Target Identification using Chemical Proteomics at Shantani

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Company Overview – Conception & Growth

Chemical-Proteomics Based Biotechnology Company

Established Year 2010
Seed-Funds from Technology Incubation Park

December 2011
Small Business Innovation Grants for New Target Discovery in T2D

April 2012
Top Indian Private VC firms Fuels the growth

July 2012
- Target ID Technology Commercialization
- Initiation of Discovery Programs

Target ID Technology Received Top Innovation Awards

- Lockheed Martin (USA) - IIGP Innovation Award 2012
- IKP Millennium Conference Innovation Prize 2014
- BIRAC Best Technology Innovation Award 2015

Premises, Problem Statement and Value

Discovery Program Identified a *bioactive* (small-molecule, peptide, protein, antibody) however the target(s), the cellular binding partners, of *bioactive* is not known

- Information of Target of *bioactive* will allow
  - Rational optimization of *bioactive*
  - Drug-Efficacy biomarker discovery, patient stratification and commercial differentiation by clarifying the action mechanism of *bioactive*
  - Early 'de-risking' of program by characterizing 'off-target' of the *bioactive*

Shantani Identify Targets of Bioactive Molecules

Program's Target ID Need

Shantani Deploy (Appropriate Technology + A Decade of Target Identification Expertise + Program Centric Business Model)

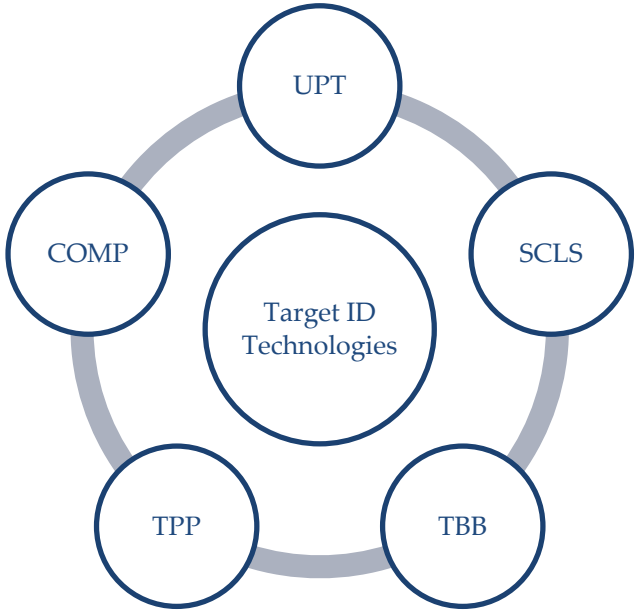
Deconvoluted Target Information

Portfolio of Target-ID Technologies

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

Our Focus = Target ID

Our Expertise in the field allows us to evaluate the 'fit-for-purpose' technology and then we deploy appropriate Technology for **right target**



Different Technologies
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

Comparative Profile of Target ID Technologies

Technolog(ies)	Key Technology Differentiator	Typical Deconvoluted Targets	Typical Time-Line (Weeks)	Comparative Cost
Proprietary Unique Polymer Technology (UPT)	'bait-molecule' derivatization not required, target enrichment based identification	8-10 (false positive rate ~40%)	2-3	$$$
Proprietary Subcellular Location Specific Target Capture Technology (SCLS)	Target Capture from Live-cell in sub-cellular location specific manner	4-6 (false positive rate ~20%)	4-8	$$$$$
Proprietary 'in-silico' Tools (COMP)	Robust, Fast and cost-effective	30-40 (false Positive rate ~80%)	1-2	$
Non-Proprietary Bead/ Biotin Based Traditional Chemical Proteomics Technology (TBB)	Target Capture from cell-lysates	12-15 (false positive rate ~40%)	2-3	$$$
Non-Proprietary Thermal-Proteome Profiling (TPP)	'bait-molecule' derivatization not required, target deconvolution base on bait-molecule induced thermal stabilization of target protein	10-12 (false positive rate ~50%)	3-5	$$$$

Shantani's Proprietary Target-ID Technology Platforms

Technologies	Final Value
Unique Polymer Technology (UPT) – Label Free Technology	• Quick Target Profiling allowing a 'Go/No Go' development decision for phenotypically screened compounds • Rapid target profiling of multiple compounds to save time and cost
SubCellularLocation Specific Target Capture Technology (SCLS) – Labelled Technology	• Precise Target Information driving rational lead development • Low False Positives saving time during target validation
Computational Supreme (Comp-S) – Label Free Technology	• Significantly Narrowed down list of targets to be validated, saving time • Assist in bringing selectivity while lead development / optimization

Unique Polymer Technology(UPT)

Unique Polymer Technology (UPT)

Key Advantages

- Derivatization of test-molecule is not needed
- Target Deconvolution can be completed within 2-3 weeks
- Low False Positive Identification Rate = Faster Validation

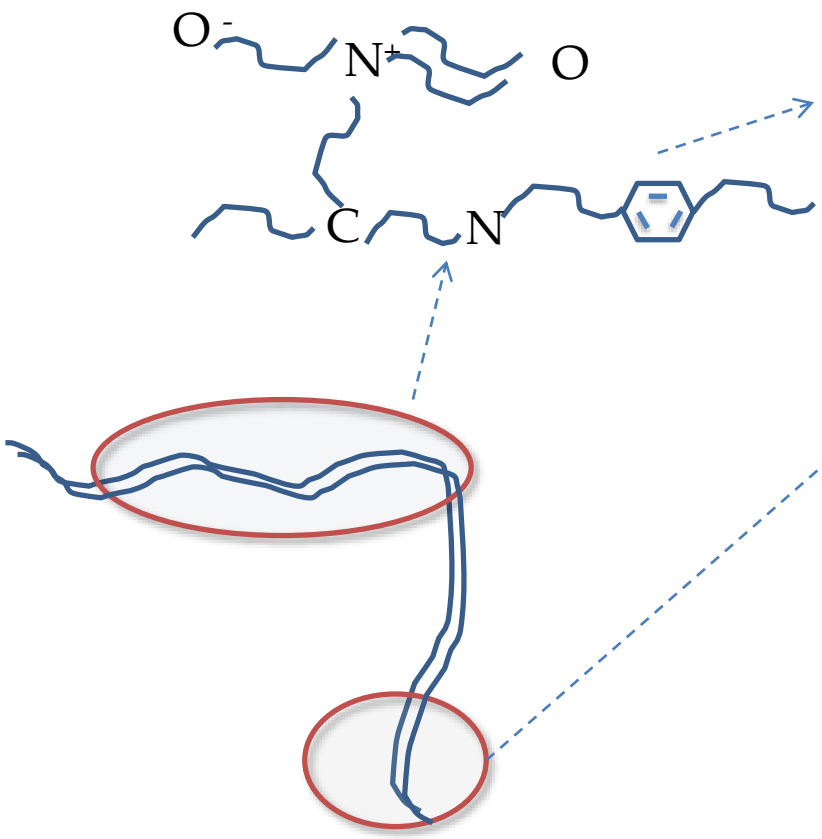
Immobilization without derivatization : Working Hypothesis

- Weak molecular interaction forces of a organic molecule can be used in immobilizing them on a surface that provides complementary weak interactions

- Sum of multiple weak interactions is strong enough to allow the molecule to stay (immobilized) on the surface

- The molecule can stay on the surface for long enough they can be used as molecule specific matrix for affinity capture of protein target

Design of Unique Polymer for Chemical Proteomics



= Unique Polymer

Multiple Possible non-covalent Affinity Binding Interactions for small-molecules

Ionic, Hydrophobic / Hydrophilic
Vander-waals, pi-pi, cation-pi, H-bonds

Optimized Surface Angel Properties for attachment of polymer to

Glass and/or Plastic in different dissolution Phase

Overall Amphiphilic in Nature

Preparation of Test-Molecule Specific Affinity Matrix

Test molecules are dissolved in appropriate solvent

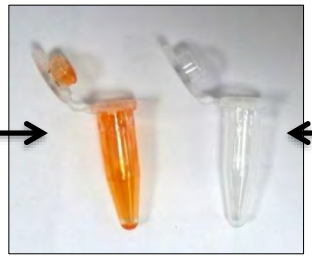
↓

Test-molecules are layered on the polymeric matrix and allowed to stay for appropriate time for immobilization

↓

Matrix is washed extensively and amount of test-molecule in washes confirms the extent of immobilization

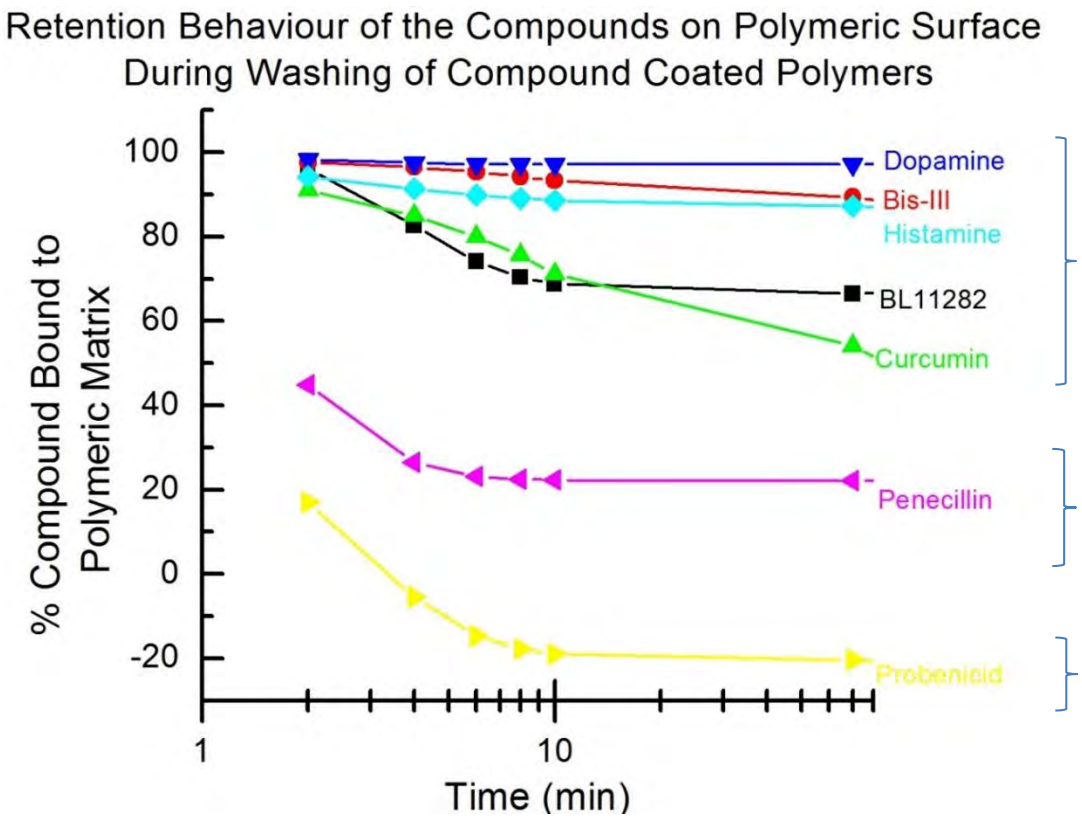
Polymeric Affinity Matrix of Bisindolylmaleimide (Bis-III)



Control Polymeric Matrix

Example: Polymer layered in a Eppendorf tube and later a bisindolylmaleimide compound was immobilized

Retention Behavior of a few Small-Molecules on Unique Polymer



Well Retained

Moderately Retained

Not Retained

Molecule Orientation on Polymer is critical

- Which interaction force is dominant and whether molecules are oriented in linear or random fashion ?

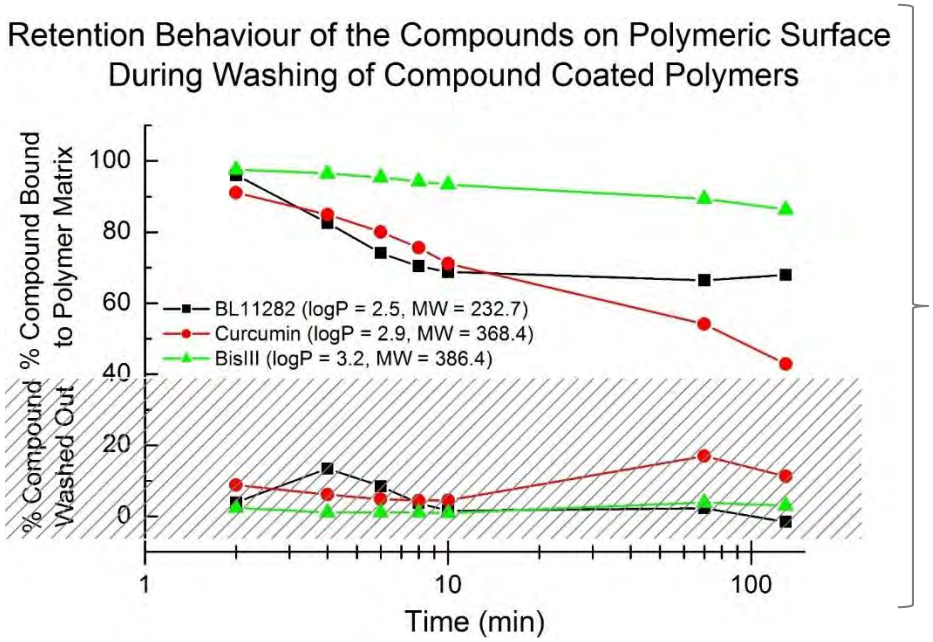
- If orientation is not random then a particular site of molecule that is responsible for its interaction with the target protein may not be exposed !!!

Let's consider one major 'weak-interaction' force at a time

Orientation of Molecule is Random in Nature

Let's consider Only Hydrophobic Interactions

If only hydrophobic interactions are playing a role in compound immobilization then different compound of about similar logP vales should be retained similarly



Retention behavior of compounds having about similar logP value is significantly different

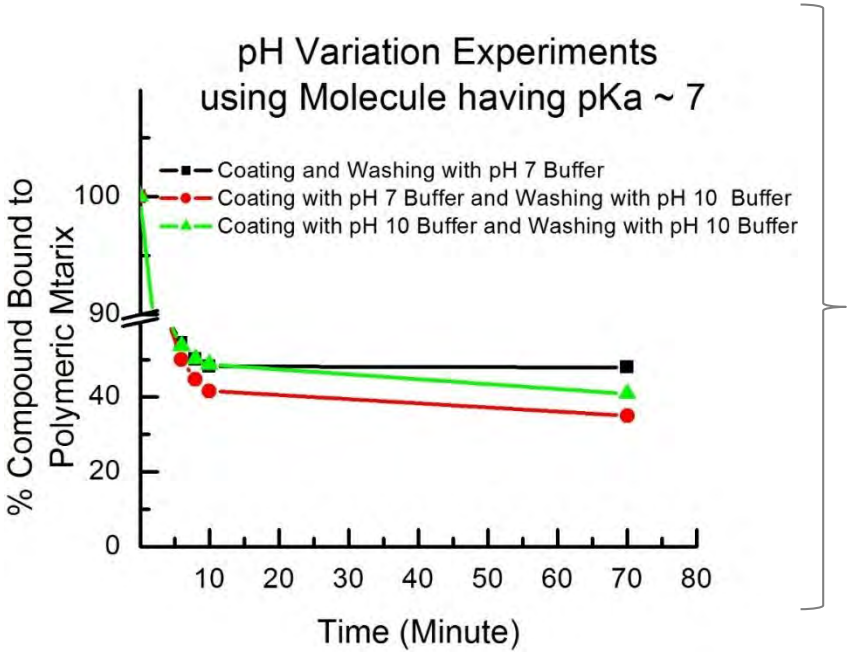
= **<u>More than one type of interaction forces are working together</u>** to retain the molecule on the polymer

= Random orientation of the molecule on polymer is plausible factor for multiple weak interactions

Orientation of Molecule is Random in Nature

Let's consider Only Ionic Interactions

If only ionic interactions are playing a role in compound immobilization then polymer coating and washing with buffer having different pH will ionize the compound differently and retention will be affected



Retention behavior of same compound coated and washed at different pH did not significantly change its retention behavior

= **More than one type of interaction forces are working together** to retain the molecule on the polymer

= Random orientation of the molecule on polymer is plausible factor for multiple weak interactions

How much amount of Molecule is needed on the polymer for good capture

- Molecules are retained but how much amount is needed on the polymer ?

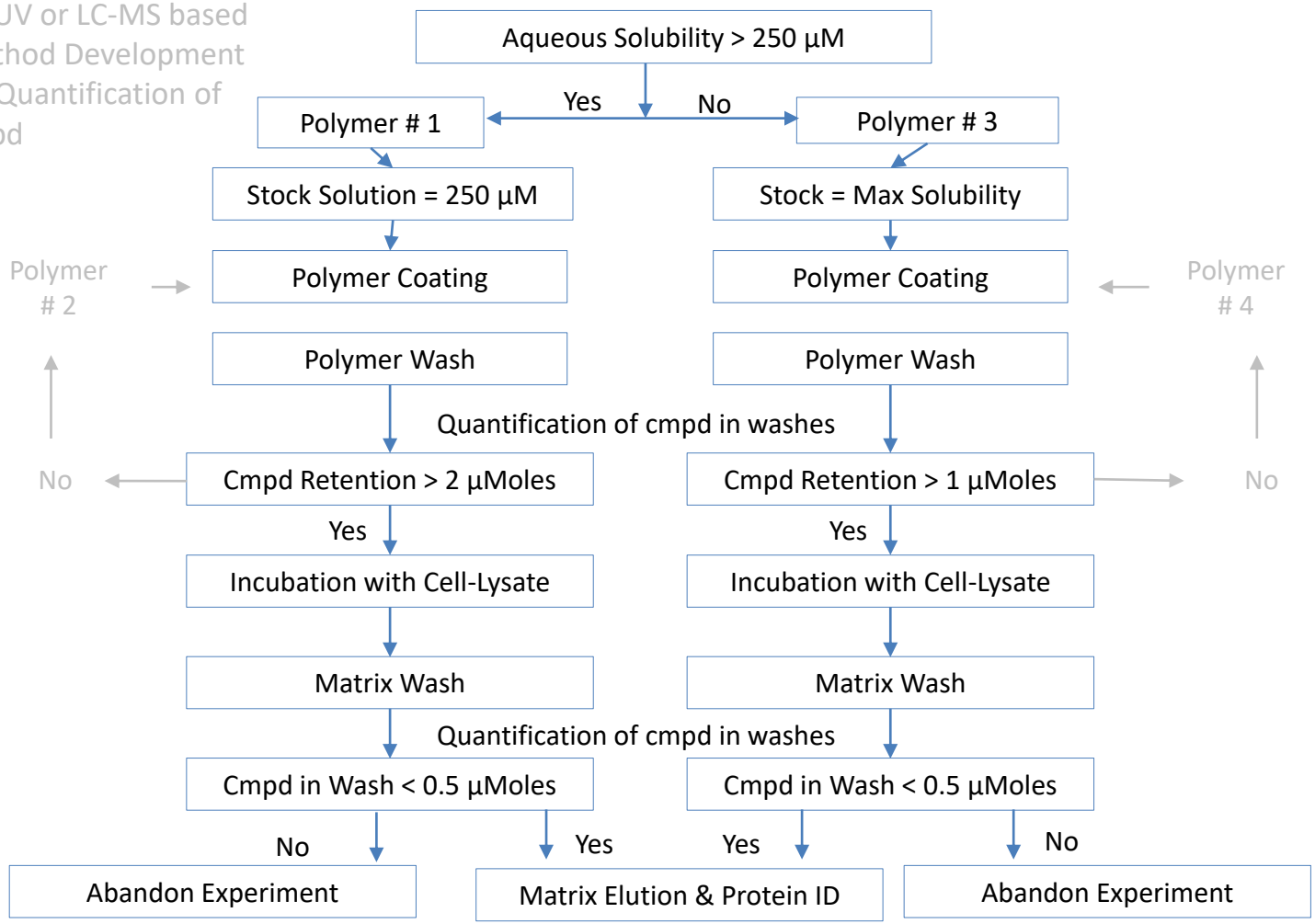
- Aqueous buffers are used to characterize the retention, what if the incubation with cell-lysate takes the molecule *'off-the-polymer'* ?

Let's Answer it by looking at Qualification Criteria of Matrix Preparation and Target Capture

Method Qualification *is a function of* Amount of Molecule Immobilized on given surface area of Polymer)

Compound Information
- Molecular Weight
- Aqueous Solubility

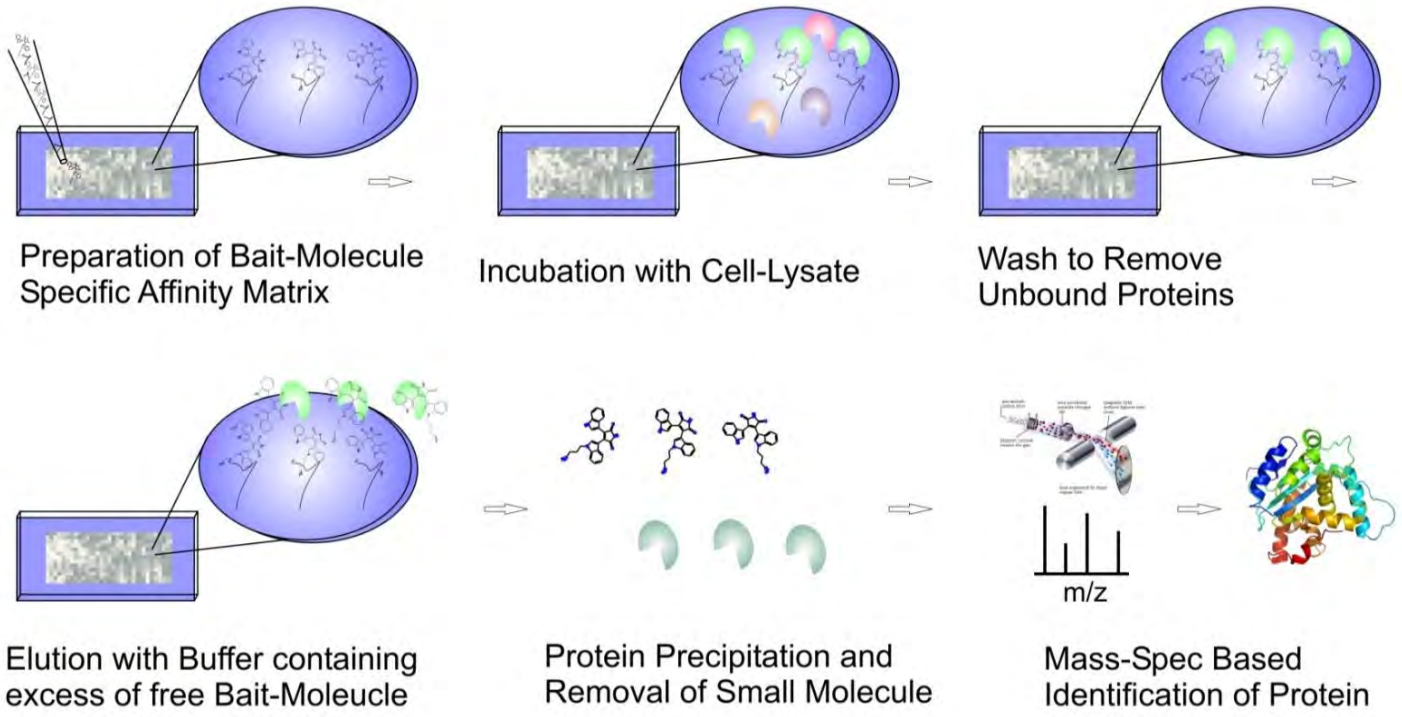
LC/UV or LC-MS based Method Development for Quantification of cmpd



Qualification Of Affinity Matrix

Qualification Of Capture

Unique Polymer Technology (UPT) Work-Flow

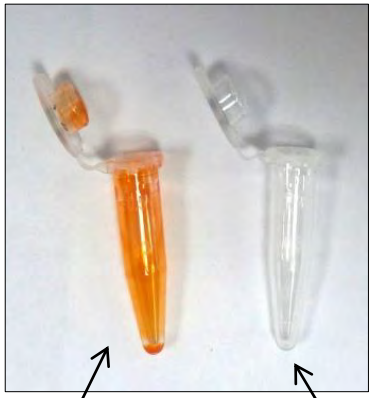


Reference: *Shantani's Proprietary Technology*.
Patent Application :PCT/IN2017/000002

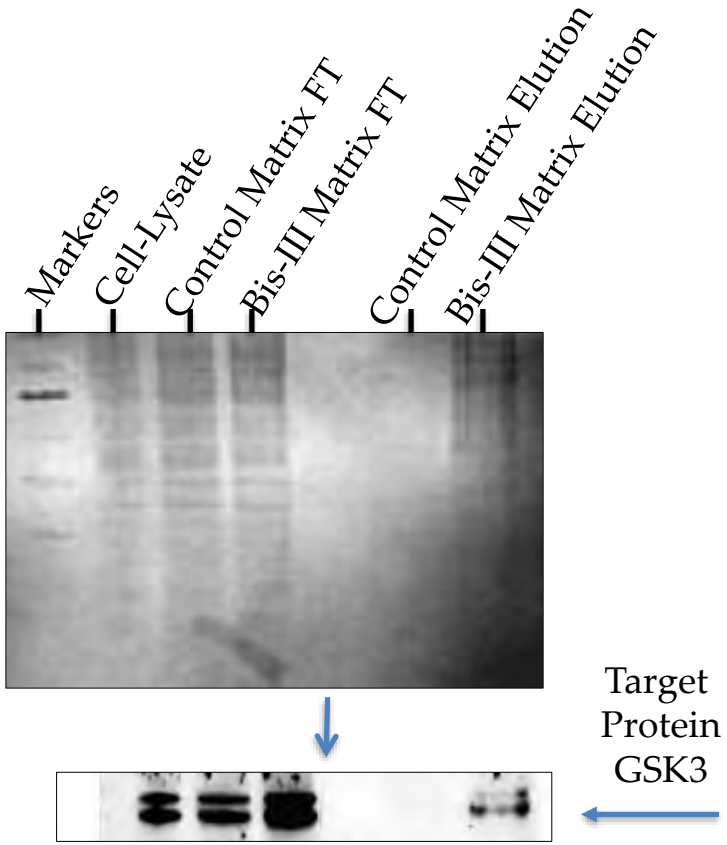
Capture of GSK3 protein, a well-established protein target (Kd ~19 nM) of Bisindolylmaleimide-III using UPT

Capture of Known Target of Bis-III using Polymer Layered in 1.5 ml tube – *Very First Capture Experiment using UPT*

Step-1) Affinity Matrix of underivatized molecule



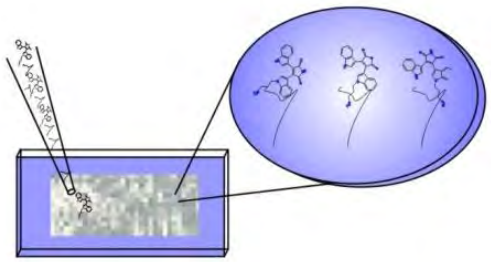
Step-2) Affinity Chromatography and SDS-PAGE and Western-blot analysis of Known Target Protein



Primary Target was Specifically Captured but capture efficiency was not great !!!

Capture of Target of Bis-III using Polymer Layered on Slide/CHIPs

Step-1) Affinity Matrix of underivatized molecule



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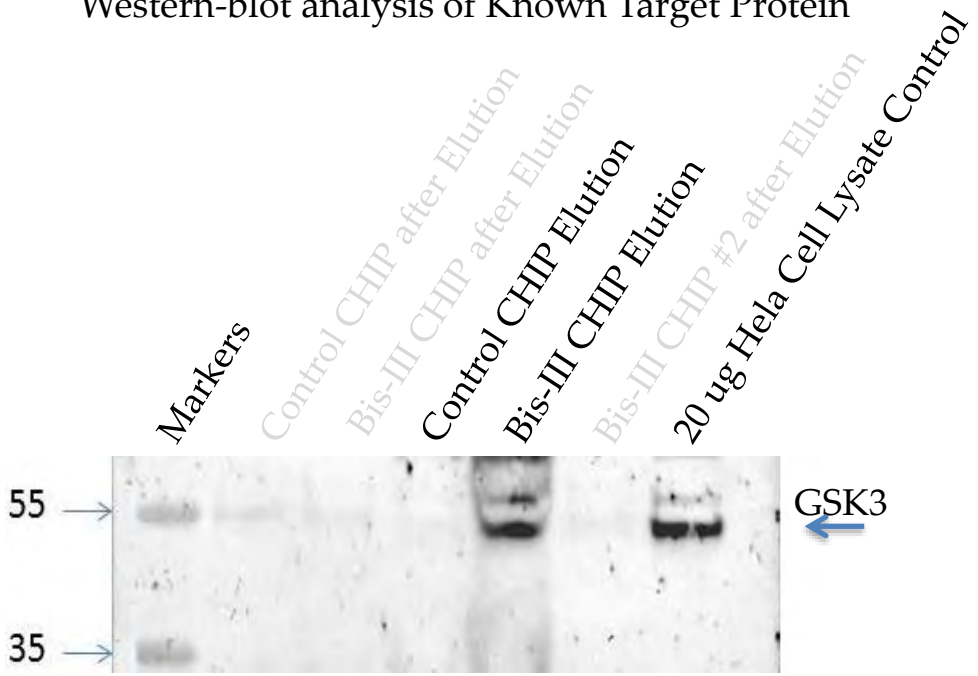

Primary Target was Specifically Captured and Amount of captured protein was significantly higher.

Other Identified Targets of Bis-III using Polymer Layered on Slide/CHIPs

Uniprot_ID	Protein Description	Maximum Number of Unique Peptides Identified	Q-Value (%)
Q13418	Integrin-linked protein kinase	7	0
Q70UQ0	Inhibitor of nuclear factor kappa-B kinase	6	0
P28482	Mitogen-activated protein kinase 1	6	0
P60891	Ribose-phosphate pyrophosphokinase 1	5	0
E9PF82	Calcium/calmodulin-dependent protein kinase type II	4	0
P49841-2	Glycogen synthase kinase-3 beta	3	0
P63208	S-phase kinase-associated protein	3	0
P51570-2	Galactokinase	3	0

Capture of protein targets of new compounds – Case Studies

Case Study # 1: Capture of Target(s) of Phenotypic Hit (Research Institute, Seattle)

- **Scenario:** Antibacterial compound not working through bacterial cell-wall disruption was identified. Target not known. Molecule can not be developed further.
- **Client Provided: Molecule -** 15 mg | **Cell-Lines –** 9 mg Bacterial Lysate
- **Time-lines at Shantani:** 1 month
- **Final Deliverable:** Very small list of highly putative targets of molecule.

Step-1) Preparation of Affinity Matrix of un-derivatized molecule & Control Matrix

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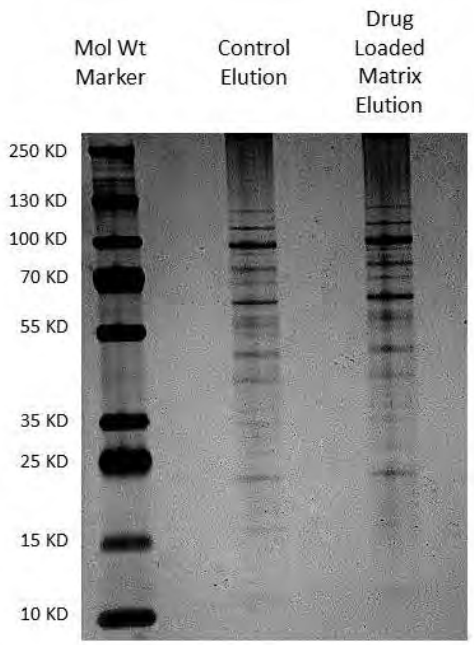
Step-2) Affinity Chromatography and SDS-PAGE Analysis →



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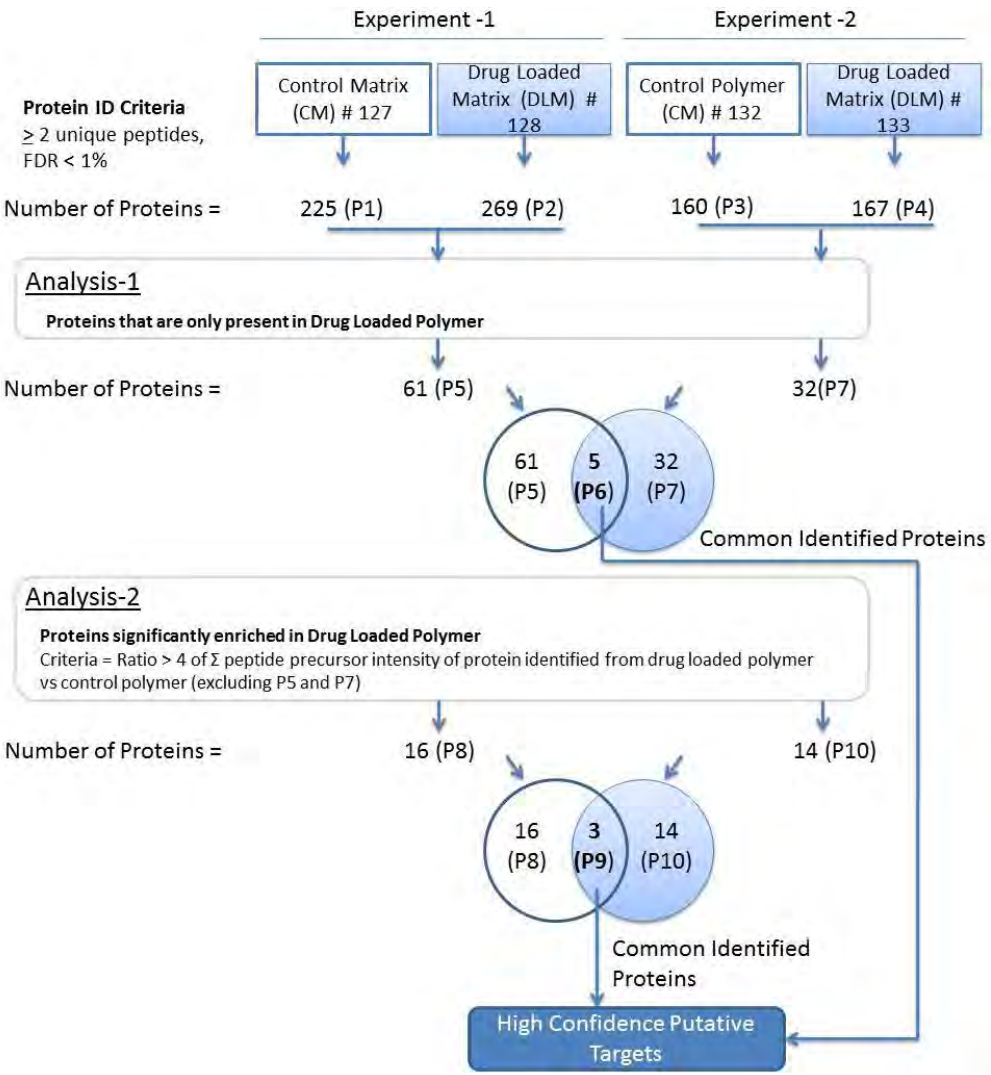
Comparative profile of proteins obtained from control and drug loaded matrix on SDS-PAGE does not appears distinct.

Mass-Spec based identification of all proteins will tell the right story.

Case Study # 1: Target Deconvolution Process



Case Study # 1: Final Deconvoluted Target

Uniprot_ID	Protein Description	Maximum Number of Unique Peptides Identified	Protein Sequence Coverage (%)	Q-Value (%)
P9WQP1	Diacylglycerol acyltransferase/mycolyltransferase Ag85B	2	8.92	0
P9WN21	Fructose-1,6-bisphosphatase class 2	3	13.81	0
P9WFV1	Leucine-tRNA ligase	3	3.82	0
I6Y7V6	Acyl-CoA ligase FadD31	2	4.52	0
P9WMJ7	Chaperone protein HtpG	2	5.87	0
I6YBZ8	3-hydroxyacyl-thioester dehydratase	6	28.97	0
P9WHV1	Gamma-glutamyl phosphate reductase	8	27.71	0
P9WMJ9	Chaperone protein DnaK	31	64.00	0

Case Study # 2: Capture of Target(s) of Phenotypic Hit (Research Institute, EU)

- **Scenario:** Molecule disrupts Notch signaling pathway, hard to derivatize, upon derivatization loosed activity, target not know.
- **Client Provided: Molecule -** 12 mg | **Cell-Lines -** RPMI-8402 & **Cell Lysate:** 9 mg
- **Time-lines at Shantani:** 2 Weeks
- **Final Deliverable:** Very small list of highly putative targets of molecule.

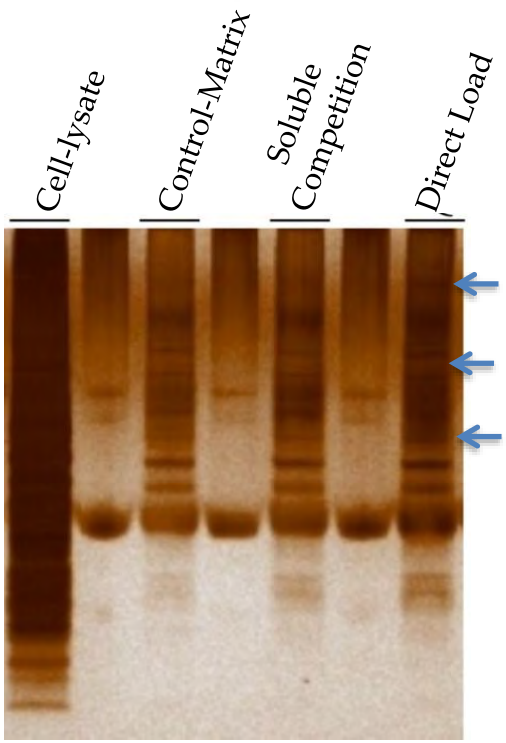
Step-1) Bait Molecule specific Affinity Matrix Preparation → **Step-2)** Affinity Matrix of un-derivatized molecule & Control Matrix → **Step-3)** Affinity Chromatography & SDS-PAGE Analysis

Polymeric Affinity Matrix of test-molecule (molecule is slightly brownish in color)

Control Polymeric Matrix

Case Study # 2: Results

Step-3) SDS-PAGE Analysis → **Step-4)** Target ID using Mass-Spectrometry → **Step-5)** Target-Deconvolution



Total Proteins Identified = ***253***

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Deconvoluted Specific Targets = 6

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

A novel non-HDAC epigenetic target was outlined and later validated by client.

Case Study # 3: Target Capture to explain the phenotype (Client – a large Indian Corporate)

- **Scenario:**
  - Molecule and its targets are established in non-human systems. In human cell-lines molecule significant inhibits PGD2 production and increases proliferation, a property of interest to client.
  - Protein target(s) of the molecule in human system are not know and hence program can not be developed further in rational manner.

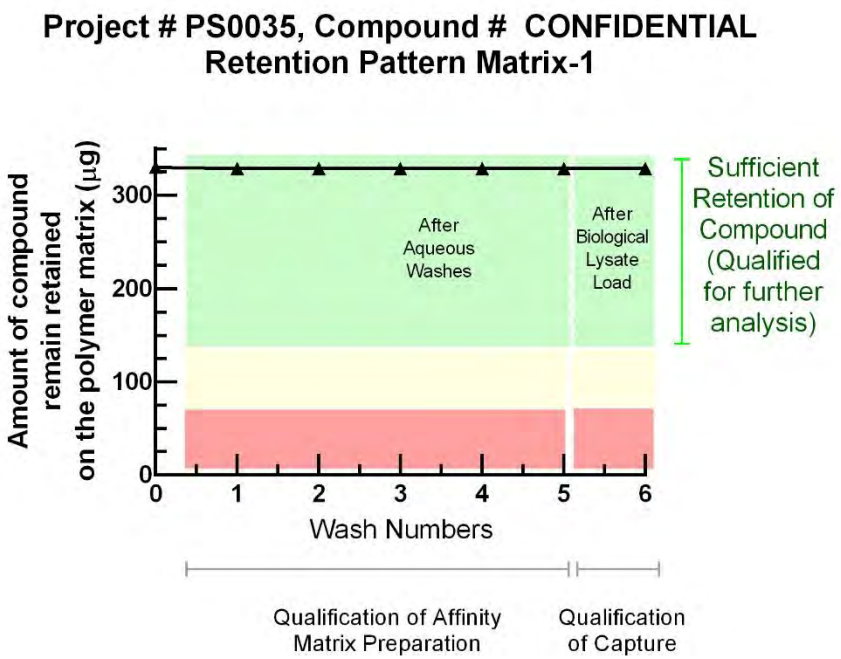
- **Client Provided:**
  - **Molecule -** 50 mg (used 12 mg)| **Cell-Lines –** CONFIDENTIAL

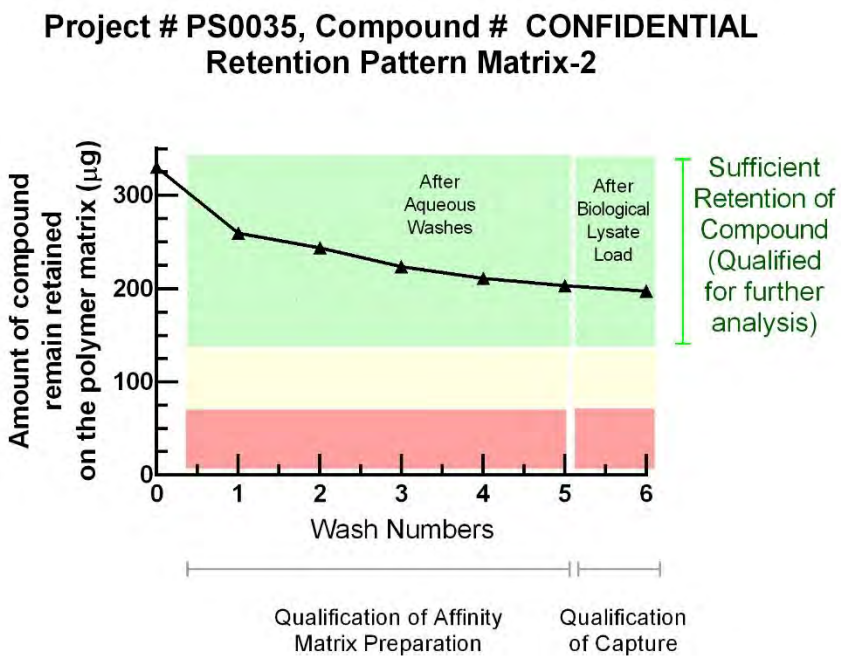
- **Time-lines at Shantani:** 5 Weeks

- **Final Deliverable:** Very small list of highly putative targets of molecule.

Case Study # 3:

Step-1) Preparation and Qualification of Test Molecule Specific Affinity Matrix





Higher amount of molecule was captured on Matrix-1 and was chosen for further experimentation.

Case Study # 3:

Step-2, 3 & 4) Affinity Chromatography, Target Deconvolution and Prioritization of Target Validation Experiments

5 Target were identified.

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Computational docking studies was utilized to further prioritize the identified putative targets for target validation efforts.

↓

List was narrowed down to 3 targets

Protein Class / Name	PDB_ID	Predicted Ki (µM)	Relative Docking Rank & Confidence
Synaptobrevin homolog	3KYQ	3.93	**90%**
ARL1	1HUR	30.90	**30%**
TRPV2	2ETA	86.49	**33%**
Exportin-T	3IBV	81.42	**18%**
huRNP	2OT8	18.53	**15%**

Case Study 3 #:

One of the Prioritized Target is ARL1 (ADP-ribosylation factor like protein 1)

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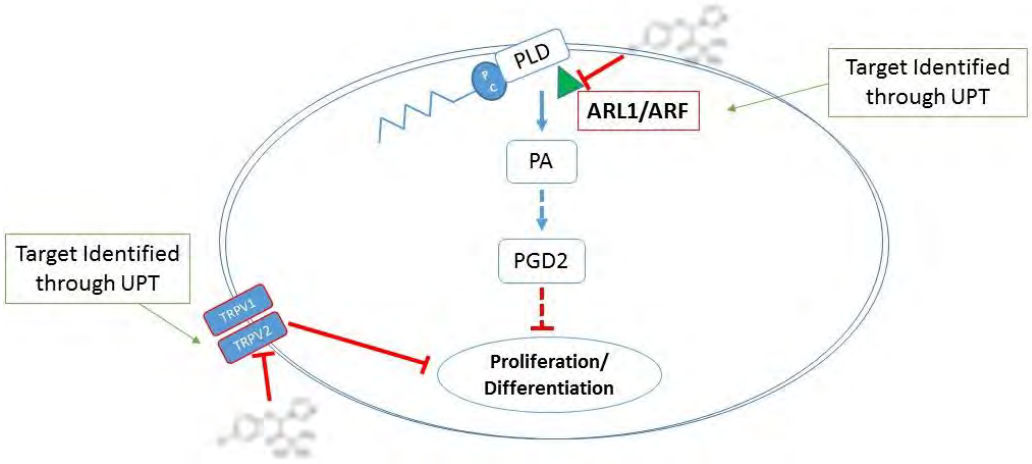
ARL1 shares 57% of amino-acid sequence with ARF1 (ADP-ribosylation factor 1)

↓

ARL1 is directly implicated in PGD2 pathway



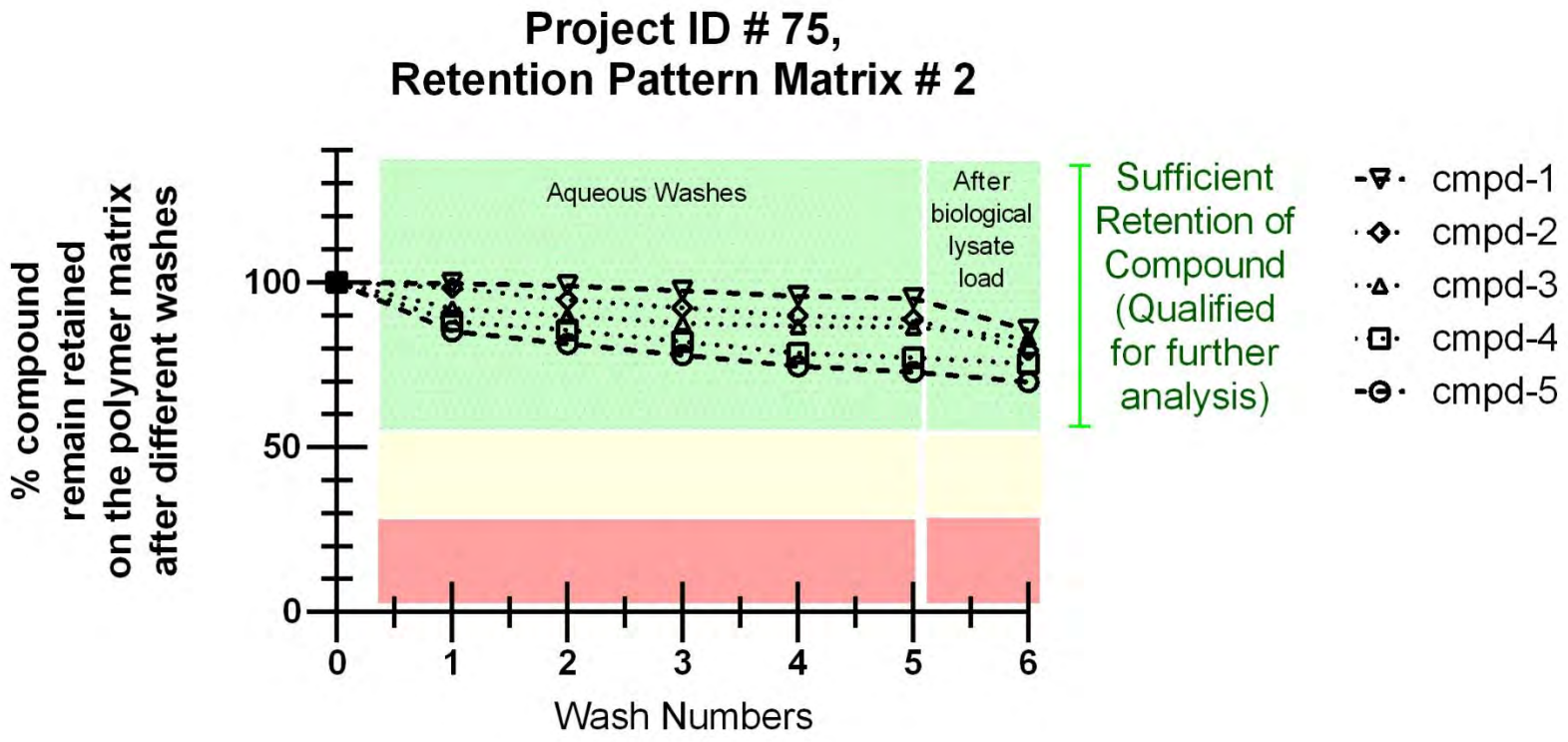
Identified Targets allowed further development of the program.

Case Study # 4 Toxicity Profiling (Client – one of the biggest global biopharma company)

- **Scenario:**
  - BACE-I inhibitor, one of the promising therapies for Alzheimer's
  - Preclinical compounds shows ocular toxicity in animal models
- **Client Provided:**
  - **Molecules -** 20 mg (Multiple molecules)| **Cell-Lines –** RPE (relevant to Ocular Toxicity)
- **Time-lines at Shantani:** 5 Weeks
- **Final Deliverable:** Very small list of highly putative targets of molecule.

Case Study - 4

Step-1) Preparation and Qualification of Test Molecule Specific Affinity Matrix



Case Study - 4

Step-2, 3 & 4) Affinity Chromatography and Target Deconvolution

12 Targets but belonging to only 2 different class of protein were identified

↓

Relevance of both the protein classes with Occular Toxicity was evaluated

↓

List was narrowed down to 3 targets

↓

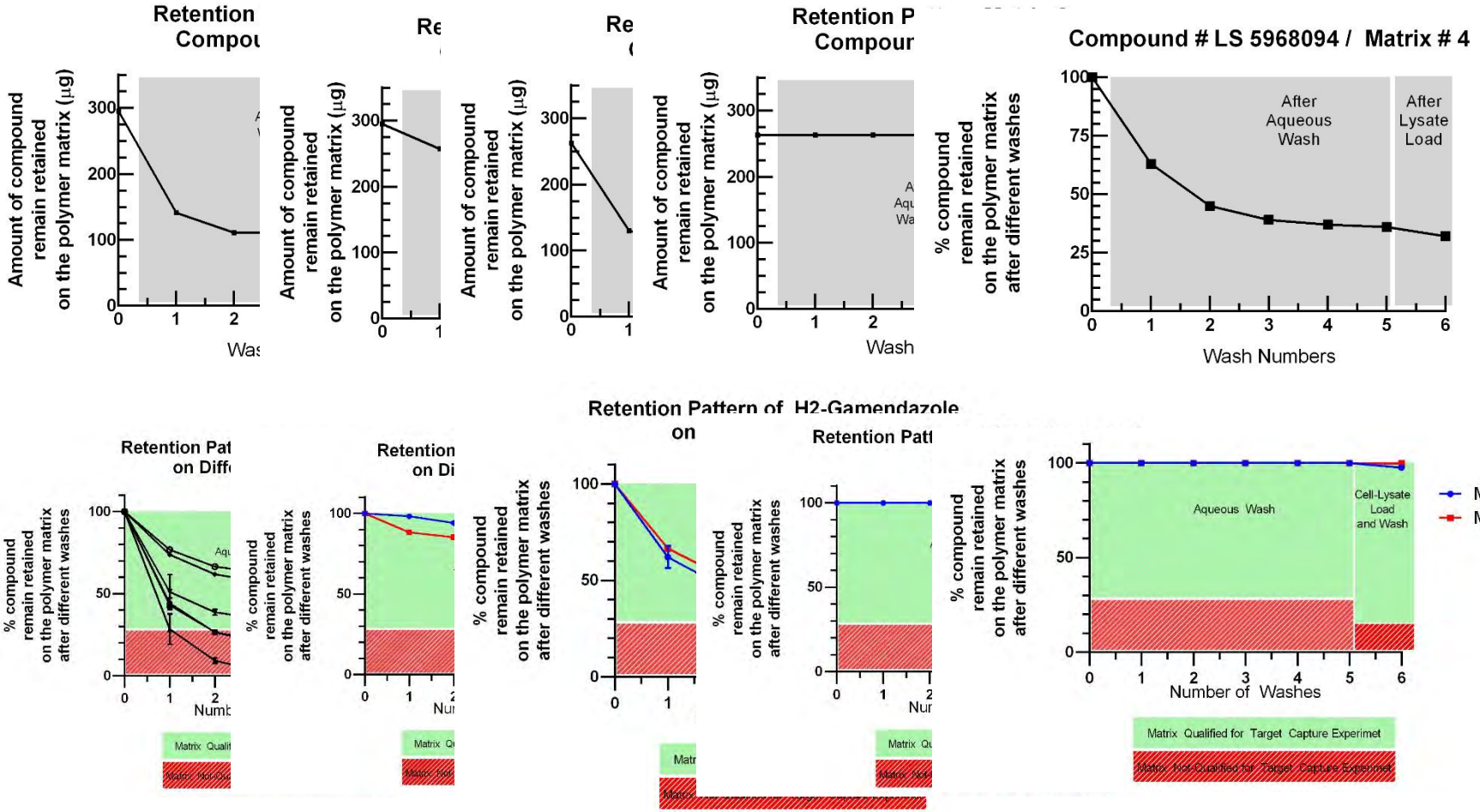
One of the Cathepsin Family Member was identified as primarily responsible for the toxicity

Value Added – Could pinpoint target protein responsible for the toxicity, 'lead-compound' selectivity process got a significant boost.

Conclusions from UPT based Chemical-Proteomics Methodologies

- 'Bait-molecule' derivation and SAR information not needed for Target ID
- Target Deconvolution can be carried out as fast as in 2 weeks
- False-positive identification rates though appears higher can be controlled by running multiple experimental replicates
- UPT can be effectively used for large and fast screening of small-molecule targets.

Several Examples

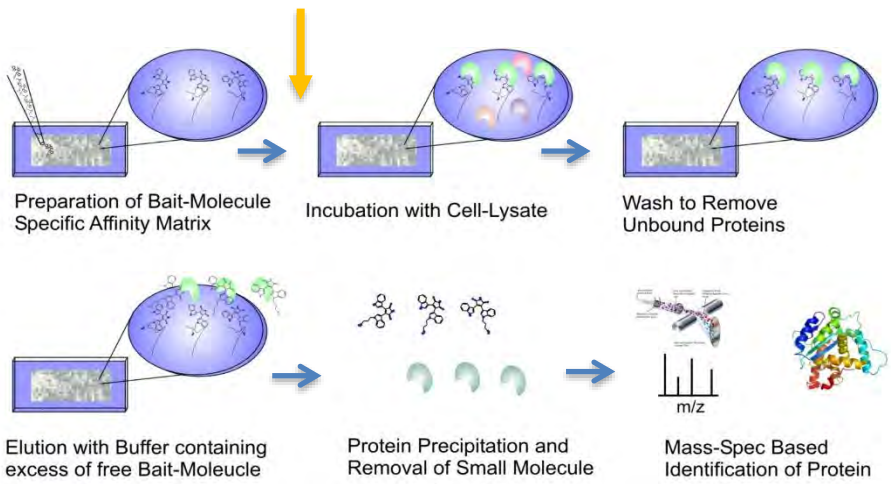


Target ID and Client Engagement Workflow

Project Information from Client

Decision on use of UPT

Go/No Go Decision Point
If molecule can not be immobilized – Project Abandoned
(Current Success Rate > 90%)



Shantani Deliver Identity of Deconvoluted Target(s)
+
Techno-commercial Target Knowledge Package (additionally charged)

If mutually decided Shantani Takes up Target Validation Work as separate project

0-2 weeks

0-5 weeks

SubCellular Location Specific Target-Capture Methodologies (SCLS)

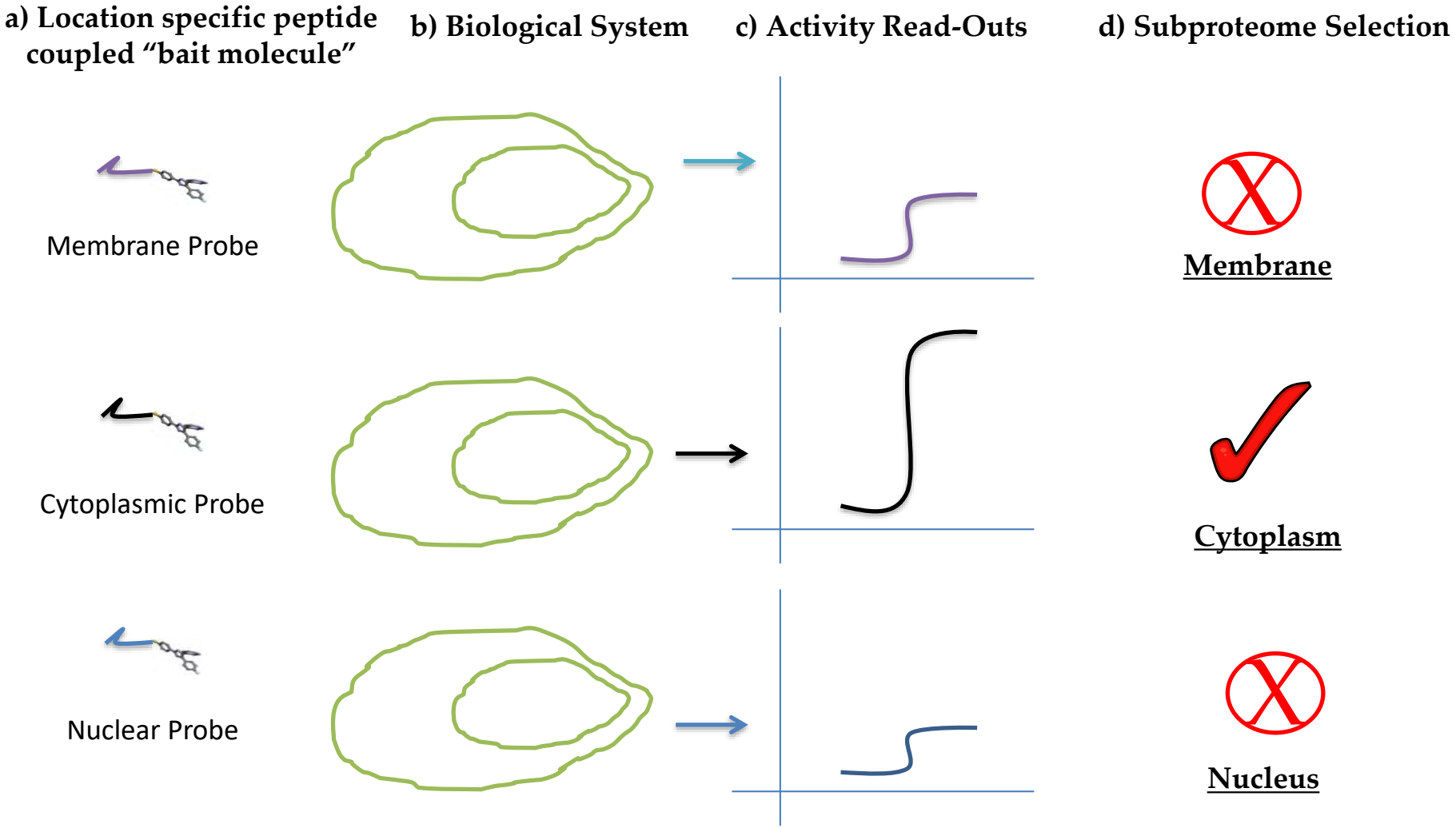
Hypothesis

Target-Capturing Probes that Can

- Identify sub-cellular compartment of molecular activity
- Be used at functionally relevant concentration for target capture
- Be Recovered from live cells

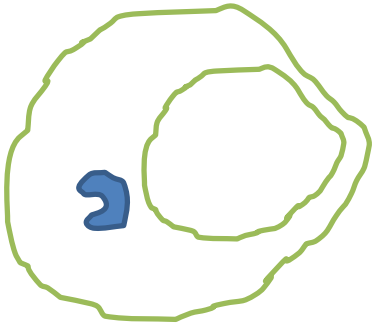
Will allow capture of rightful targets of small-molecule from physiologically relevant live-cell systems

Step-1: Identification of Sub-Cellular Location

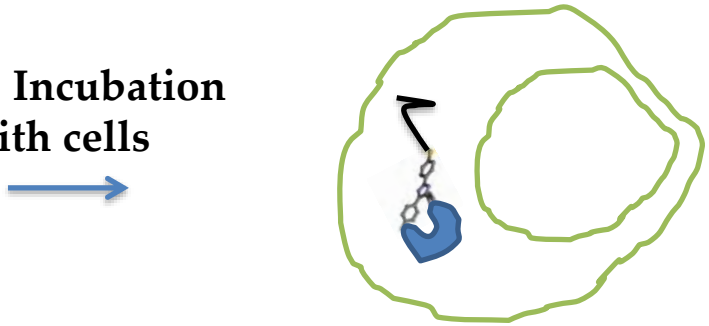


Step-2: Target Capture from Sub-Cellular Location and Target Identification

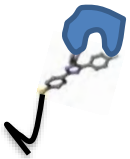
a) Cytoplasmic probe

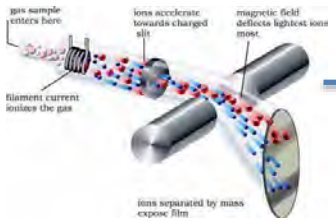


b) Incubation with cells

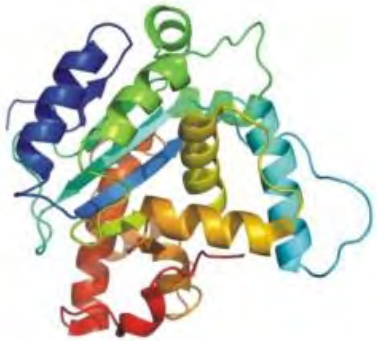


c) Lysis and affinity isolation



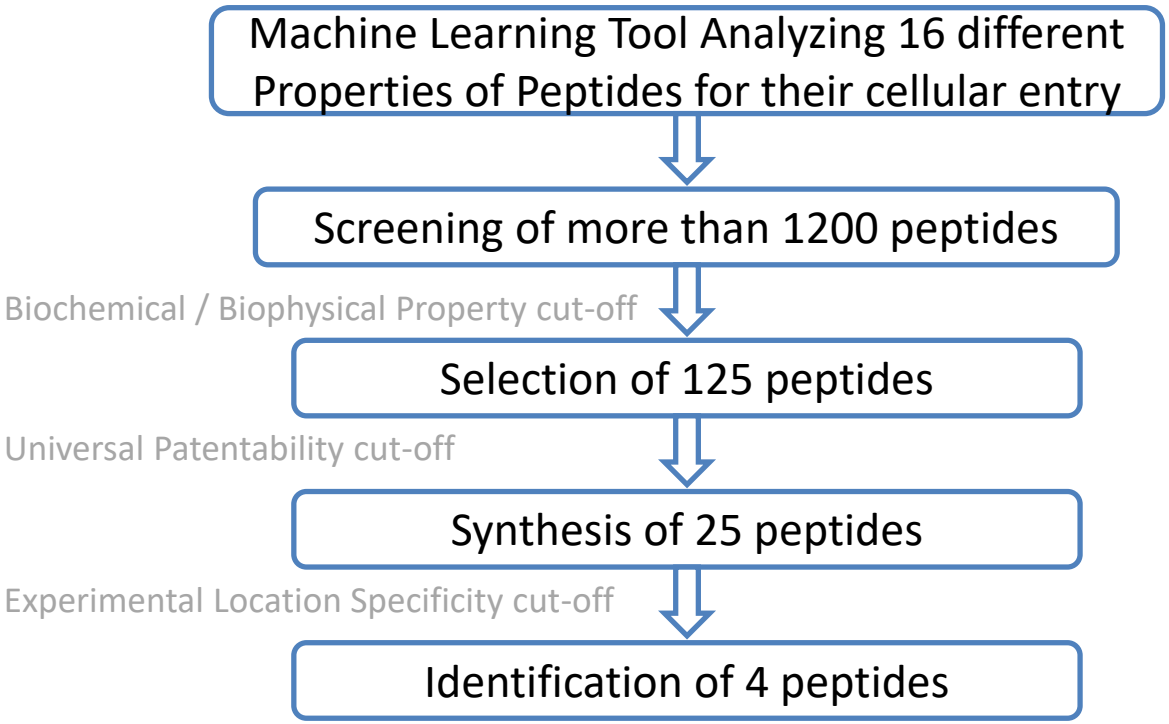


d) Mass-spectrometry



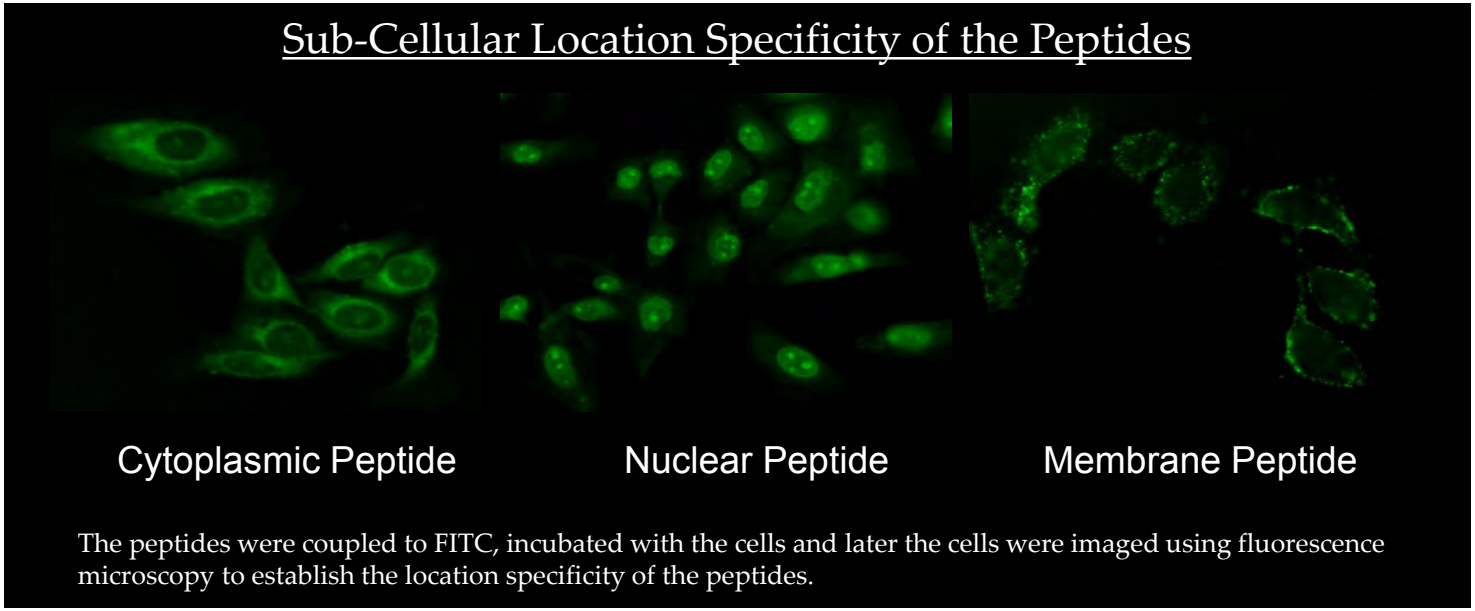
e) TARGET

Background: Development of Sub-cellular Location Specific Peptides



Properties of Peptides

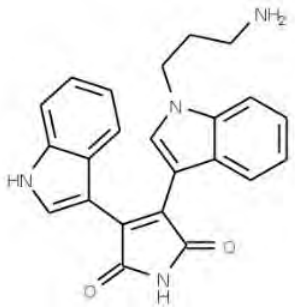
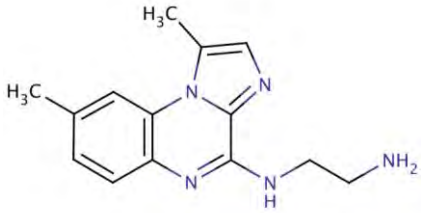
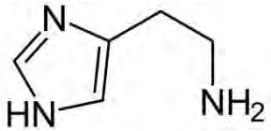
- Penetrate wide variety of mammalian cells and remain confined in sub-cellular location specific manner
- Not toxic to the cells
- No known peptidase sequence - Do not degrade in the cell
- 6-10 amino-acids long - Do not form secondary structures



SCLS –Validation Experiments

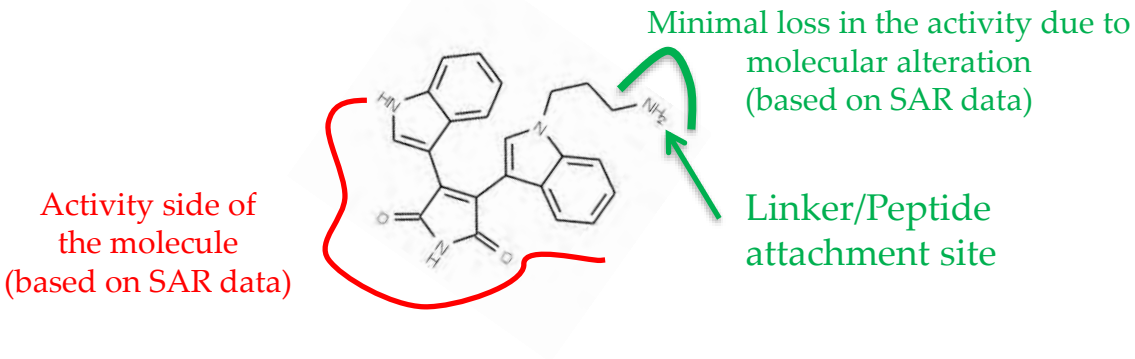
Proof of Concept Experiments (PoCs)

Three known molecules with known targets and sub-cellular target location were chosen for PoC Experiments

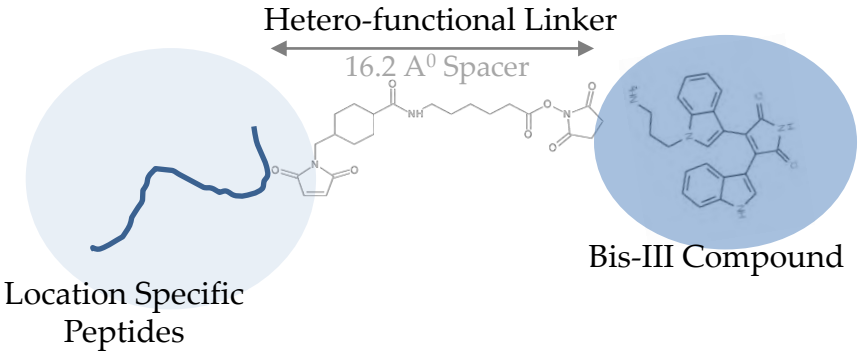
Molecule	Bis-III	BMS345541	Histamine
			
Target	GSK3-beta	IKK-2	H1-Receptor
Location	Nucleus / Cytoplasm	Cytoplasm	Membrane
Functional Activity	Cytotoxic	Inhibition of LPS Induced Nitric –Oxide Production	Increase Intracellular Calcium

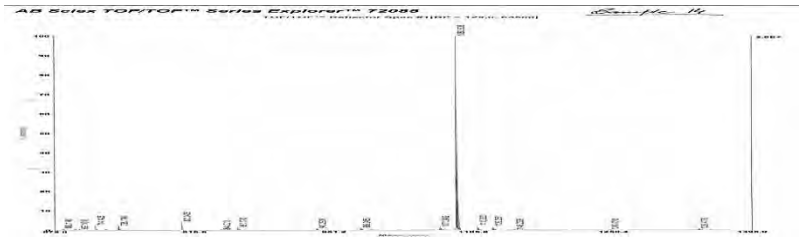
Preparation of Bis-III Probes

Step-1: Analysis of Structure Activity Relationship (SAR) for identifying site for peptide coupling



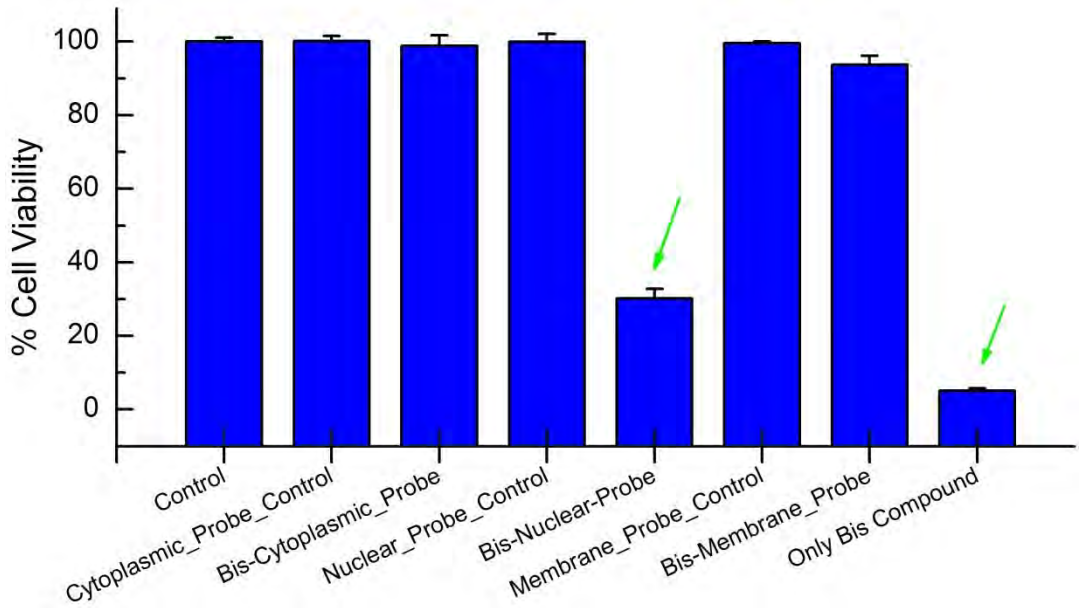
Step-2: Coupling of Location-Specific Peptide with Bis-III followed by HPLC based purification and Mass-Spectrometry Based Characterization of Probe





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

Capture of Target from Nuclear Fractions

Probes + Nuclear Fractions of He La Cells

↓

Incubation with probe antibody

↓

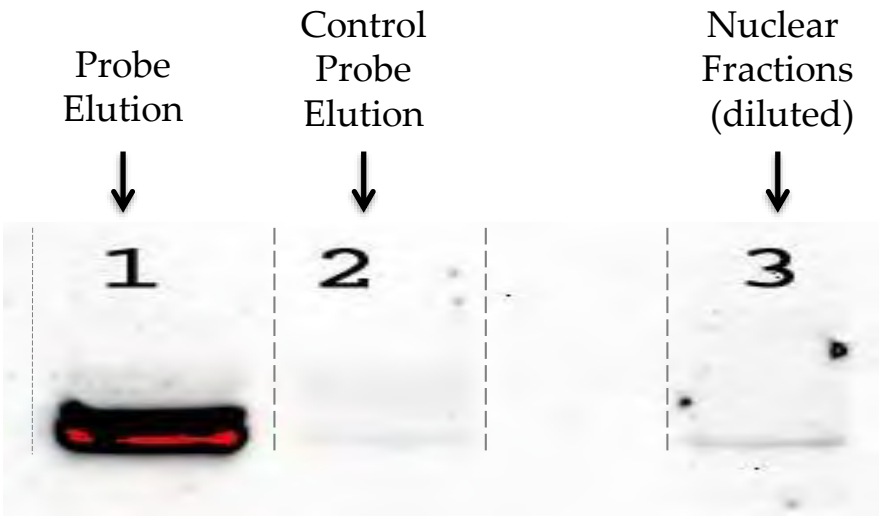
Washing of antibody

↓

Western Blot Analysis of Elution for Target Protein

Probes = Coupled with Bis-III

GSK3-beta ⇨



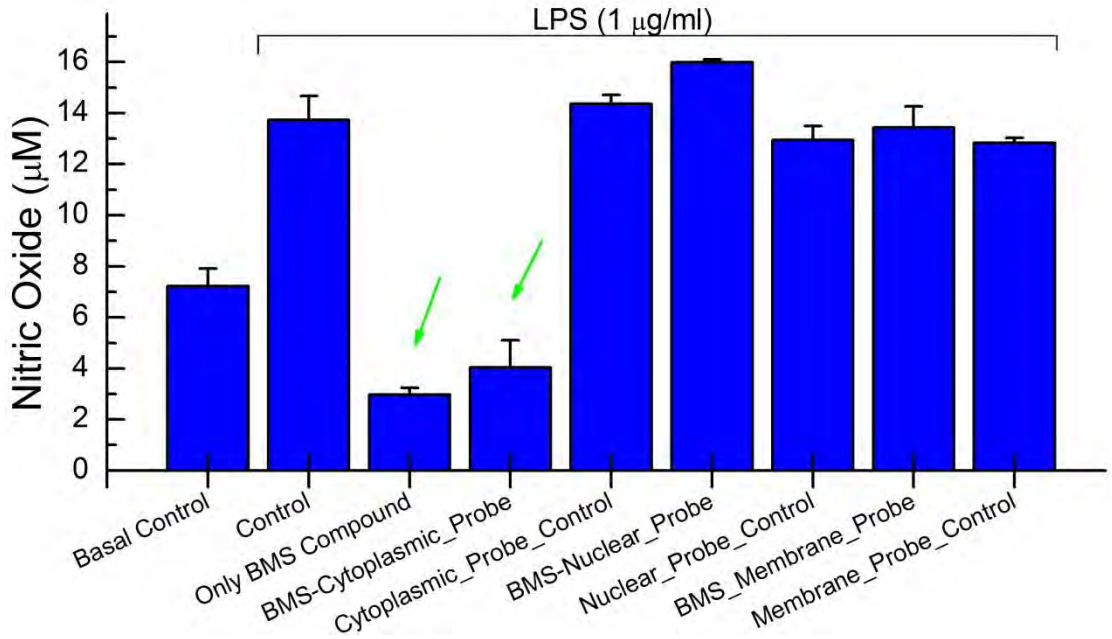
Target was specifically captured with Probe.

Sub-cellular Location Specific Functional Activity of BMS345541 Probes

BMS345541 was coupled to three sub-cellular location specific proprietary peptides

→

Functional Assay: Inhibition of LPS induced Nitric Oxide Production in RAW cells



- Basal Control = No induction of Nitric Oxide by LPS
- BMS compound = 12.5 μM
- All peptide probes and control probes = 25 μM

BMS probes that were targeted to the cytoplasm exhibited similar activity as the free BMS compound

→

Target located in cytoplasm is responsible for the activity

Capture of Target from Cytoplasmic Fractions

Probes + Cytoplasmic Fractions of RAW Cells

↓

Incubation with probe antibody

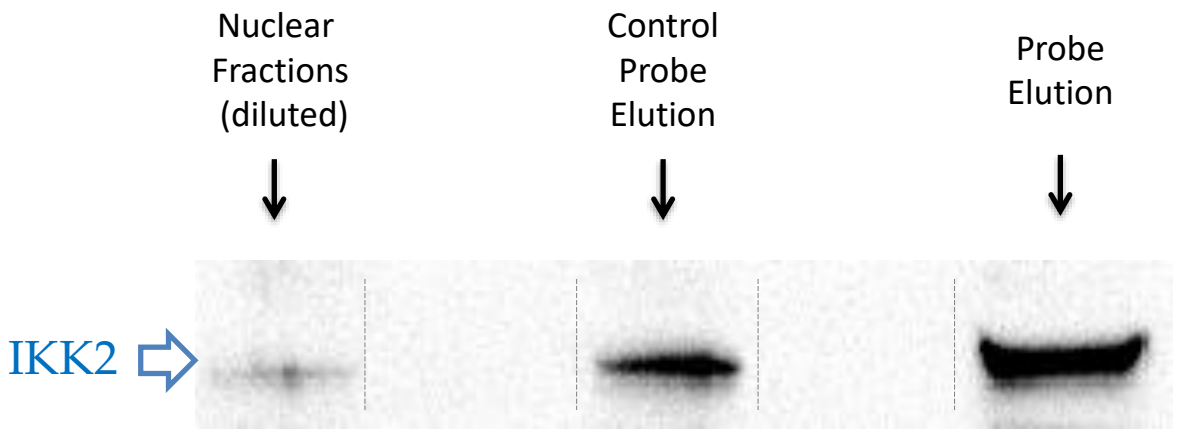
↓

Washing of antibody

↓

Western Blot Analysis of Elution for Target Protein

Probes = Coupled with BMS345541



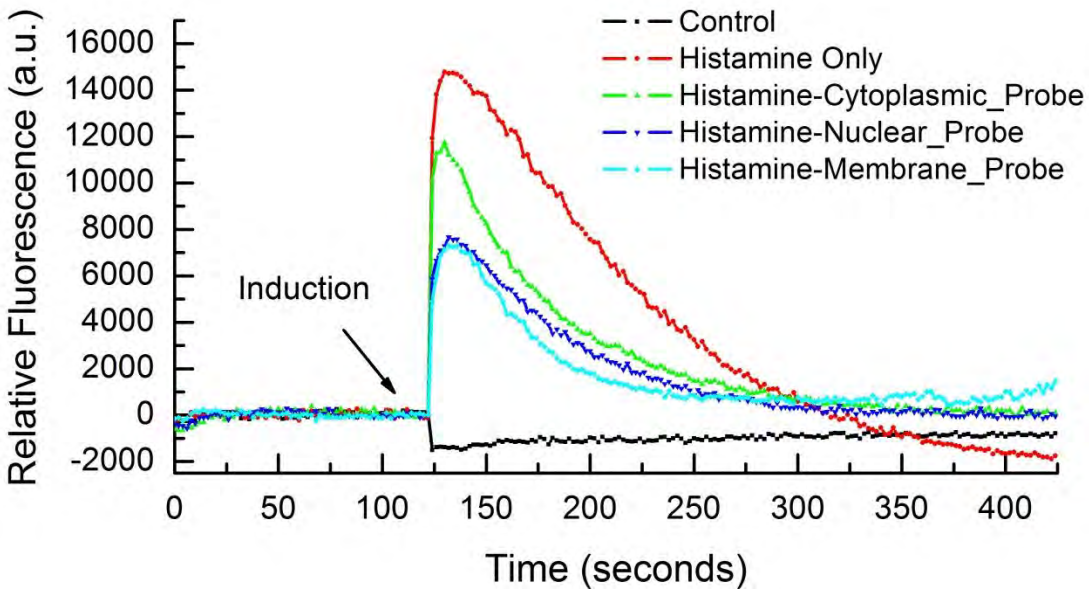
Target was specifically enriched with Probe.

Sub-cellular Location Specific Functional Activity of Histamine Probes

Histamine was coupled to three sub-cellular location specific proprietary peptides

→

Functional Assay: Increase in intracellular calcium in HeLa Cells



Histamine-compound and all probes at 200 μM concentration.

All the histamine probes showed the activity

→

Every peptide probe shows the activity, i.e. target is located on the membrane because every probe will either hit or cross the membrane activating increase in calcium

Capture of Target from Cytoplasmic Fractions

Peptide Probes + Membrane Fractions of He La Cells

↓

Incubation with peptide antibody

↓

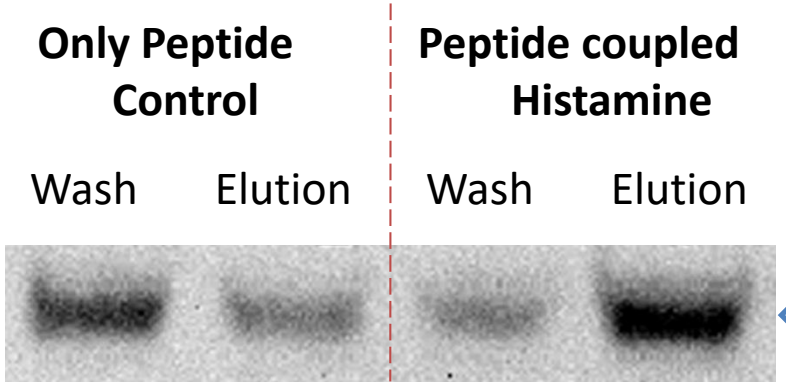
Washing of antibody

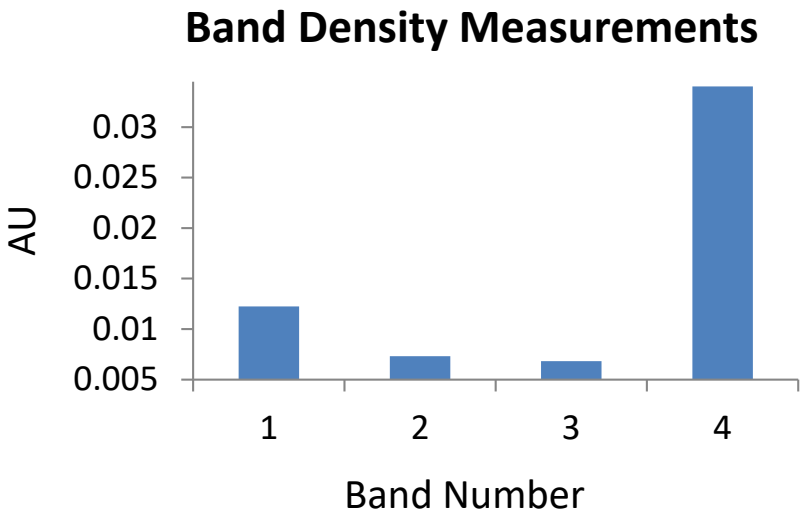
↓

Elution of antibody with Histamine

↓

Western Blot Analysis of Elution





Target was specifically captured with Probe.

SCLS
vs
Traditional Bead Based Method

Direct Comparison: SCLS vs Traditional Bead Based Method – Molecule # 1

Bait-molecule = Bis-III

Workflow Comparison

	SCLS	Bead Based
Biological System	Physiologically Relevant Live-cells	Cell-Lysate
Target Capturing Probe	Nuclear Specific Probe of Bis-III (based on the functional activity)	Bis-III immodilized on sepharose 6B Epoxy beads
Affinity Interaction	55 µM Bis-III Probe in live cell	25 µl Bis-III on beads with cell lysate
Affinity Pull-Down	Antibody Against the Probe	Bis-III on beads
Protein Analysis	SDS-PAGE followed by in-gel trypsin digestion and Mass-spec based protein identification	SDS-PAGE followed by in-gel trypsin digestion and Mass-spec based protein identification

Results on next slide

Direct Comparison: SCLS vs Traditional Bead Based Method – Molecule # 1

Criteria	SCLS	Traditional Bead Based
Primary Target Identified	YES	YES
Confidence in Target Identity	Strong (4 unique peptides identified)	Weak (only 1 unique peptide identified)
Total Number of Deconvoluted Protein Targets	15	16
Capture from Native Cellular Environment	YES	NO
Capture using Functionally Relevant Ligand	YES	NO
Number of Target(s) (with Known Strong Affinity to Molecule) Identified	4	1
Number of Target(s) (with Known Low Affinity to Molecule) Identified	2	3
Dynamics Range of Capture	Medium (Primarily identify high affinity binding partner)	High (Identify high & low affinity binding partners)
False Positive Capture	Low	High

SCLS provides better target deconvolution.

Direct Comparison: SCLS vs Traditional Bead Based Method – Molecule # 2

Bait-molecule = SB202190, Known Target = p38

Workflow Comparison

	SCLS	Bead Based
Biological System	Physiologically Relevant Live-cells	Cell-Lysate
Target Capturing Probe	Cytoplasm Specific Probe of SB202190 (based on the functional activity)	SB202190 immodilized on sepharose 6B Epoxy beads
Affinity Interaction	25 μM Bis-III Probe in live cell	25 μl Bis-III on beads with cell lysate
Affinity Pull-Down	Antibody Against the Probe	Bis-III on beads
Protein Analysis	SDS-PAGE followed by in-gel trypsin digestion and Mass-spec based protein identification	SDS-PAGE followed by in-gel trypsin digestion and Mass-spec based protein identification

Results on next slide

Targets Identified for SB202190

Protein Annotation	No. of Unique Peptides Identified	Sequest Xc For highest Matched peptide	Tandem e value For highest Matched peptide	Specificity Ratio
Mitogen-activated protein kinase 14 isoform 2 (p38)	9	5.51	0.000	1.00
Isoform 1 of Glycogen synthase kinase-3 beta (GSK3-beta)	5	5.49	0.000	1.00
Lactoylglutathione lyase (GLO1)	5	5.31	0.003	1.00
Isoform 1 of Casein Kinase I delta (CKId)	3	4.35	0.000	1.00
STE20/SPS1-related proline-alanine-rich protein kinase (STK39)	2	6.13	0.000	1.00
Isoform Alpha-2 of Mitogen-activated protein kinase 9 (JNK2)	5	6.54	0.005	0.83

Robust Target Identification

Direct Comparison: SCLS vs Traditional Bead Based Method – Molecule # 2

	SCLS	Bead Based Method
Number of Deconvoluted Target	6	18
Validation Time (assuming one target / month, validation at in-vitro/cellular level only)	6 months	18 months

Better Target Deconvolution = Faster Validation = Program Go/No-Go Decision = Time and Cost Saving

Validation of Identified Target

Protein Annotation	Interaction Efficiency
Mitogen-activated protein kinase 14 isoform 2 (p38)	Inhibition 95.95% (Kinase Panel) IC50 = 38 nm
Isoform 1 of Glycogen synthase kinase-3 beta (GSK3-beta)	Inhibition 68.65% (Kinase Panel)
Lactoylglutathione lyase (GLO1)	ND
Isoform 1 of Casein Kinase I delta (CKId)	Inhibition 88.72% (Kinase Panel)
STE20/SPS1-related proline-alanine-rich protein kinase (STK39)	Inhibition 63.25% (Kinase Panel)
Isoform Alpha-2 of Mitogen-activated protein kinase 9 (JNK2)	Inhibition 72.25% (Kinase Panel)

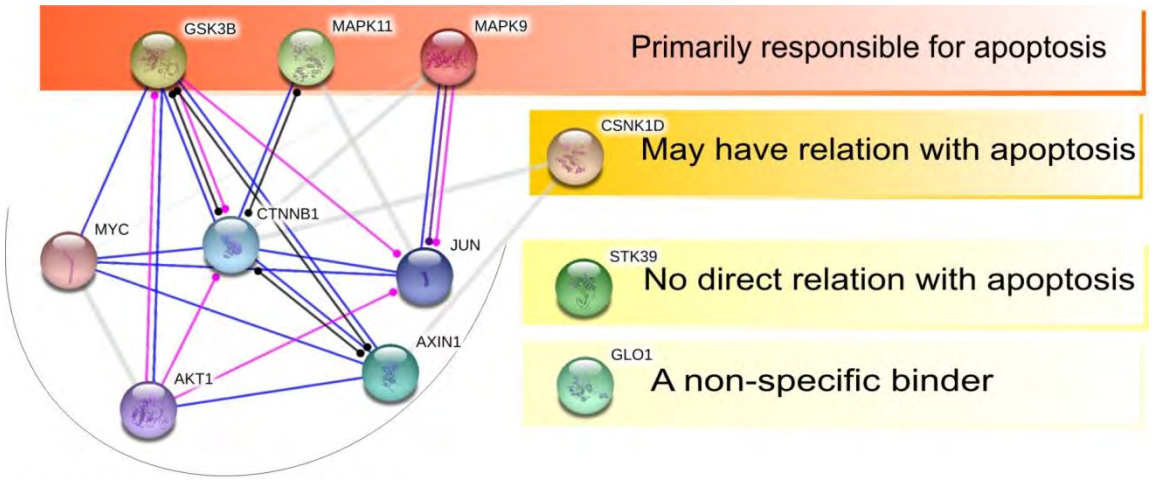
Identified Targets were True Positive Targets of SB202190

From Quantitative Target Information to Action Mechanism and Tox Prediction

For SB202190 compound the identified proteins were rank-ordered based on their abundance and interaction ability and were mapped on to the canonical pathways to predict that molecule may induce apoptosis

Pathway Knowledge Base

Identified Proteins if inhibited together will lead to Apoptosis.



Protein Borrowed for apoptosis network analysis

SB202190 Does Induce Apoptosis

A selective inhibitor of p38 MAP kinase, SB202190, induced ...
www.sciencedirect.com/science/article/pii/S0925443900000454
by H Karahashi - 2000 - Cited by 36 - Related articles
A selective p38 MAP kinase (p38 MAPK) inhibitor, **SB202190**, **induced apoptotic** cell death of a macrophage-like cell line, J774.1, in the presence of ...

Induction of Apoptosis by SB202190 through Inhibition of ...
www.jbc.org/content/273/26/16415.full.pdf
by S Nemoto - 1998 - Cited by 279 - Related articles
... addition, SB202190 was able to potentiate apoptosis induced by Fas(APO-1) ... These re- sults indicate that **SB202190 induces apoptosis** through activation of ...

Induction of Apoptosis by SB202190 through Inhibition of ...
www.jbc.org/content/273/26/16415.full
by S Nemoto - 1998 - Cited by 279 - Related articles
Jun 26, 1998 - In contrast, expression of p38α induced cell death mildly. These results indicate that **SB202190 induces apoptosis** through activation of ...

A selective inhibitor of p38 MAP kinase, SB202190, induced ...
www.ncbi.nlm.nih.gov/pubmed/11040446
by H Karahashi - 2000 - Cited by 36 - Related articles
Oct 18, 2000 - A selective inhibitor of p38 MAP kinase, **SB202190**, **induced apoptotic** cell death of a lipopolysaccharide-treated macrophage-like cell line, ...

Induction of apoptosis by SB202190 through inhibition of ...
www.ncbi.nlm.nih.gov/pubmed/9632706
by S Nemoto - 1998 - Cited by 279 - Related articles
J Biol Chem. 1998 Jun 26;273(26):16415-20. **Induction** of **apoptosis** by **SB202190** through inhibition of p38beta mitogen-activated protein kinase. Nemoto S(1) ...

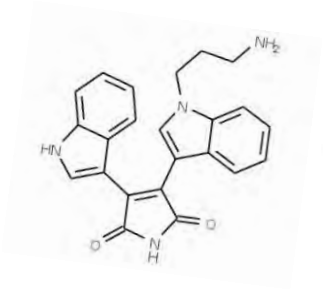
Computation Workflow

Computational Supreme (Comp-S)

Key Advantages

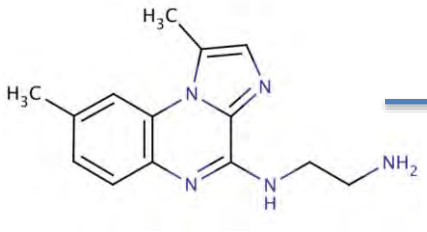
- Utilizes Structural and Functional Features of 'test-molecule' when comparing historical database
- Quick and cost-effective
- Low False Positive Identification Rate = Faster Validation

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

Identification of Target of Novel Molecule

Identification and Validation of Target and Understanding MoA of a Novel Imidazoline

Molecule: BL11282 Stimulates insulin secretion in unknown fashion.

a) BL11282 does not block ATP-dependent K^+ channels
b) BL11282 activity appears to be sensitive to the inhibition of PKA
c) The increase in intracellular calcium upon BL11282 administration makes only a minor contribution to insulin secretion
d) BL11282 directly influences exocytosis process…..

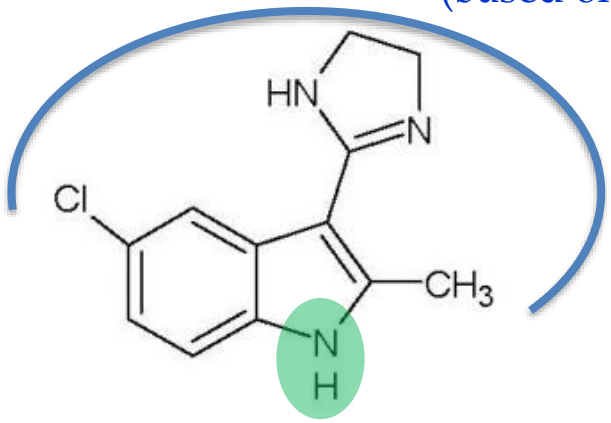
BL11282

- The Cellular Binding Partners of BL11282 are not know and its action mechanism is elusive hence the molecule can not be rationally optimized…

In this PoC we will utilize our chemical-proteomics tools and strategies to identify and validate primary targets of BL11282

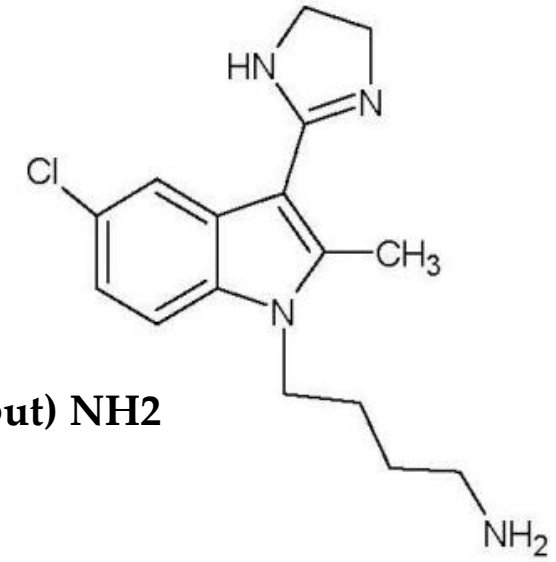
Synthesis of BL11282 derivative for preparation of target capturing probe

Active site of the molecule
(based on SAR data)



BL11282

Molecule Derivatization /
Peptide attachment site

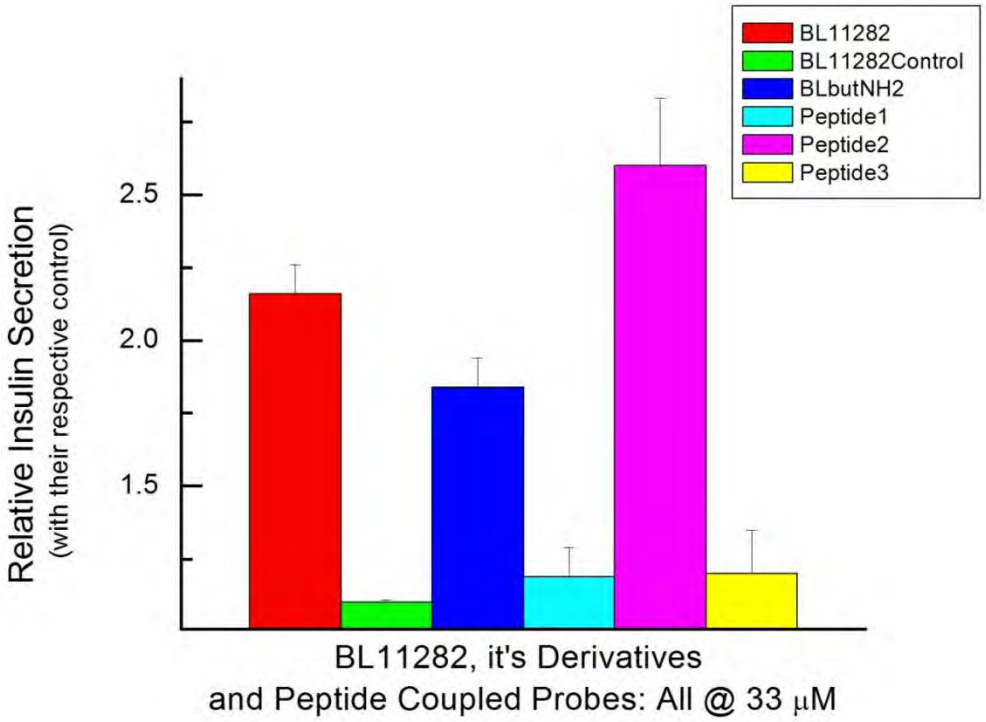


BL-(but) NH2

References: BL11282 - Structure-Activity Relationship (SAR) data

- Bioorganic and Medicinal Chemistry 15: 3284-3265 (2007)
- Bioorganic and Medicinal Chemistry 15: 6782-6795 (2007)

Functional Activity of BL11282 derivatives and Target Capturing Probes

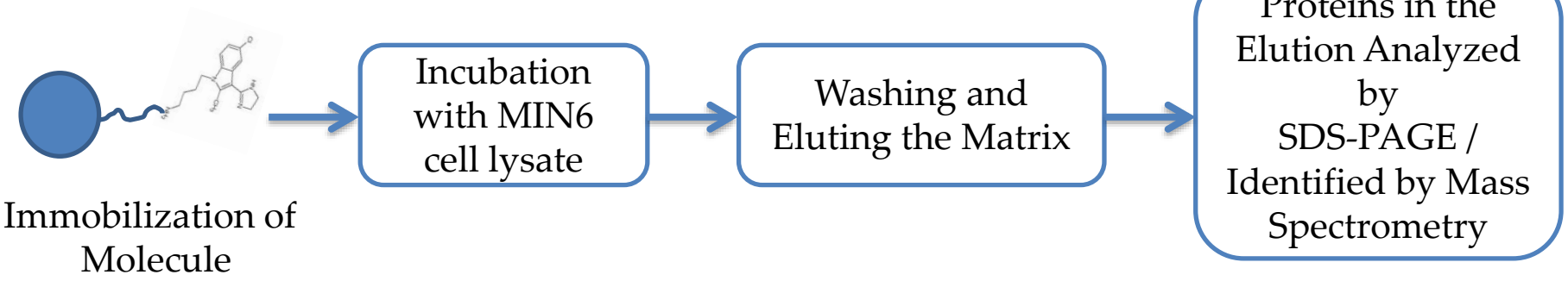


Peptide 1, 2 and 3 are three different peptides that targets three different locations of the cell.

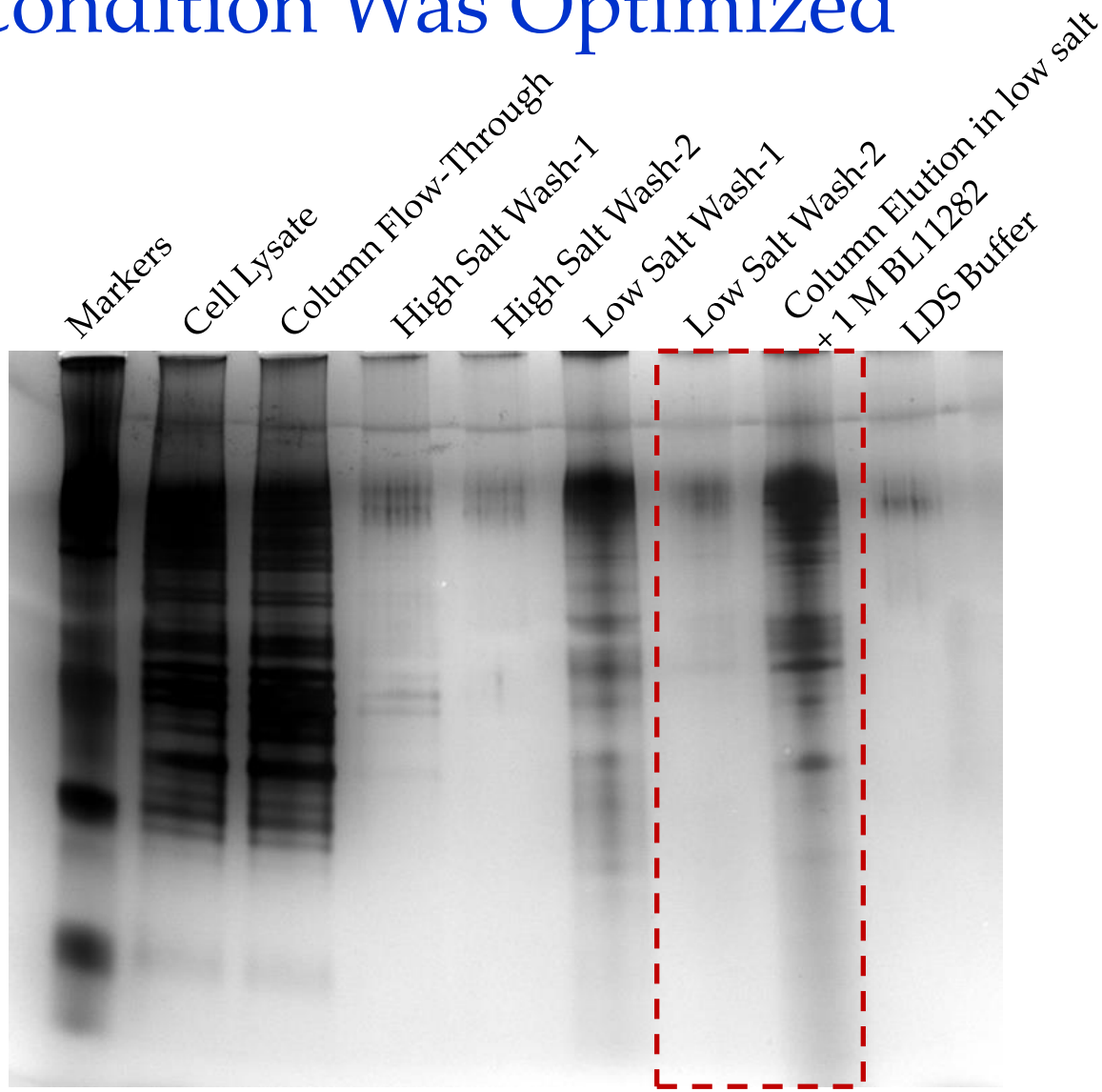
BL11282 was coupled to these peptides and functional activity (insulin secretion ability) of the constructs was evaluated.

Sub-Cellular Location of the Target was Identified

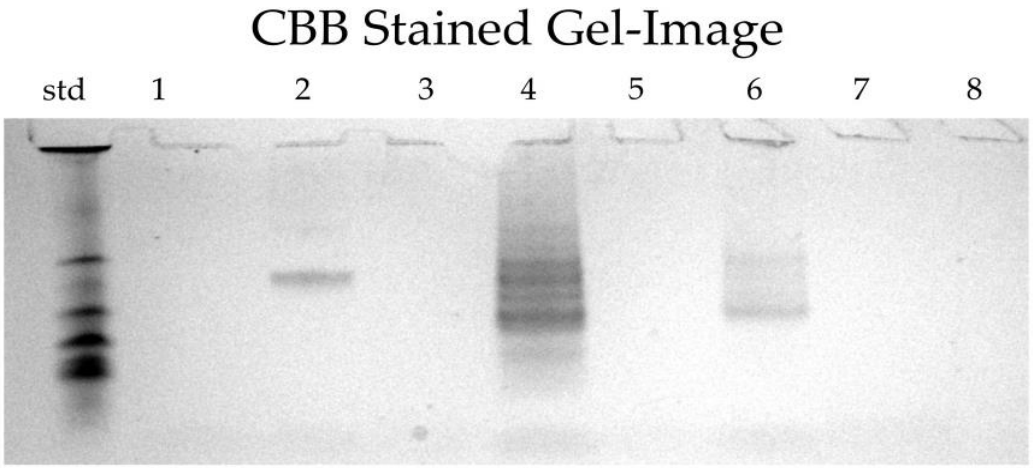
Utilization of Traditional Bead Based Method for Target Pull-Down from Specific Cellular Fraction



Distinct Elution Profile over Wash-Condition Was Optimized



Distinct Protein Profile Over Control Experiments



Lane 0 = Std
Lane 2 = Control Matrix
Lane 4 = Elution from BL11282 Matrix
Lane 6 = Elution from BL11282 Matrix (cell-lysate pre-incubated with BL11282)
Lane 1,3, 5, 7 & 8 = Buffer

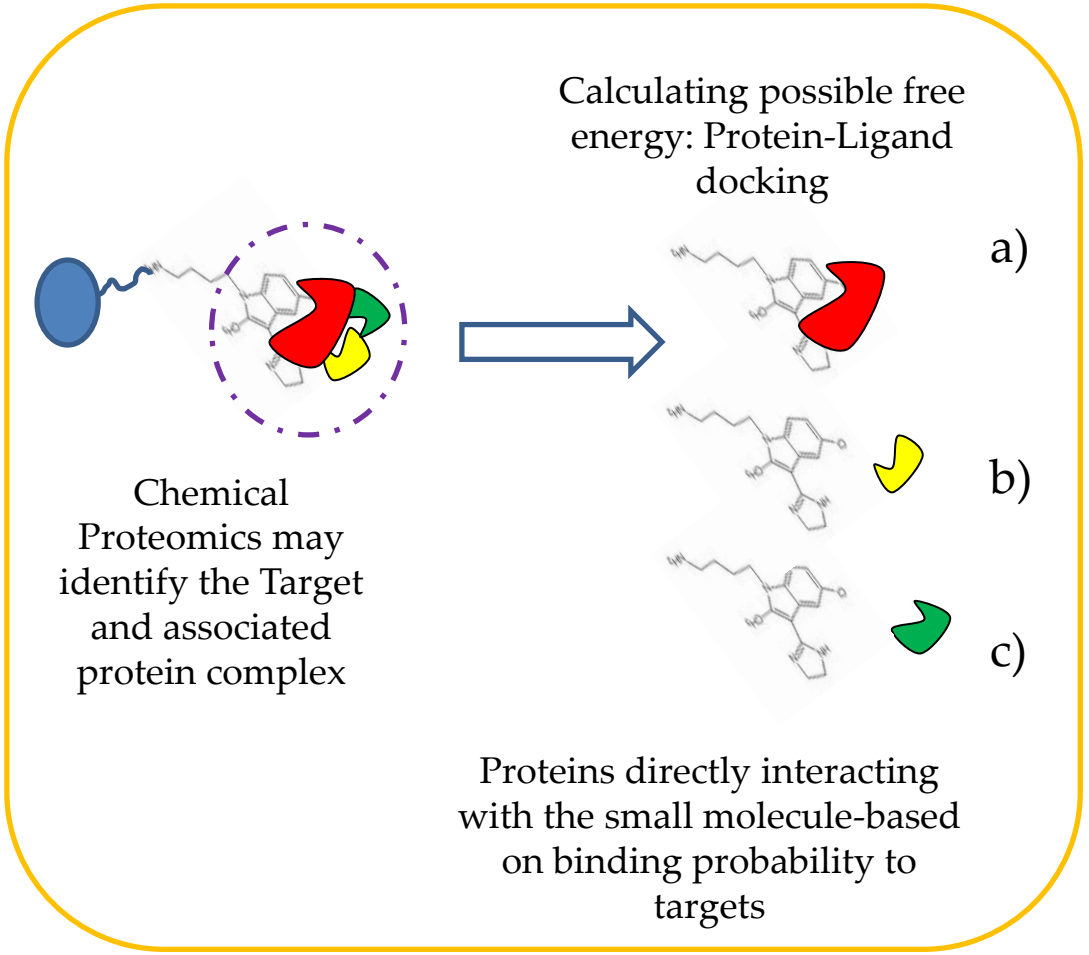
*Each of these lanes are proteins pooled from three different experiments and at least two such runs were carried out for protein identification. Protein bands were cut, in-gel trypsin digested and identified using mass-spectrometry based workflow.
Note: The proteins were separated only for 7 minutes on SDS-PAGE gels.

Deconvoluted Proteins were Subjected to '*in-silico*' docking analysis with BL11282

Small-molecule affinity based chromatographic methods will elute **protein complexes associated** with the target protein rather than **The Only Target Protein.**

↓

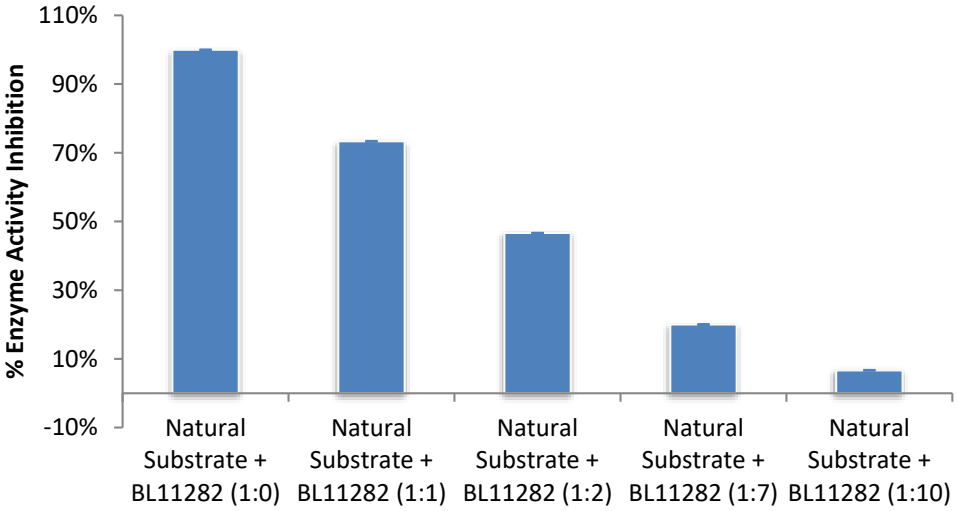
Proteins that may be directly interacting with the small-molecule can be prioritized based on their binding probability to the targets



Prioritization of Proteins using Predicted Free-Energy of Interaction

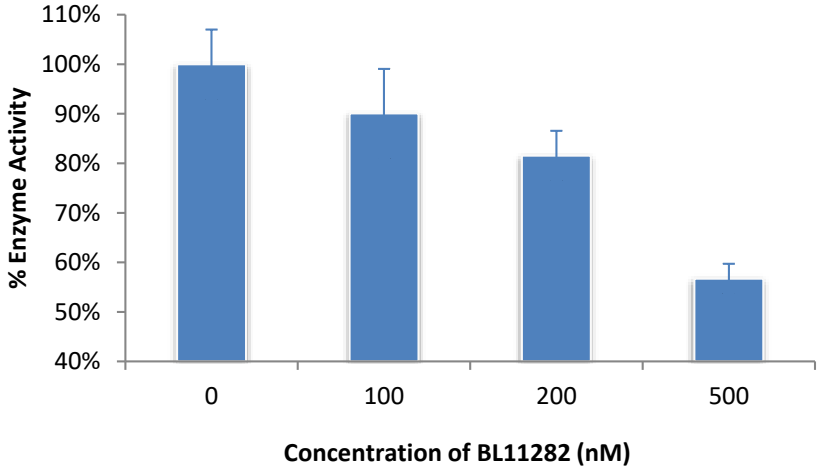
Top 10 Target Proteins	Predicted Free Energy (kcal/mol)	Predicted (Kd) µM
1	-8.71	0.41
2	-8.61	0.49
3	-8.23	0.93
4	-8.03	1.30
5	-7.94	1.50
6	-7.54	2.96
7	-7.52	3.07
8	-7.43	3.60
9	-7.44	3.77
10	-7.4	3.79

BL11282 Inhibits Target 1 and 2 – Biophysical Validation

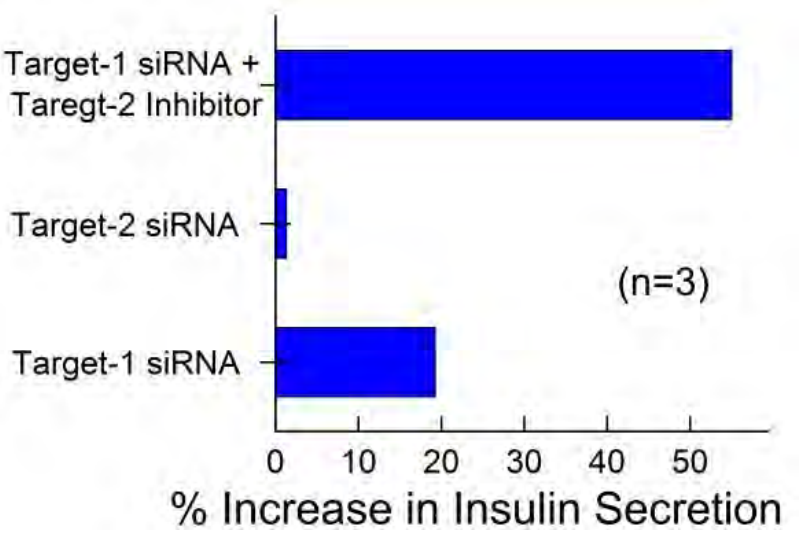


Inhibition of Target – 1 by BL11282
(Spectrophotometric Assay Monitoring Product of the Enzyme Reaction)

Inhibition of Target – 2 by BL11282
(ELISA based Cell Based Assay)



siRNA and Pharmacological Inhibition Based Functional Validation of Target(s)

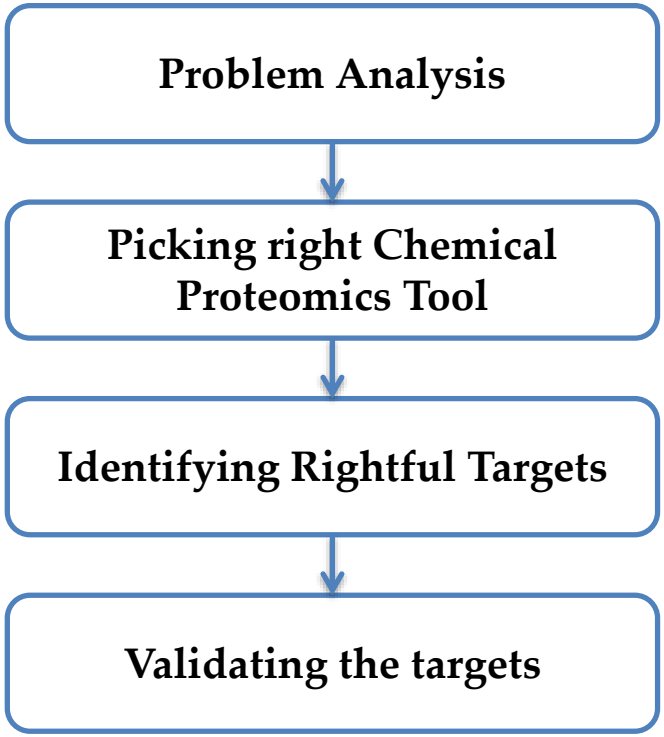


- Cell Type – MIN6 (Mouse Insulinoma)
- Target knock down by siRNA was optimized and established separately.
- Data are plotted after subtracting respective control.

Both the targets if inhibited together increases insulin secretion !!!

Conclusions From BL11282-Chemical Proteomics

- Study of molecule SAR allowed preparation of functionally active target capturing probe
- Appropriate chemical proteomics tools were used to identify cellular binding partners of BL11282
- Identified binding partners were thoroughly analyzed using in-silico tools and their putative relation with phenotype to deconvolute the targets
- Prioritized targets were validated using biophysical and molecular biology tools



Shantani Identify Targets and MoA of Bioactive Molecules

Program's Target ID Need

Shantani Deploy (Appropriate Technology + A Decade of Target Identification Expertise + Program Centric Business Model)

Deconvoluted Target Information

Our Key Strengths

- Globally Competitive Science
- High Ethical and Professional Standards
- Networked Operational Model for Cost-Effectiveness

Our Customer's Profile

East Coast-USA based one of the Largest Pharmaceutical Company

World's Largest Chemical Producer HQ - Germany

Unilever

THE UNIVERSITY OF TEXAS MD Anderson Cancer Center

IDRI

KU MEDICAL CENTER The University of Kansas

reclaimrx recover and regenerate

EPFL ÉCOLE POLYTECHNIQUE FÉDÉRALE DE LAUSANNE

ITC

ICGEB

praj Innovate • Integrate • Deliver

national centre for cell science An Autonomous Institution of the Department of Biotechnology, Govt. of India

CSIR-Central Drug Research Institute, Lucknow

BARE FEET Analytics

SHIV NADAR UNIVERSITY

NATIONAL INSTITUTE OF VIROLOGY PUNE

POONAWALLA GROUP SERUM INSTITUTE OF INDIA LTD.

BIONEEDS INDIA PRIVATE LIMITED INTEGRATED DISCOVERY, DEVELOPMENT AND REGULATORY SERVICES

SEAGULL BioSolutions Pvt. Ltd.

Shantani R&D Center @ Innovation Park

Thank You.

Connect for further discussions

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