µM) or low (EC_{50} > 100 µM) potency, and nuisance compounds. The hit rate for hits, confirmed hits and each of the five clusters is shown in (E).

Screening with Delta

Primary Screen — of focused and fragment libraries

Secondary Screen — of HTS hits to prioritize for downstream biophysical tools

Orthogonal Screen — to inform hit expansion/lead optimization activities

Delta provides structural insight at a throughput practical for primary and secondary screening, improving decisions made early in the screening process.

Biodesy Delta System

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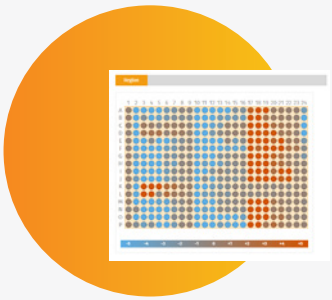

Structural insight in seconds

- Designed for your screening, follow-up and SAR workflow
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- Intuitive software and integrated robotics for simple experimental setup and walk-away operation

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