Small-Molecule Drug Target Identification/Deconvolution Technologies

Case-Studies

Shantani™

Advancing Technologies and Applications of Proteome Analysis

Shantani Target ID Technology Tool Box

Target Deconvolution is not Trivial =
A single Tool / Technology May Not necessarily solve the problem

Our Focus = Target ID

We Utilize Several Proprietary and Generic Technologies for **Right Target**

1) UPT = Unique Polymer Technology, 2) **SCLS** = Subcellular Location Specific Target Capture Technology, 3) **COMP** = 'in-silico' target ID workflows, **4) TBB** = Traditional Bead/Biotinylated Molecule Based Method, 5) **TPP** = Thermal Proteome Profiling

Case Study # 1: A Drug Discovery Program from a Seattle, USA based Organization

Scenario

- Phenotypically Screened 'Hit' compound, Target not known, 'Hit' can not be rationally optimized
- For Target ID Typical bead/biotinylation based affinity chromatography can not be performed because Molecule is not easy to derivatize

Shantani's Solution

- Shantani's Proprietary Unique Polymer Technology that can immobilize underivatized molecule for affinity chromatography
- Eventually Target(s) that can be linked to observed phenotype were identified

Unique Polymer Technology (UPT)

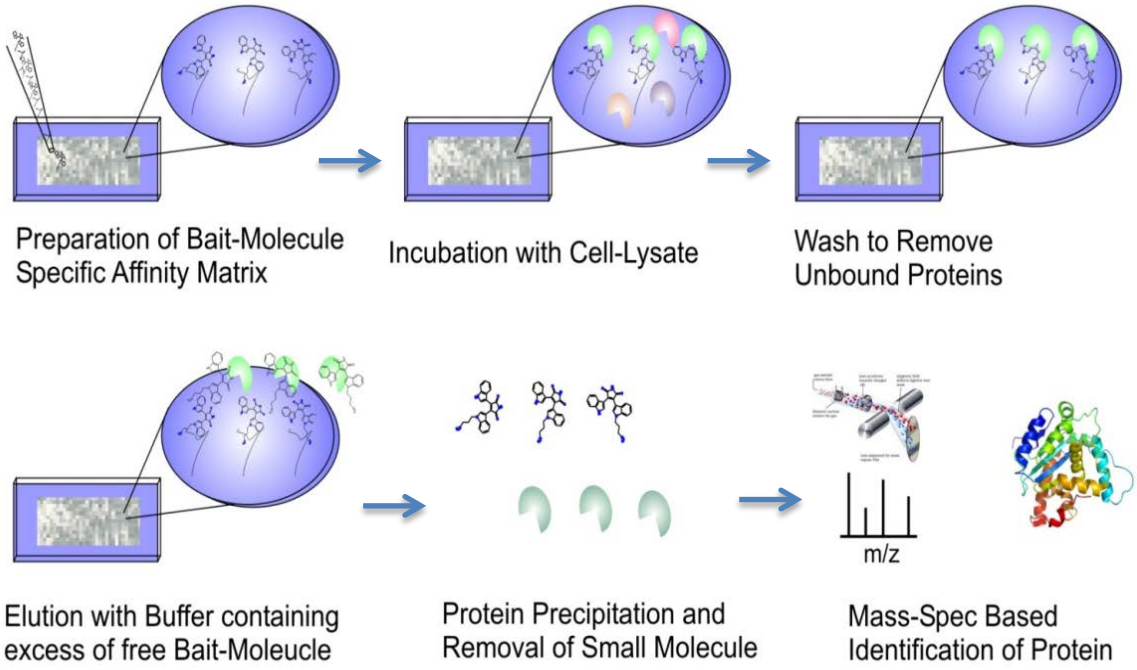
Uniqueness

1. Molecule Derivatization not needed.
2. Target Identification can be completed in 2-3 weeks.

Working Principle

Molecule transiently get immobilized on Specifically Engineered Polymeric Surface through multiple non-covalent interaction with Polymer. Thus formed molecule specific affinity matrix is used in capturing the target from cell-lysates.

Typical Target ID Workflow



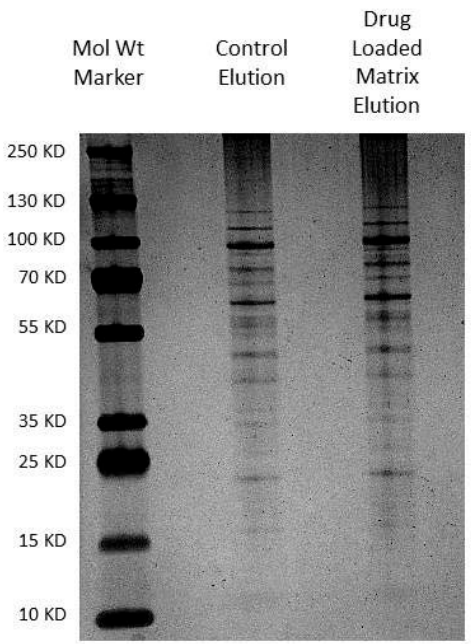
Case # 1:

Client provided | **Shantani used**

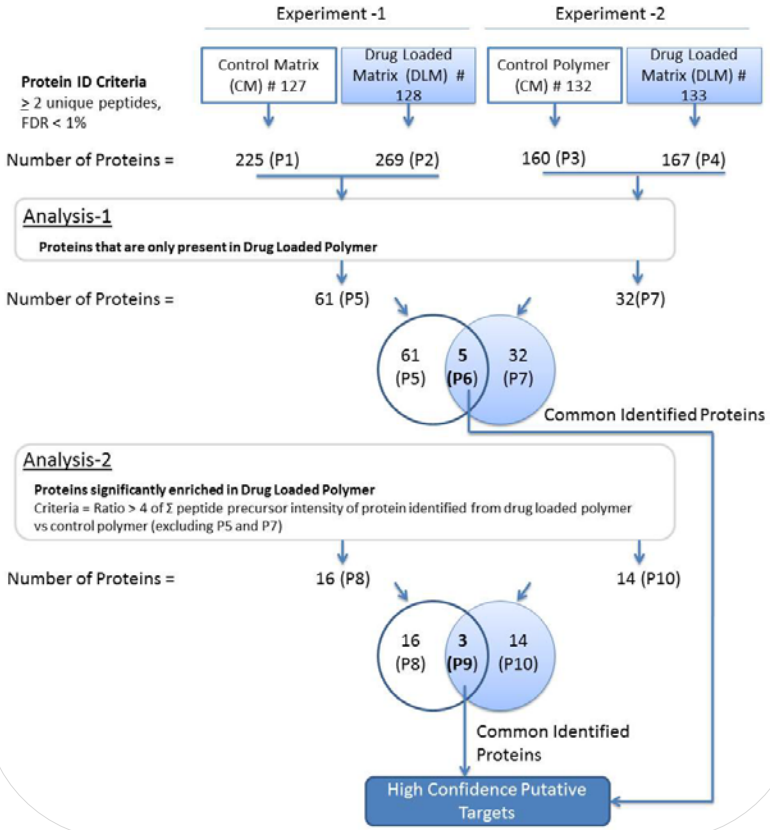
9 mg of Lysate + 15 mg of Molecule → UPT → Deconvoluted Targets

1 month

Typical SDS-PAGE Separated Protein Profile from Control and Molecule Loaded Matrix

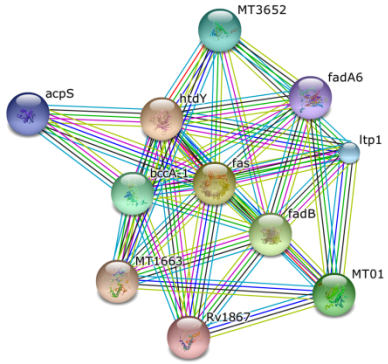


Typical Target Deconvolution Process



Eventual Value and 'GO' Decision

Information of Target was used for Action Mechanism Analysis and few targets were shortlisted for validation



Case Study # 2: A Large Indian Corporate looking for new regenerative medicine

Scenario

- Bioactive compound, at lower concentration induces proliferation of cells but at 20X concentration induced cell death Target not known/MOA not clear 'Hit' can not be rationally optimized
- Some structure-activity relationship data available but no path forward.

Shantani's Solution

- Shantani was consulted for Project
- Shantani used traditional bead based (TBB) methods and identified the targets
- 'in-silico' target validation was performed and targets were rank ordered based on predicted Kd values
- Identified targets with predicted lower affinity were linked with cell-death and higher affinity (strong affinity) with proliferation

Traditional Bead Based Methodology (TBB)

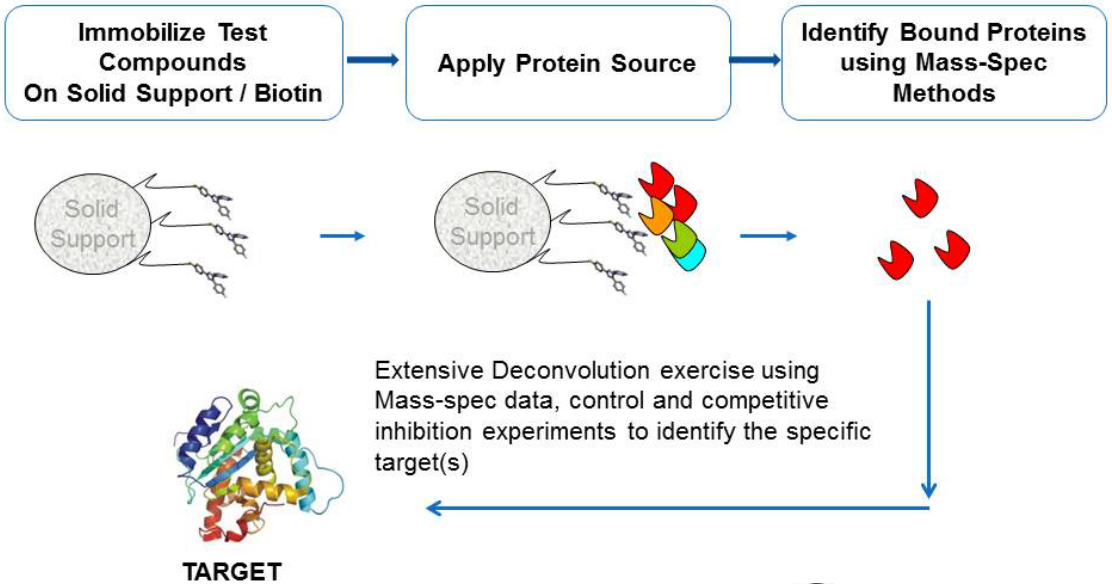
Uniqueness

Can identify targets having broad range of affinity with molecule (Kd ~ nanomolar to micromolar)

Working Principle

Molecules are immobilized on the solid support to prepare molecule specific affinity matrix. Affinity chromatography is carried out from relevant cell-lysate to capture the target proteins on solid support. Later captured proteins are identified using mass-spectrometry and deconvoluted.

Typical Target ID Workflow



Case # 2:

Client

Compound Combinatorial Library

Shantani (5 weeks)

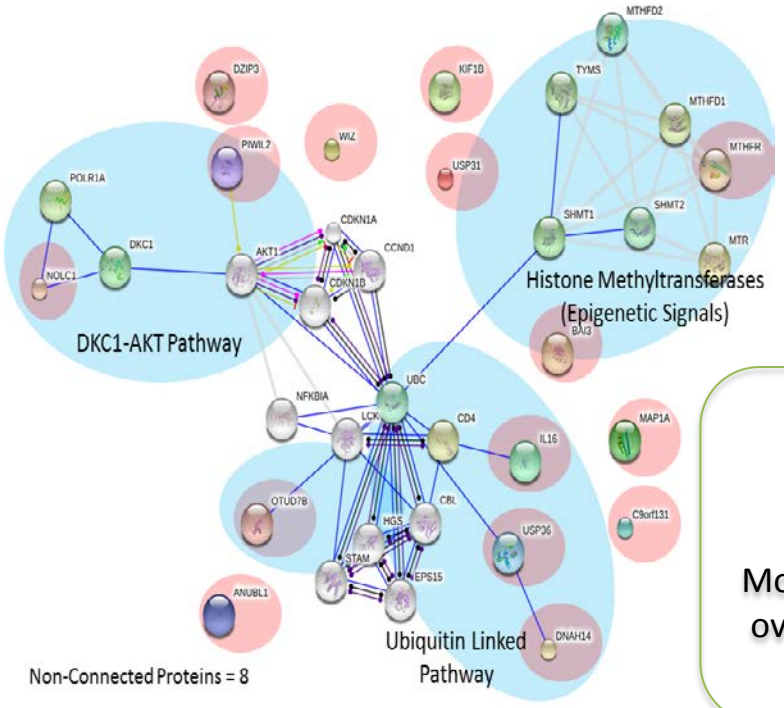
SAR analysis & Synthesis of primary amine derivative

Shantani (2 weeks)

Confirmation of functional activity of derivatized molecule

Shantani (3 weeks)

Target Identification, 'in-silico' target validation & MOA analysis



Eventual Value and 'GO' Decision

Molecules selectivity was optimized over identified target proteins that were linked to cell death

Identified proteins (red circled) with predicted higher kd were involved in cell death and with lower kd were linked to proliferation.

Case Study # 3: A European SME

Scenario

- Phenotypically Screened 'Hit' compound, Target not known, 'Hit' can not be rationally optimized
- For Target ID Typical bead/biotinylation based affinity chromatography can not be performed because a slight change in molecular structure looses the activity of the compound

Shantani's Solution

- Shantani's Proprietary Unique Polymer Technology that can immobilize underivatized molecule for affinity chromatography
- Eventually Target(s) that can be linked to observed phenotype were identified

Case # 3:

Client

'Hit' compound

Shantani (1 week)

Preparation of affinity matrix using UPT

Shantani (1 week)

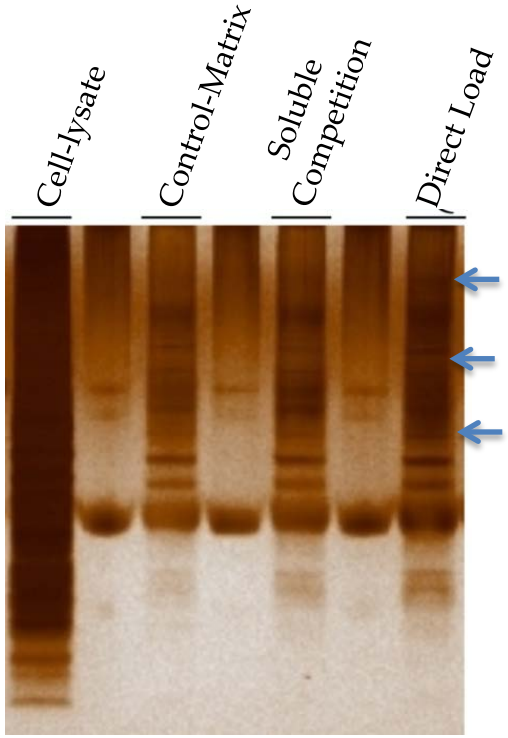
Affinity Chromatography and Target ID

Total Proteins Identified = *253*

Shantani (1 week)

Target Deconvolution

Deconvoluted Specific Target = **5**



Typical Enrichment of the Target in Direct Load Experiment – Silver Stain Profile

Protein Class / Name	Specificity Ratio
Methyl Transferase	1
Fibronectin Binding Protein	1
Mitochondrial Transport Protein	1
GTPase activating Protein	1
Adapter Protein for T-Cell Signalling	1

Eventual Value and 'GO' Decision

Identified a Novel Epigenetic Target
Target was validated by client using overexpression and Binding Studies

Case Study # 4: A Internal Study

Scenario

- Molecule Bisindolylmaleimide-III (Bis-III) interacts with GSK3-β and induce apoptosis
- GSK3-β is present both in cytoplasm and nucleus. For clarity on MOA and newer compound design location of target is needed
- SAR of the Bis-III molecule is well-established

Shantani's Solution

- Shantani's Proprietary subcellular location specific target capturing target methodology
- Target location was identified and for conformation target was captured from specific sub-cellular location

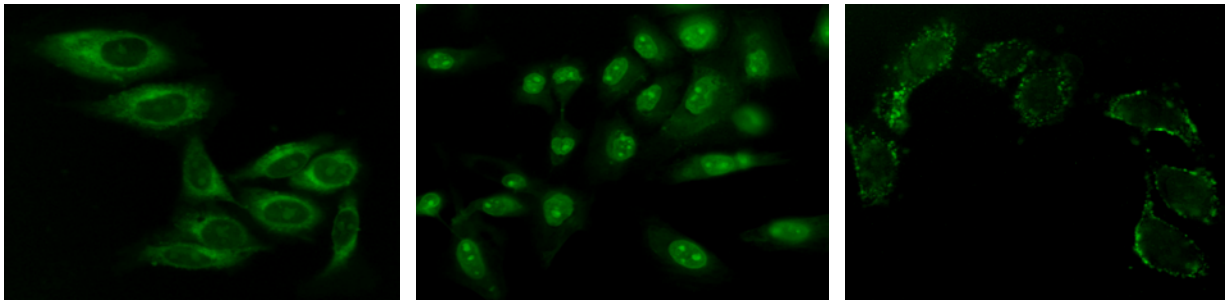
Subcellular Location Specific Target Identification Technology (SCLS)

Uniqueness

1. Very low false positive identification rate ~20%
2. Can identify targets in subcellular location specific manner in a live-cell set-up

Working Principle

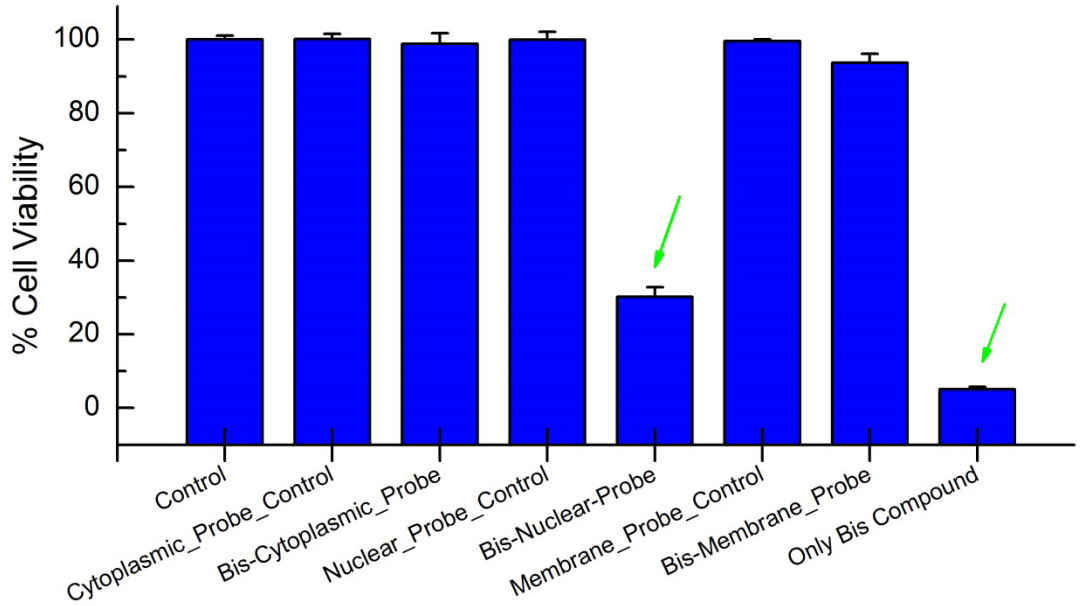
1) Bait –molecules are coupled to proprietary sub-cellular location specific probes
2) These probes in separate experiments drag the molecule in different specific sub-cellular locations and functional activity of the molecule is measured
3) Sub-cellular location that shows maximum activity is chosen as 'target-rich' compartment and target captures experiments are performed from 'target-rich' locations



Cytoplasmic Probe **Nuclear Probe** **Membrane Probe**

Case # 4: Sub-cellular Location Specific Functional Activity of Bis-III Probes

Bis-III was coupled to three sub-cellular location specific proprietary peptides → Cytotoxic Assay in HeLa Cells



Bis-compound and all probes and control probes at 80 μM concentration.

Bis-III probes that were targeted to the nucleus exhibited similar activity as the free Bis-III → Target located in nucleus is responsible for the activity

Case # 4. Capture of Target from Nuclear Fractions

Probes + Nuclear Fractions of He La Cells

↓

Incubation with probe antibody

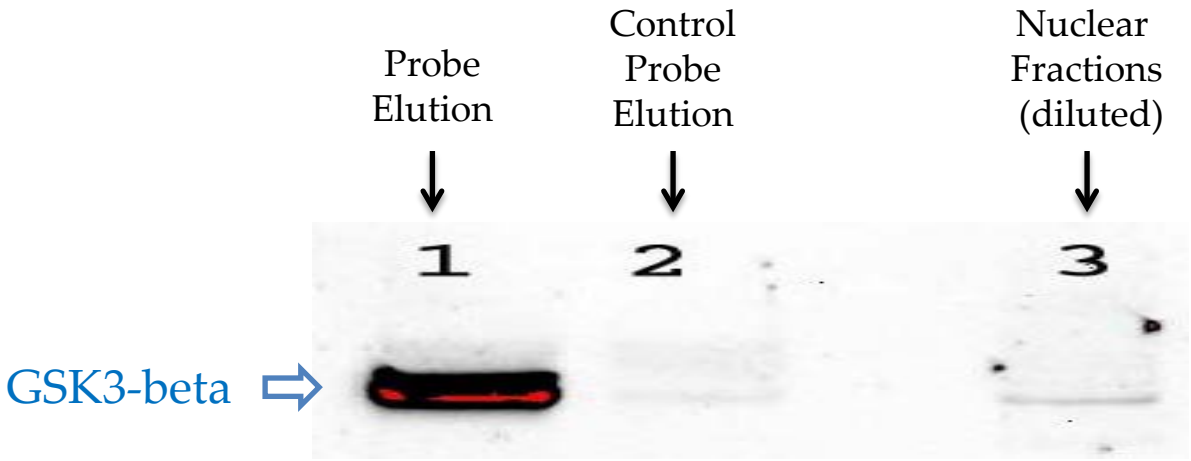
↓

Washing of antibody

↓

Western Blot Analysis of Elution for Target Protein

Probes = Coupled with Bis-III



Target was specifically captured with Probe.

Eventual Value and 'GO' Decision

Establishing sub-cellular location of the target clarified the MOA and new compound design included the additional parameters that molecule should target nucleus

Case Study # 5 Identification of Target Using Computational Approaches

Scenario

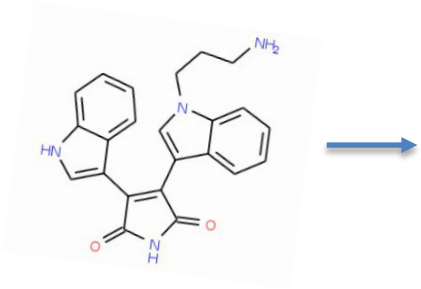
- Several Computational Approaches are available for identifying the Targets of Small Molecule that utilizes structural information of the molecule and compares it with the historical database (similar structure + Target)

- These approaches generate a long list of targets (most of them are false positive) and a few target for validation can not be prioritized

Shantani's Solution

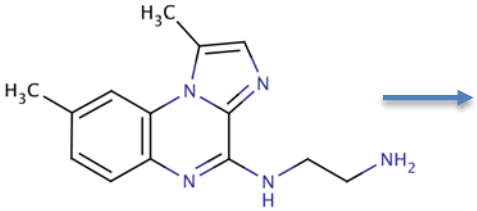
- Shantani's Proprietary computational approach identify a few but the rightful target

Case # 5: Deconvolution of Targets of a Few Known Small Molecules using Shantani's COMP workflow



Bisindolylmaleimide-III

	# of Target Identified	# of Actual Target (Kd < 500 nm)	Success Ratio (Total / Actual Target)
Algorithm-1	26	1	4%
Algorithm-2	4	2	50%
Algorithm-3	16	2	13%
Shantani-Algorithm	2	1 (Identified Target =PKC-α)	50%



BMS-345541

	# of Target Identified	# of Actual Target (Kd < 500 nm)	Success Ratio (Total / Actual Target)
Algorithm-1	17	1	6%
Algorithm-2	8	2	25%
Algorithm-3	19	2	11%
Shantani-Algorithm	2	1 (Identified Target =IKK-β)	50%

Eventual Value and 'GO' Decision

Shantani's COMP workflow allows identification of a few but rightful targets

Connect for further discussions

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