This note explains various steps involved in deconvoluting the right target(s) of small molecule using Shantani's proprietor workflow.

## Step-1 Derivatization of 'Bait Molecule'

1) Analyze SAR (Structure-Activity Relationship) of the bait molecule
2) Define a point of derivatization for minimal activity loss

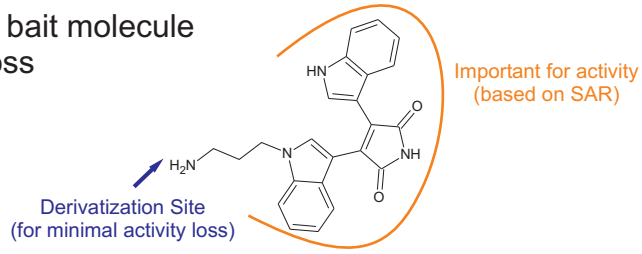


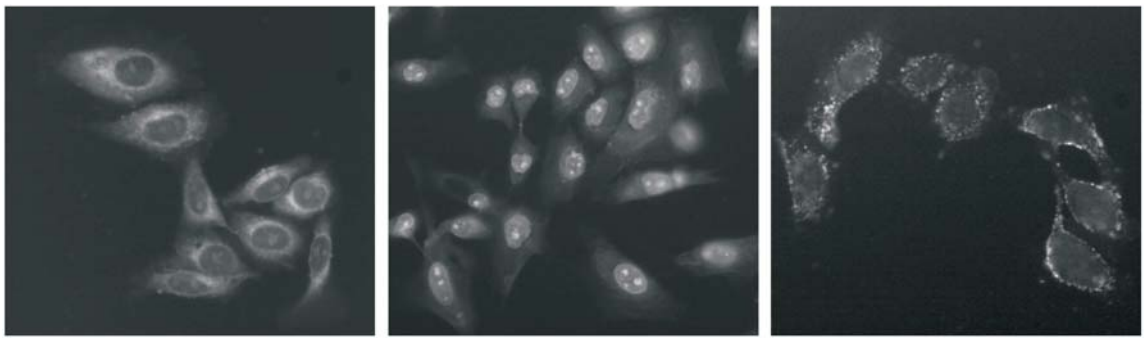

Figure 1. Example - 'bait molecule' Bisindolylmaleimide III
Choosing the right site for derivatization

## Step-2 Preparation of Subcellular Location Specific Target Capturing Probes

Subcellular location specific target capturing probes are prepared by covalently coupling the bait molecule with the proprietary peptides of Shantani.

These peptides have following characteristics:

(1) Penetrate wide-variety of mammalian cells and then remain confined to its subcellular location (membrane, cytoplasm and nucleus)
(2) 6-10 amino acids long and do not form secondary structures
(3) Have no known peptidase sequence and designed to remain protected from 'xeno-degradation' machinery of the cells
(4) Are not toxic to the cells



Cytoplasmic Peptide | Nuclear Peptide | Membrane Peptide

Figure 2. Peptides were coupled to FITC (fluorescein) and incubated with the cells. Later cells were imaged using fluorescence microscopy to establish subcellular location specificity of the peptide probes

## Step-3 Functionality Assay of Probes

Target capturing probes are incubated with the cells and functional activity of these probes compare to un-coupled bait molecule (free molecule) is assessed.

Carrying out functionality assay of target capturing probes serve three purposes

1) Establishes that the bait molecule has not lost its bioactivity after peptide coupling
2) Determines the cellular location of the target (activity from a particular cellular location is used as judgment for the purpose (see figure 3))
3) Establishes an optimum concentration range of the target capturing probes that should be utilized during target capture experiments

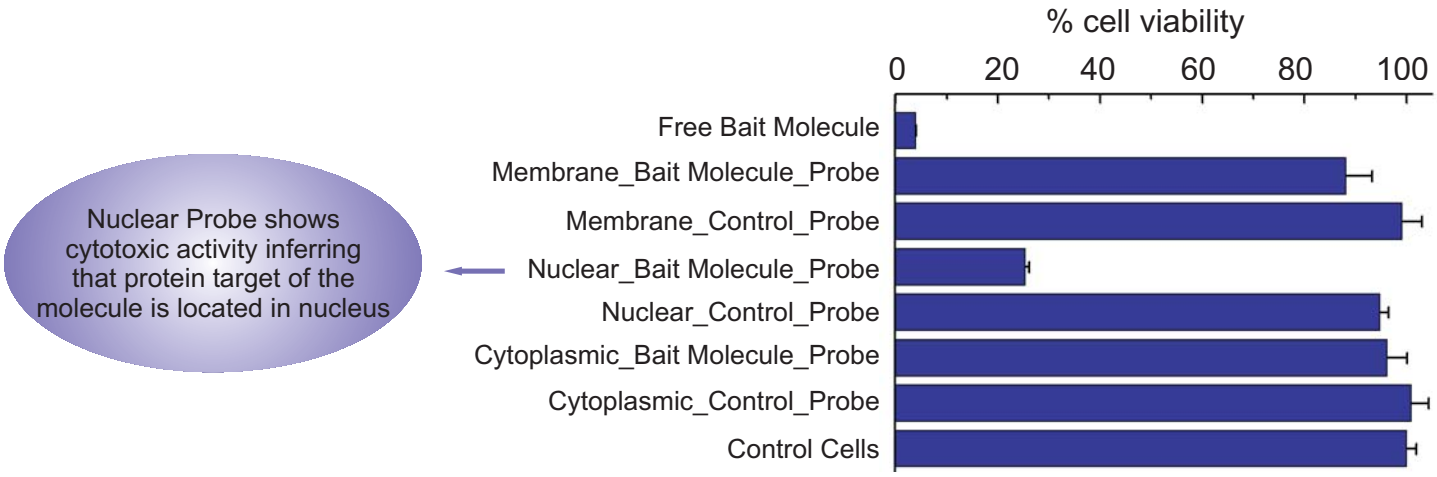
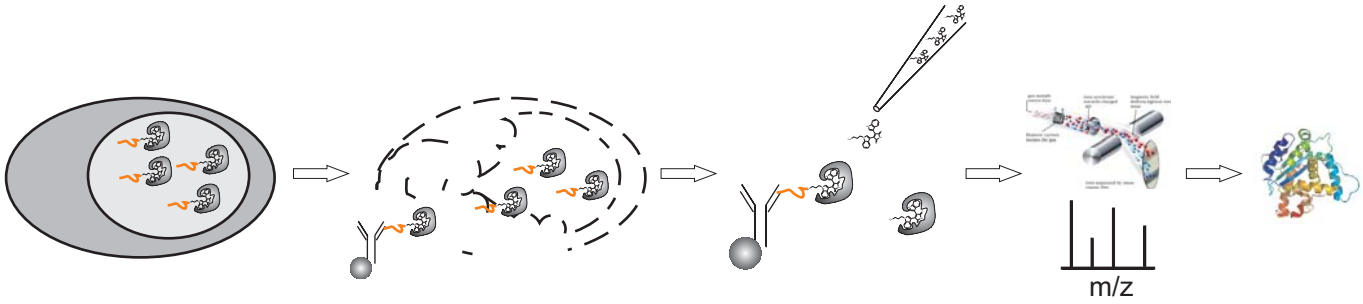



Figure 3. Example - Bisindolylamaleimide-III is a cytotoxic compound. It was coupled to the subcellular location specific peptide probes and cytotoxic activity of the probes were monitored.

## Step-4 Target Capture and Identification Experiments




Incubation of Functionally Relevant Target Capturing Probes with Live Cells

Cell-Lysis using proprietory buffer and Isolation of Target Capturing Probes using antibody against the peptide

Elution of Target Proteins from antibody column by displacing it with free molecule

Identification of Target Proteins using mass-spec-trometry based methods

## Step-5 Target Deconvolution

Target capture experiments in step-4 are performed along with two control experiments. In first control experiment, a similar location specific peptide but not coupled to 'bait molecule' is used for the target capture experiment. And in second control experiment, the cells are first incubated with free 'bait molecule' to competitively inhibit the capture of target to the target capturing probes. Later proteins are identified in similar manner as described in Step-4. Using a methods described in (Saxena et al. J. Proteome Research 2008) proteins that are significantly enriched in Step-4 experiments compare to control experiments are considered as specific binding partners of the bait molecule.

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## Contact Us

Shantani Proteome Analytics Pvt. Ltd.
100 National Chemical Laboratory Innovation Park
Dr. Homi Bhabha Road, Pune, MH, India 411 008
Phone: +91-20-64103918, +91-20-64009991, +91-997 544 7489
Email: bd@shantani.com
Online: www.shantani.com