Target Identification using Unique Polymer Technology (UPT)

Overview of Company, Technology & New Case Studies of UPT

Small-molecule Target-ID Premises, Problem Statement and Value

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

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

Our Focus = Target Identification

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

Our >10 years expertise allows us to evaluate the 'fit-for-purpose' technology for every program
and
then we deploy appropriate Technology for **right target** from a portfolio of Technology

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

Unique Polymer Technology (UPT)

Technology Details & Case Studies

Unique Polymer Technology (UPT)

Key Advantages

- Derivatization of test-molecule is not needed
- Target Deconvolution can be completed within 3-5 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

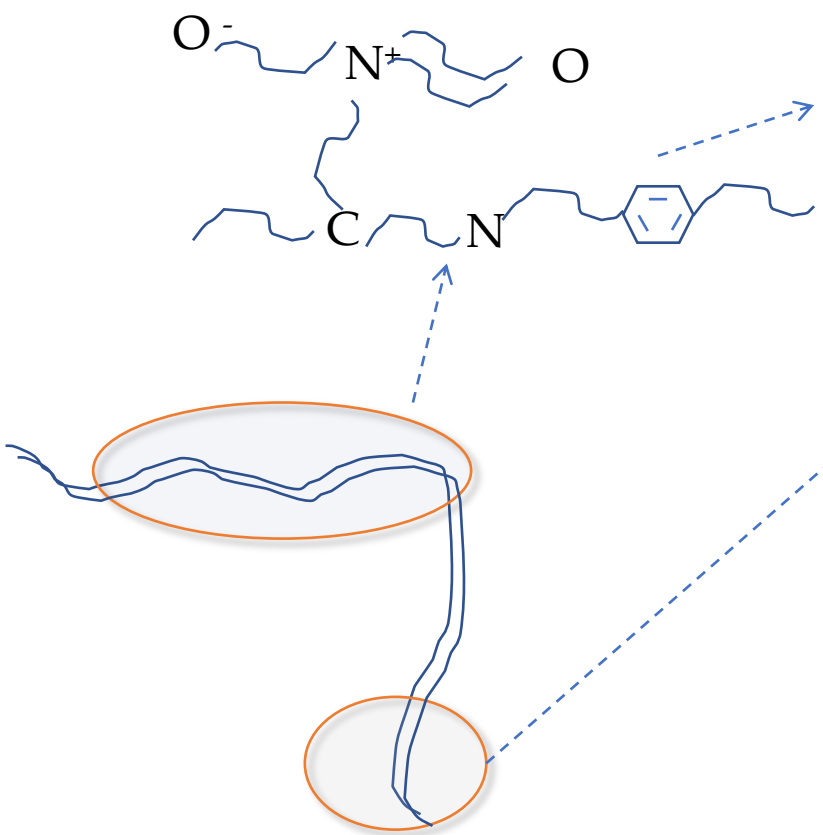
↓

- 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

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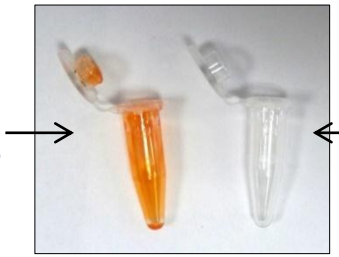
Matrix is washed extensively and amount of test-molecule in washes confirms the extent of immobilization

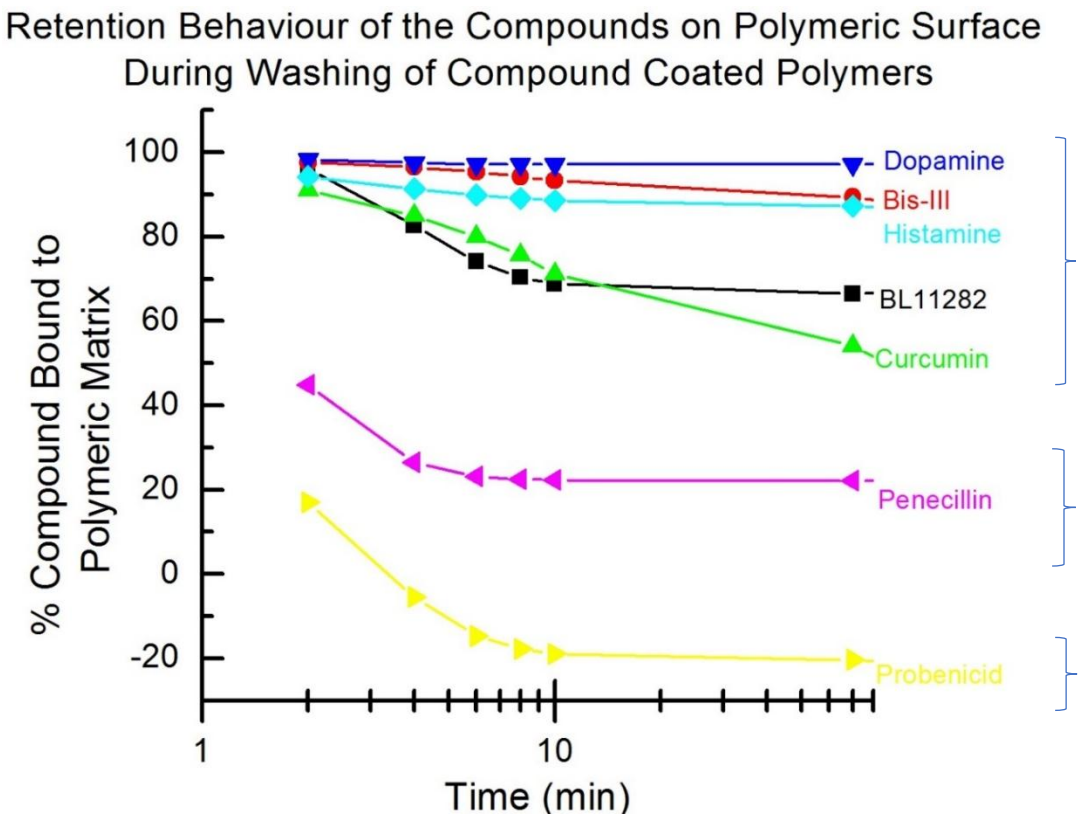
Polymeric Affinity Matrix of Bisindolylmaleimide (Bis-III)



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 ?

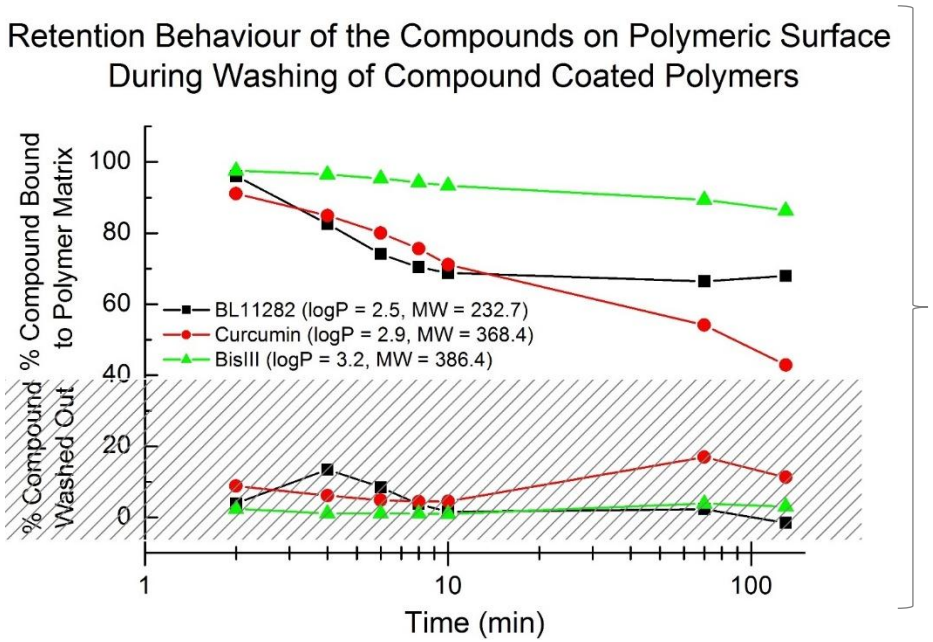
- If orientation is not random then a particular site of molecule that is responsible for its interaction with the target protein may not be exposed !!!

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

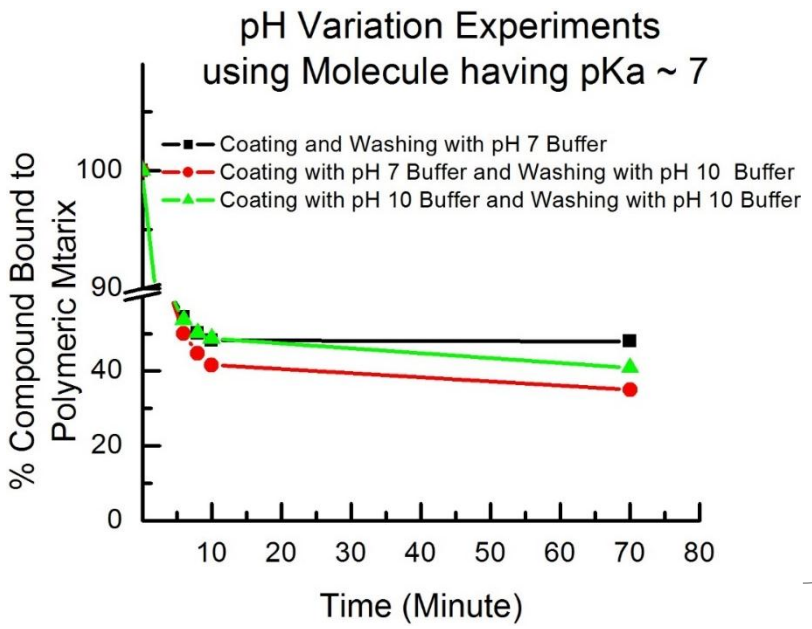
= **More than one type of interaction forces are working together** to retain the molecule on the polymer

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

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 ?

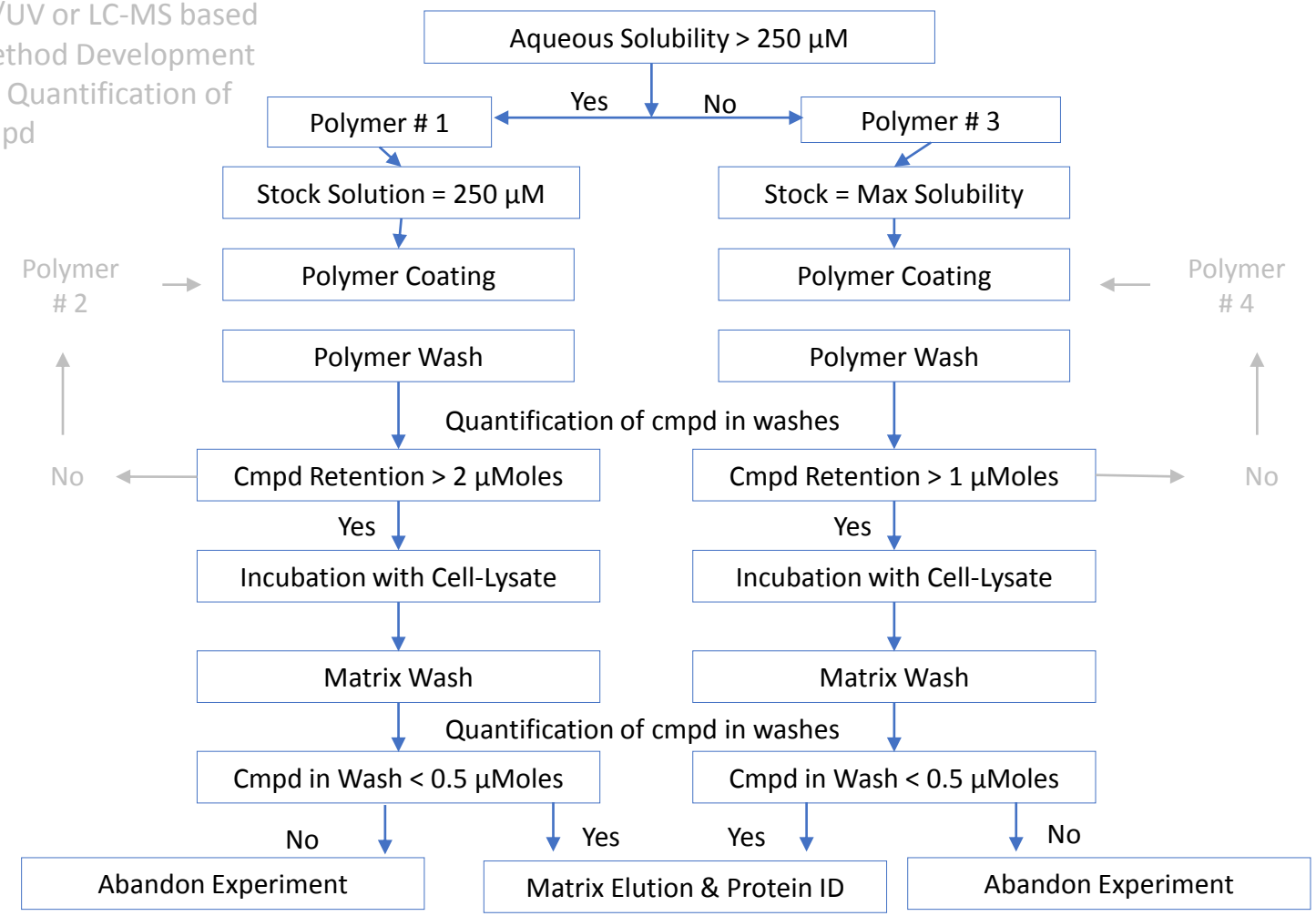
- Aqueous buffers are used to characterize the retention, what if the incubation with cell-lysate takes the molecule *'off-the-polymer'* ?

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

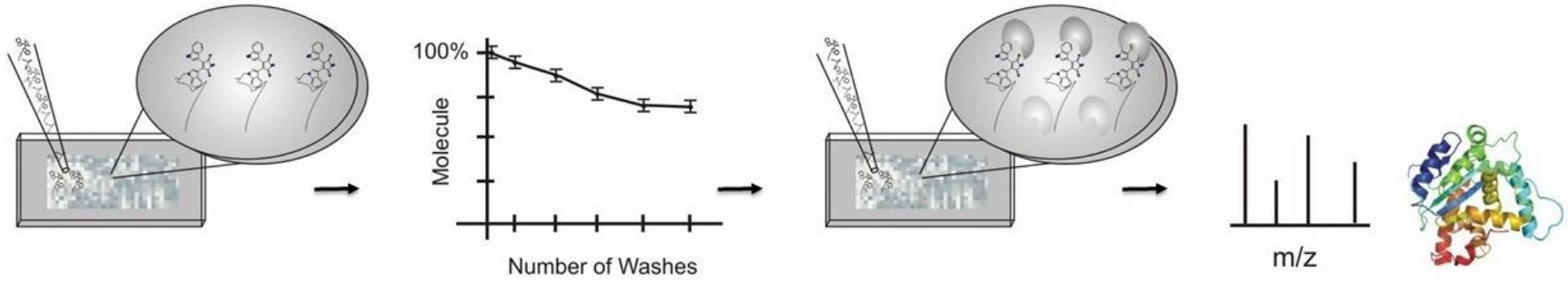
Method Qualifcation *is a function of* Amount of Molecule Immobilized on given surface area of polymer

Compound Information
- Molecular Weight
- Aqueous Solubility

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



Technology Workflow



Immobilization of molecule on unique polymer

Confirmation of molecule retention on unique polymer (By quantifying the molecule in polymer wash after immobilization)

Molecule Specific Affinity Chromatography (By Incubating biological lysate with molecule immobilized polymer)

Mass-Spectrometry Based Identification of Protein

References:

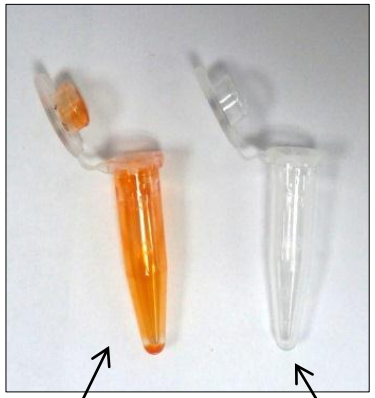
1. *Hati S. et al.* **Nature-Scientific Report** (2016) 6:32213.
2. *Bathula C. et al.* **Org Biomol Chem** (2016) 14:8053-63.
3. *Saxena C.* **Expert Opinion on Drug Discovery** (2016) 11:1017-1025.
4. Patent Application :**PCT/IN2017/000002,** Priority Date January 2016

Technology Validation

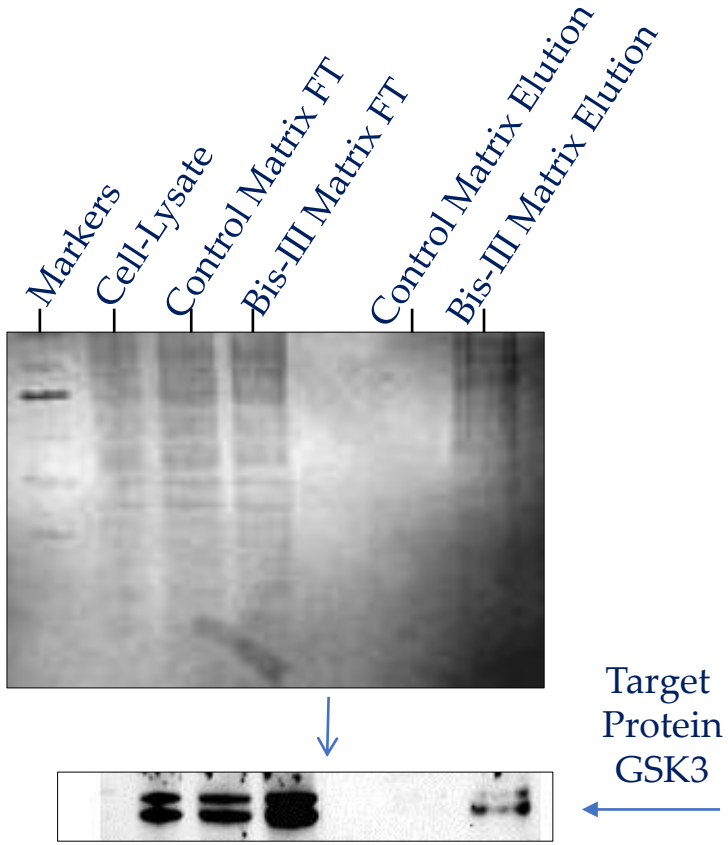
Capture of GSK3 protein, a well-established protein target (Kd ~19 nM) of Bisindolylmaleimide-III using UPT

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



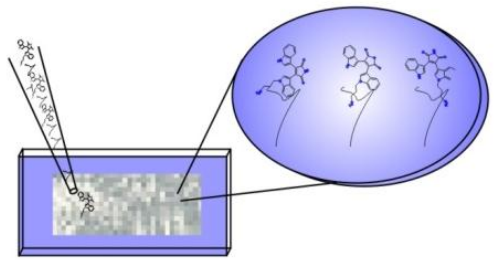
Step-2) Affinity Chromatography and SDS-PAGE and Western-blot analysis of Known Target Protein



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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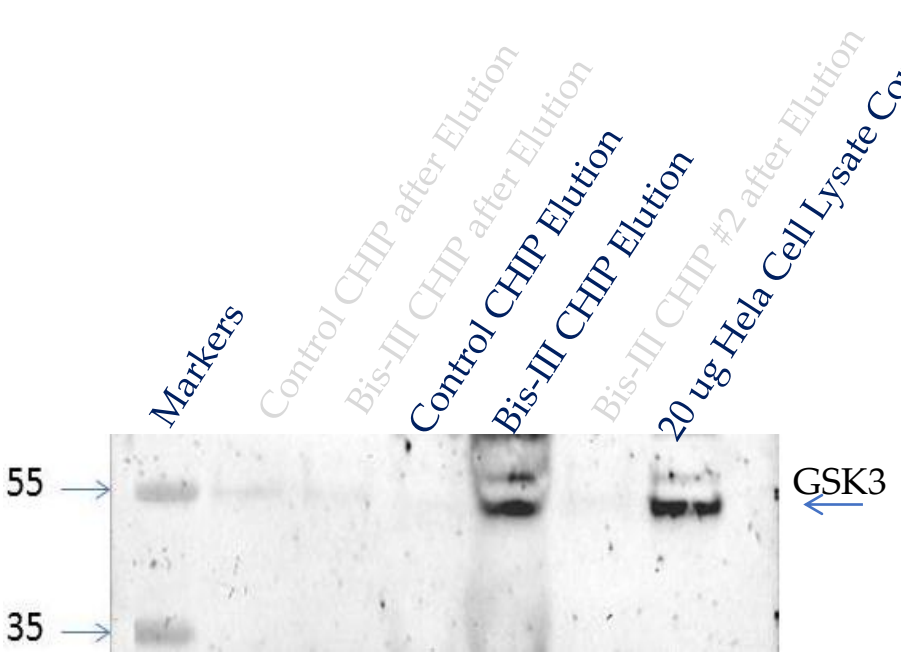


Primary Target was Specifically Captured and Amount of captured protein was significantly higher.

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

Conclusions from UPT based Chemical-Proteomics Methodologies

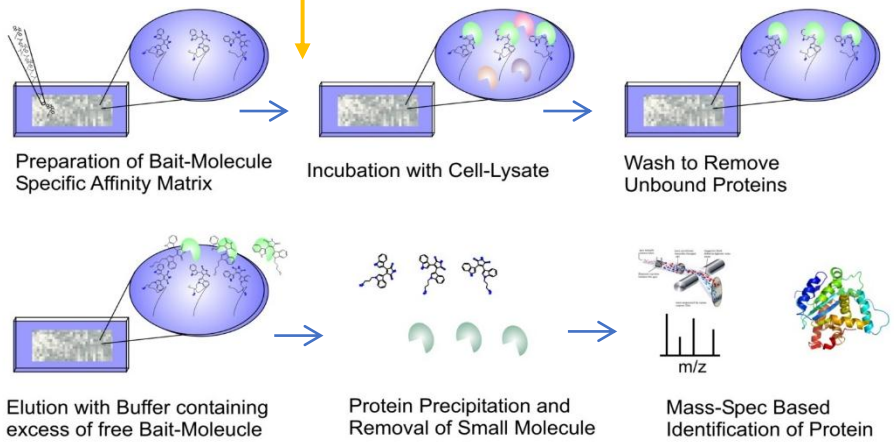
- UPT method is capable of identifying targets of Small Molecules.
- 'Bait-molecule' derivation and SAR information not needed for Target ID.
- Target Deconvolution can be carried out as fast as in 5 weeks.
- False-positive identification rates can be controlled by running multiple experimental replicates.

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

Our Key Strengths

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

Shantani R&D Center @ Innovation Park

Thank You.

Connect for further discussions

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Advancing Technologies and Applications of Proteome Analysis