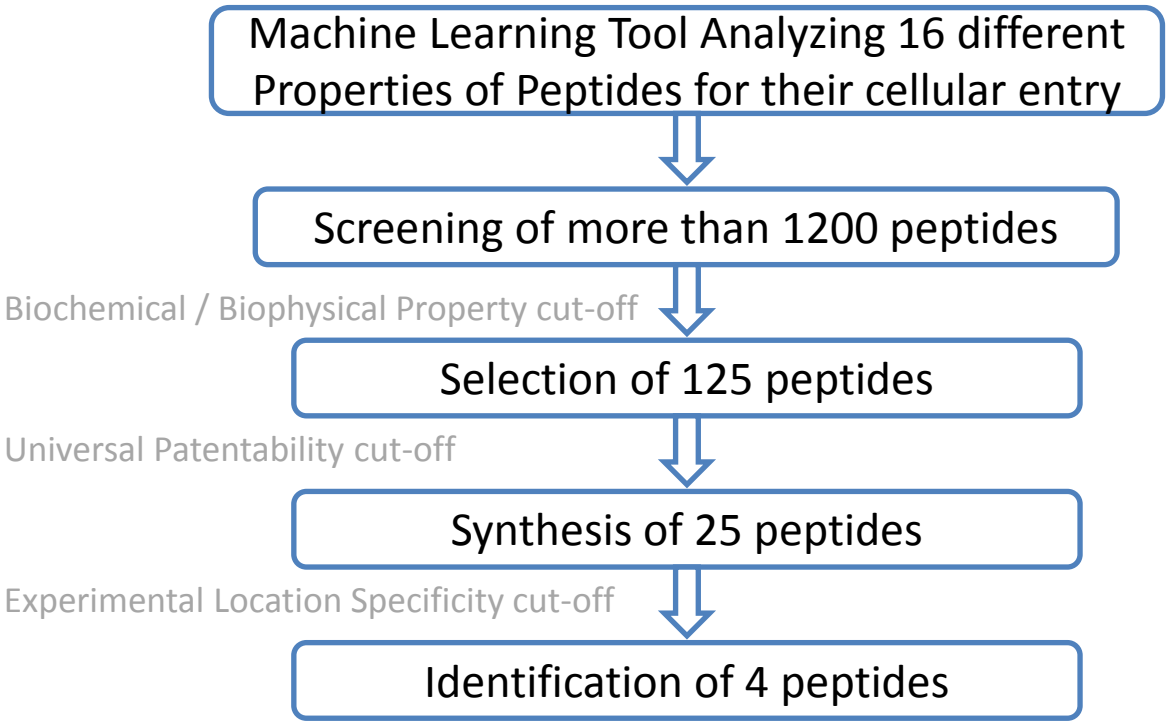
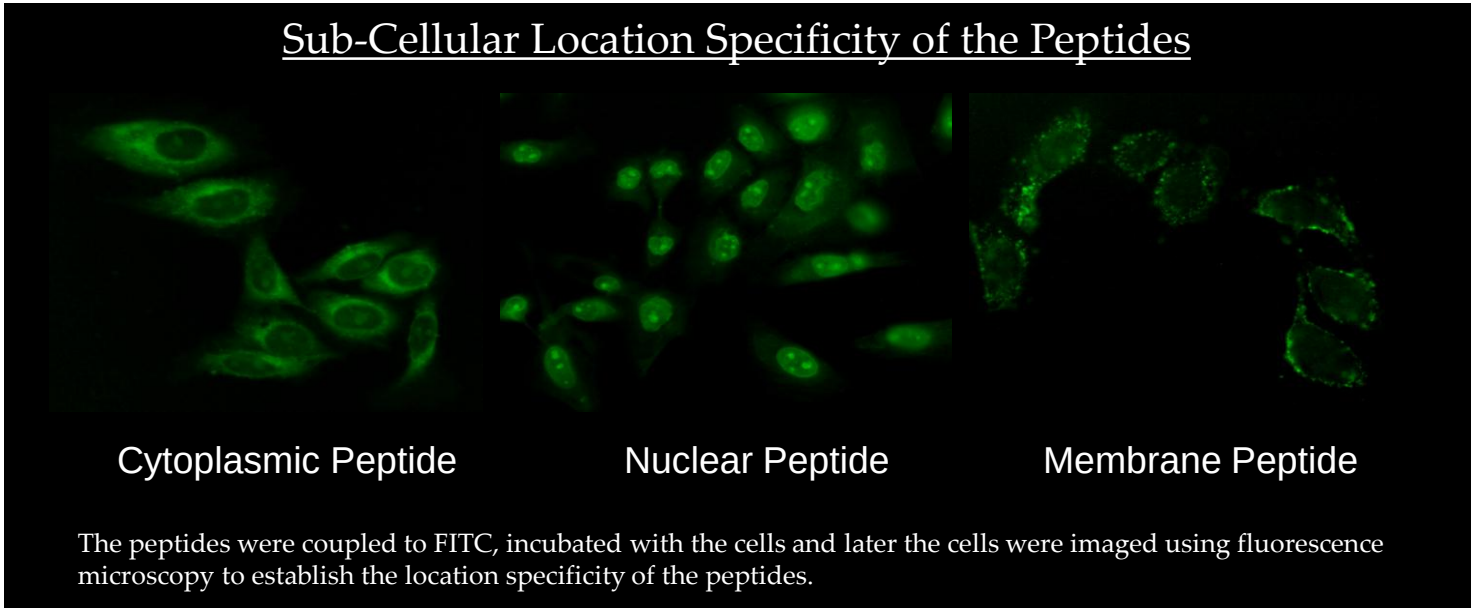
Subcellular Location Specific Peptide Probes

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



Subcellular Location Specific Protein Delivery

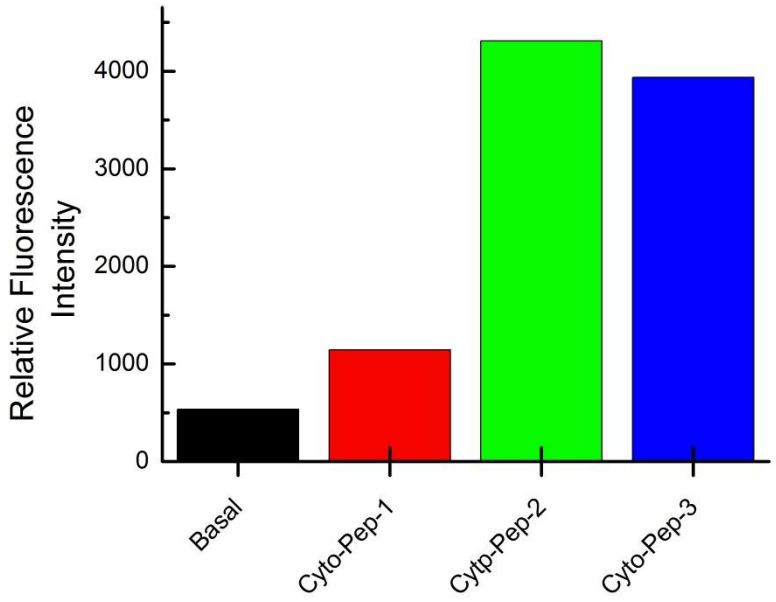
Selection of Peptide for Cytoplasmic Delivery of Protein

Cells were incubated with 80 µM of three different FITC labelled proprietary cytoplasmic peptide in PBS or Media for 1 hr

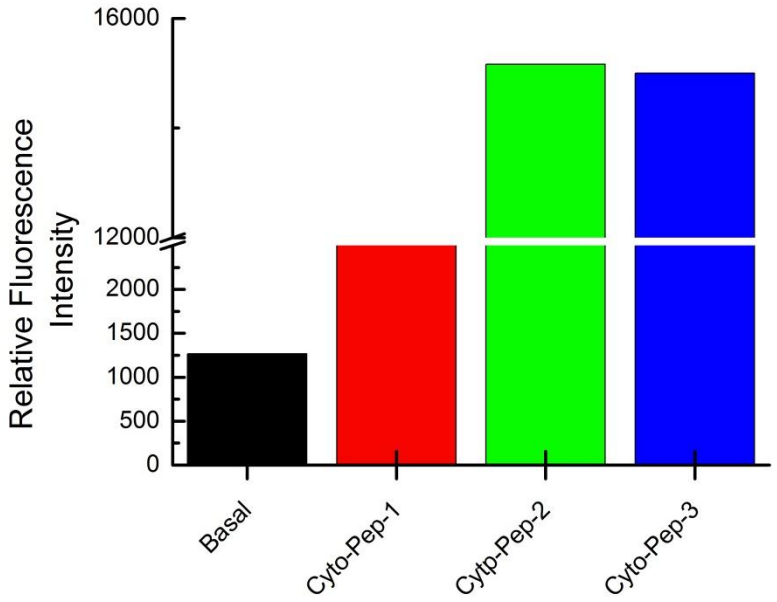
Cells were washed 3X with PBS and 2X Dilute Acid to remove any hydrophobically bound peptide to membrane and 1X PBS

Fluorescence of FITC was read in multiplate reader

Peptide Incubation in Cell Culture Media



Peptide Incubation in PBS



Coupling and Delivery of BSA to Cytoplasmic Compartment using Cyto-Pep-2

FITC-Coupled Cyto-Pep-2 was Coupled to BSA using appropriate Linker

↓

Unbound peptide was removed using gel-filtration chromatography

↓

80 µM of FITC-Peptide-BSA was incubated with the cells for 30 minutes in PBS

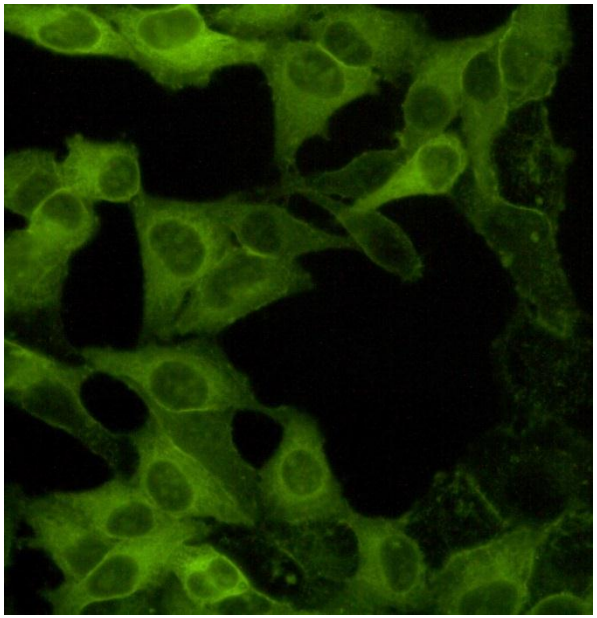
↓

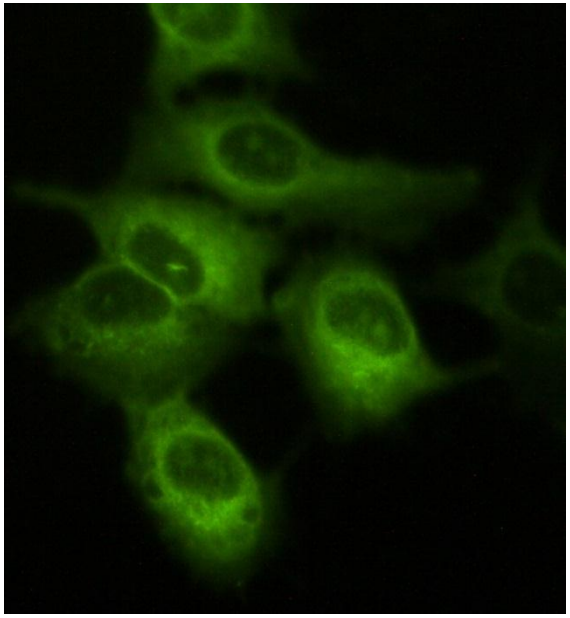
Cells were washed 3X with PBS, 2X with diluted acid and finally 1X with PBS

↓

Finally, cells were fixed with formaldehyde and later analyzed using fluorescence microscopy

Successful Delivery of BSA in Cytoplasmic Compartment





Representative FOVs at 50X magnification from a typical experiment (n=3)

SubCellular Location Specific Target-Capture Methodologies (SCLS) for Small Molecule Target Identification

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

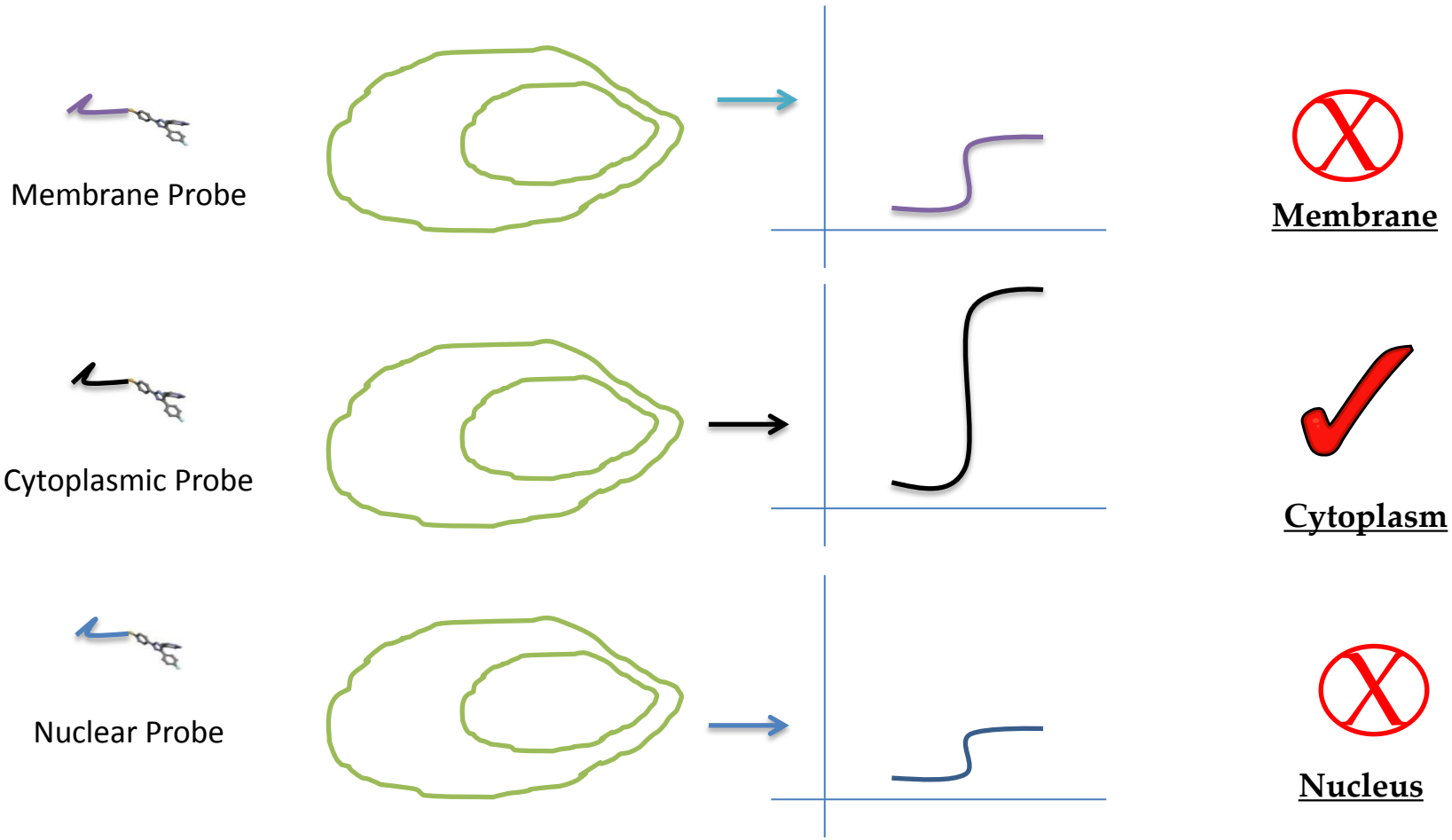
Step-1: Identification of Sub-Cellular Location

a) Location specific peptide coupled "bait molecule"

b) Biological System

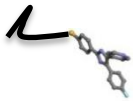
c) Activity Read-Outs

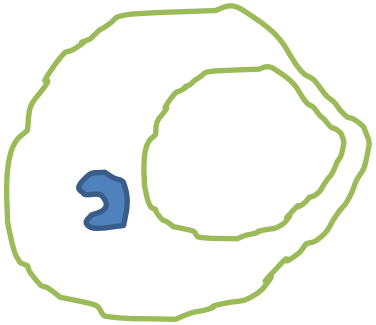
d) Subproteome Selection



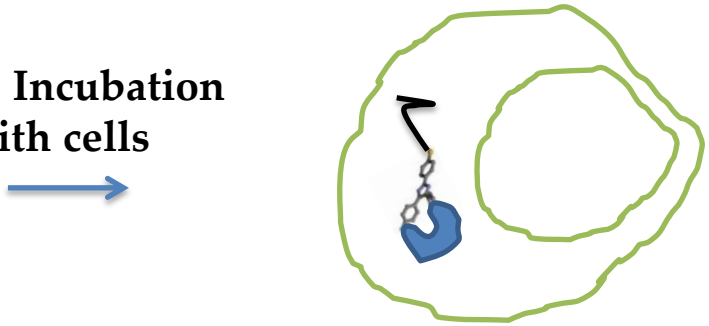
Step-2: Target Capture from Sub-Cellular Location and Target Identification

a) Cytoplasmic probe

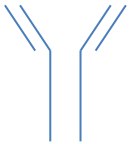


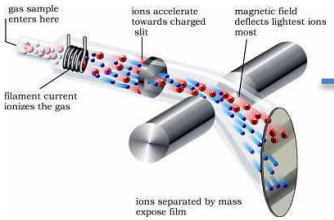


b) Incubation with cells

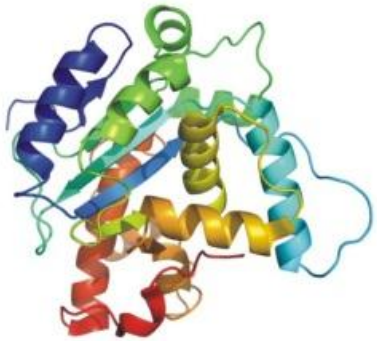


c) Lysis and affinity isolation





d) Mass-spectrometry

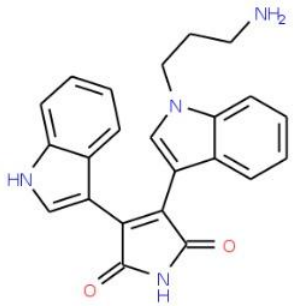
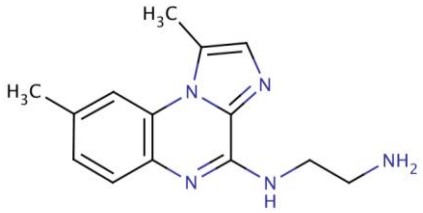
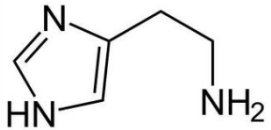


e) TARGET

SCLS –Validation Experiments

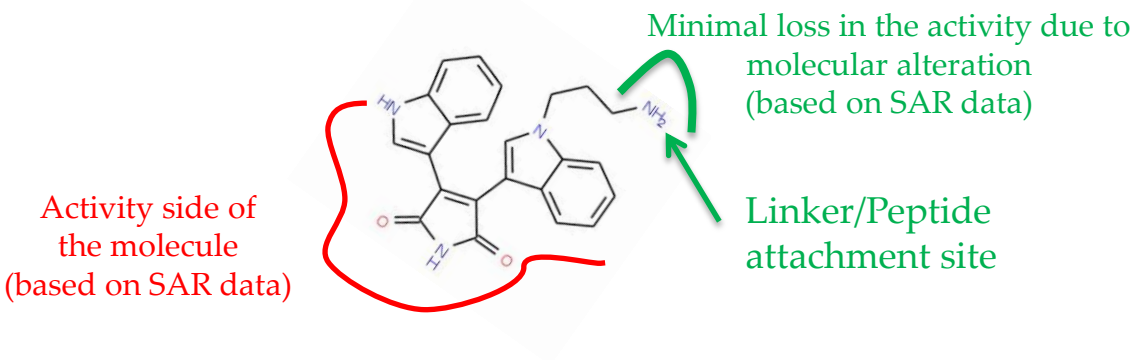
Proof of Concept Experiments (PoCs)

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

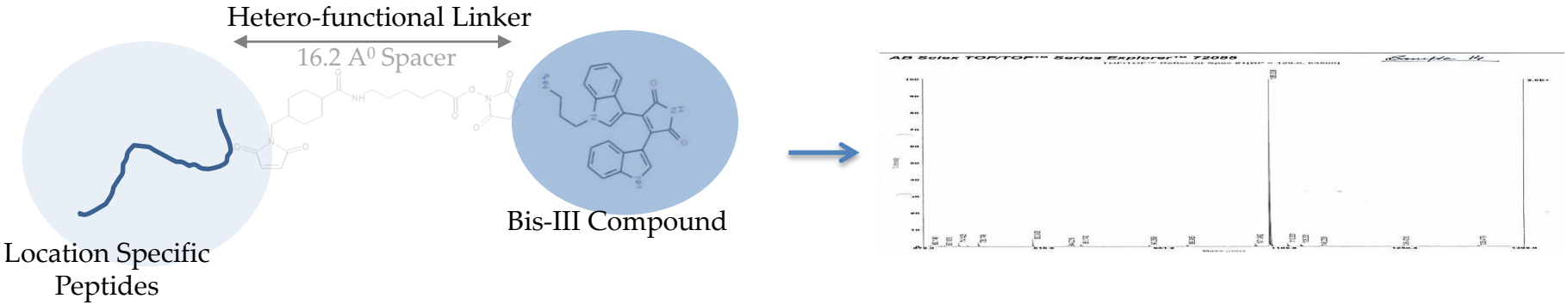
Molecule	Bis-III	BMS345541	Histamine
			
Target	GSK3-beta	IKK-2	H1-Receptor
Location	Nucleus / Cytoplasm	Cytoplasm	Membrane
Functional Activity	Cytotoxic	Inhibition of LPS Induced Nitric –Oxide Production	Increase Intracellular Calcium

Preparation of Bis-III Probes

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

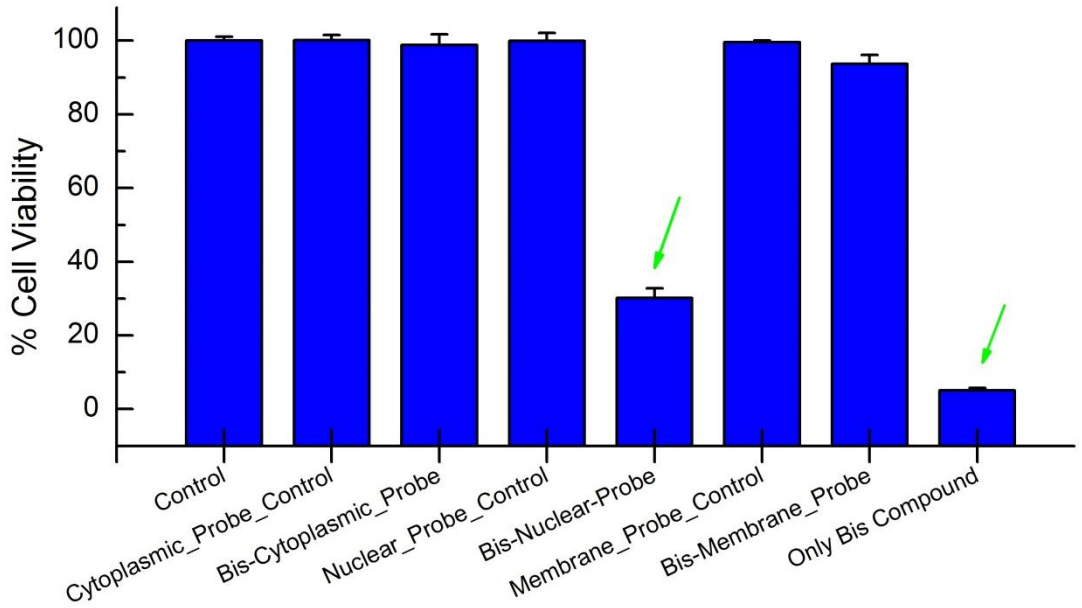


Step-2: Coupling of Location-Specific Peptide with Bis-III followed by HPLC based purification and Mass-Spectrometry Based Characterization of Probe



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

↓

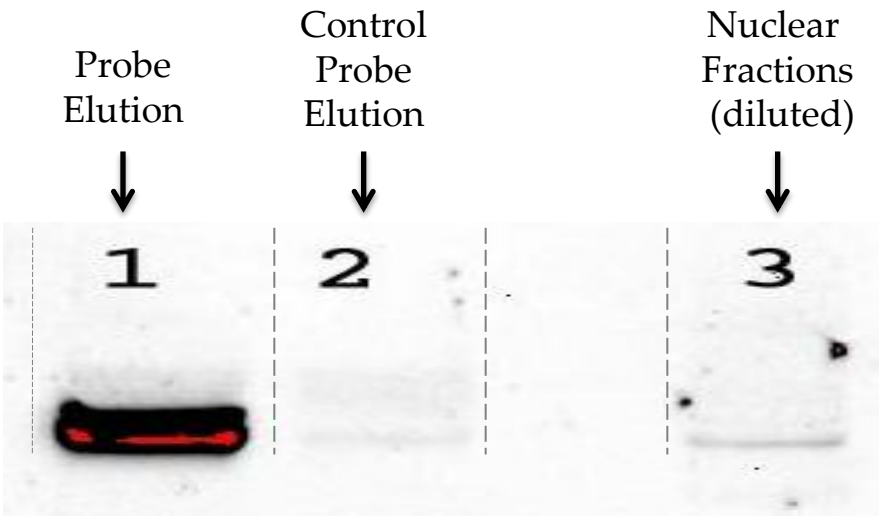
Washing of antibody

↓

Western Blot Analysis of Elution for Target Protein

Probes = Coupled with Bis-III

GSK3-beta ⇨

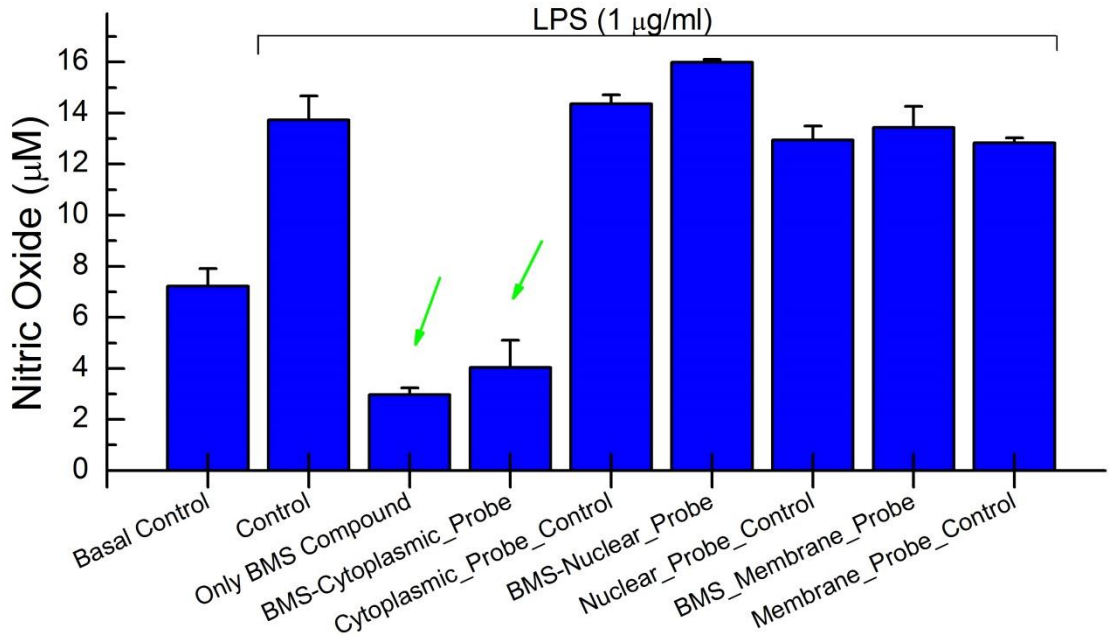


Target was specifically captured with Probe.

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

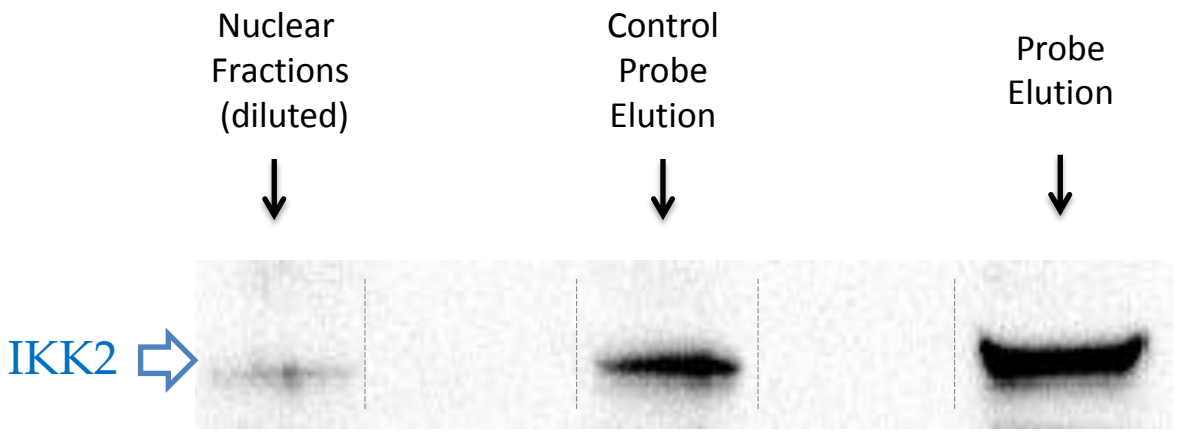
↓

Washing of antibody

↓

Western Blot Analysis of Elution for Target Protein

Probes = Coupled with BMS345541

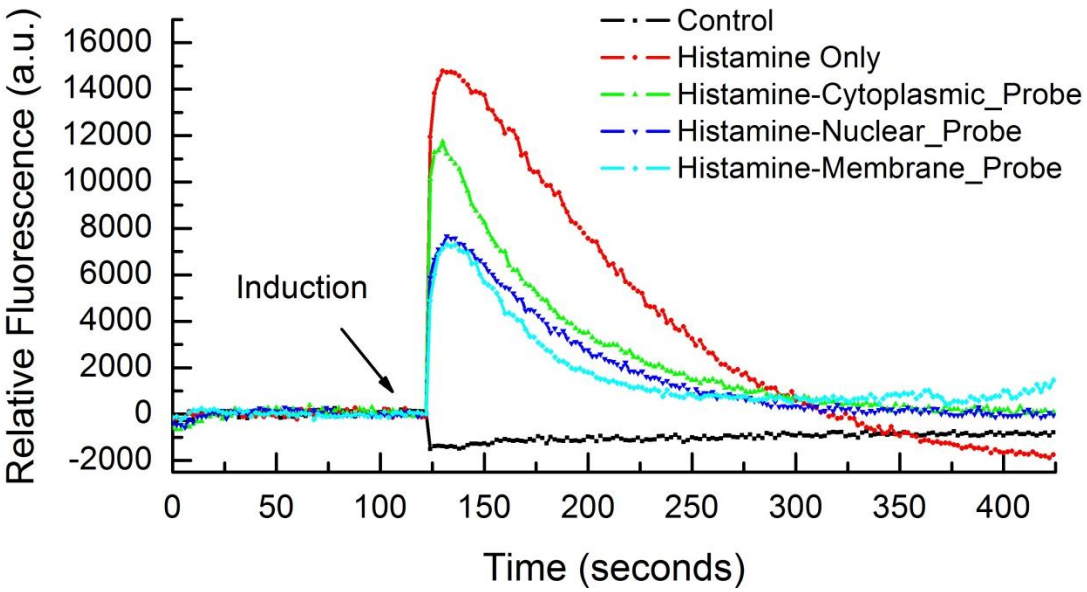


Target was specifically enriched with Probe.

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

↓

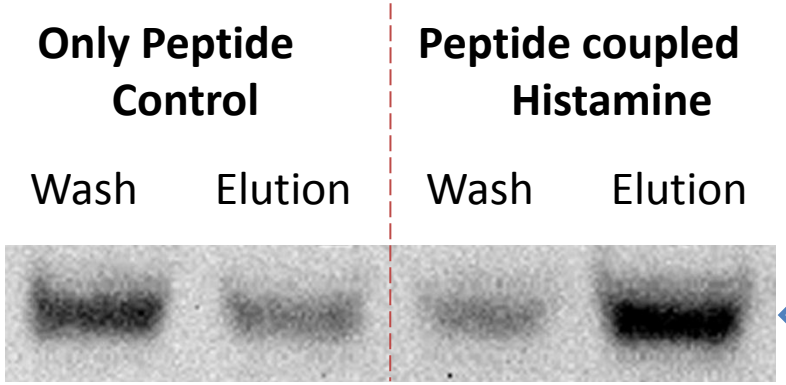
Washing of antibody

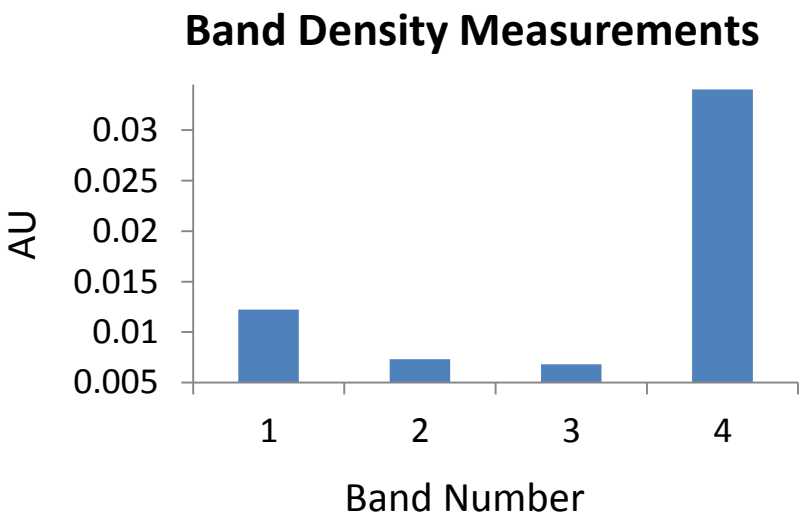
↓

Elution of antibody with Histamine

↓

Western Blot Analysis of Elution





Target was specifically captured with Probe.

Thank You.

Connect for further discussions

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