This note describes the application of the technology in identifying/de-convoluting true positive targets of a non-derivatized 'test' molecule BIS-III.

Background and Overall Goal

Bisindolylmaleimide-III (Bis-III) a known inhibitor of GSK3 protein and it induces apoptosis in the cancerous cell-lines. At 1µM, Bis-III inhibits 93% of PKCα kinase activity and also inhibits many other protein kinases including, S6K1, MAPKAP-K1, RSK2 and MSK1 with similar potency. Additionally, it inhibits PDK1, an important kinase in the insulin signaling pathway.

In following experiments Shantani's technology was utilized to identify primary and secondary targets of BIS-III.
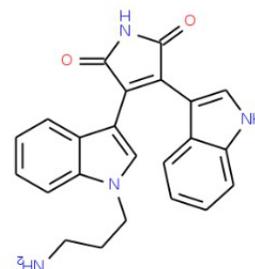
Step-1) Immobilization of 'Bis-III' on Shantani's proprietary polymer matrix

1. Based on BIS-III compatibility with the polymer matrix; a 10 ml soluble stock solution of 0.3 mM BIS-III was prepared in HPLC water.

2. The molecule was quoted on the matrix in small amount (1 ml) and allowed to dry at RT. The coating and drying of membrane was carried out till the complete 10 ml solution of Bis-III was coated on the membrane.

Step-2) Capture and Identification of Targets

The molecule coated matrix was incubated with cell lysate for 2 minute. Excess lysate was removed and proteins were eluted with 2 ml elution buffer (1 mM Bis-III in TBST). Proteins were acetone precipitated, extracted and measured. Two similar experiments were performed - one for western blot analysis and another for Mass spec analysis.

Protein concentration from both control and test experiment was normalized and probed for GSK3-beta protein using western blot method.
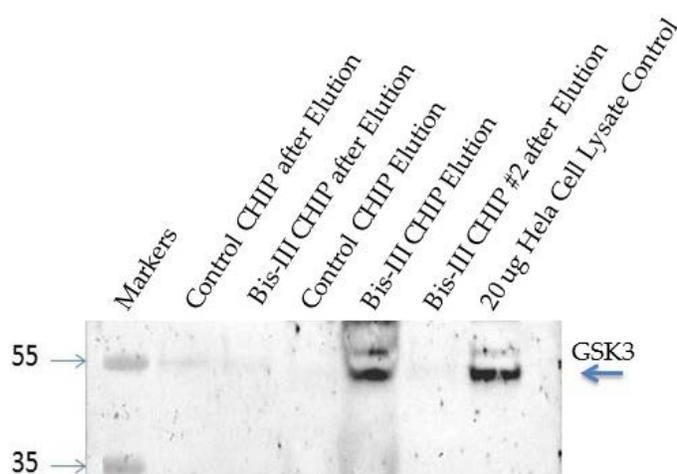
The target protein, GSK3-beta was specifically enriched on the Bis-III bound matrix.

Step-3) Deconvolution of Targets

Following the protocol (UPT - Technical Notes) proteins were identified using the mass-spectrometry based methods and specific targets were de-convoluted. Table 1 summarizes the outcome of target deconvolution experiments.

Uniprot ID	Protein Description	Maximum Number of Unique Peptides Identified	Protein Sequence Coverage (%)	Q-Value (%)
Q13418	Integrin-linked protein kinase	7	18.14	0
Q70UQ0	Inhibitor of nuclear factor kappa-B kinase	6	20.85	0
P28482	Mitogen-activated protein kinase 1	6	21.94	0
P60891	Ribose-phosphate pyrophosphokinase 1	5	20.44	0
E9PF82	Calcium/calmodulin-dependent protein kinase type II	4	10.46	0
P49841-2	Glycogen synthase kinase-3 beta	3	11.42	0
P63208	S-phase kinase-associated protein	3	19.63	0
P51570-2	Galactokinase	3	10.96	0

The primary target, GSK3-beta, of BIS-III was effectively captured using the described workflow. At the same time secondary targets of the molecule were also identified.