Mora than 60% of the marketed medicines works through target proteins that are membrane bound. Traditional Chemical Proteomics method, where 'bait molecule' typically is immobilized on solid surface, remain limited by its inability to capture membrane bound targets. This note describes the application of the technology in capturing membrane bound targets.

Background and Overall Goal

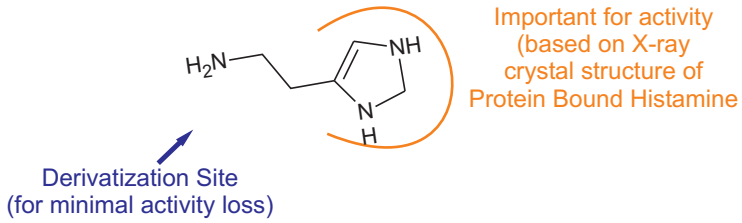
Histamine regulates several physiological functions including immune responses and also serves as neurotransmitter. It interact with specific GPCRs (G-protein coupled receptors) histamine receptors H1 to H4 to exhibit its effect.

In following experiments Shantani's technology was utilized to capture the Histamine H1 receptor target from HeLa Cells. Along side target was also captured using the traditional chemical proteomics methods by coupling the Histamine to solid support (beads). Here we provide a comparative evaluation of two methods in capturing the Histamine H1 receptor.

Development of Subcellular Location Specific Target Capturing Probes

Based on the X-ray crystal structure of protein bound histamine α-amine position of histamine was chosen as the derivatization site.

Figure 1. Histamine derivatization site
(note: Histamine also interact with the specific aspartic acid of H2 receptors and protonation of α-amine is implicated as critical for that interaction. However to capture the H1 receptors α-amine site was used for derivation. Later it is shown that functional activity of the molecule was not altered.



Later, Shantani's subcellular location specific target capturing probes were prepared by following coupling scheme. After coupling Histamine coupled peptide probes were purified using HPLC and characterized using Mass-spectrometry.

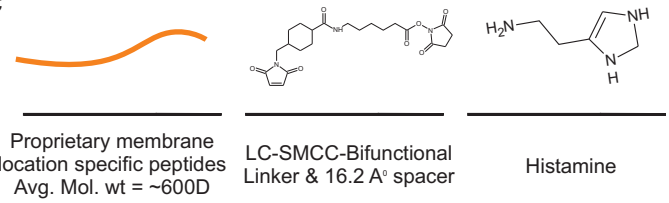
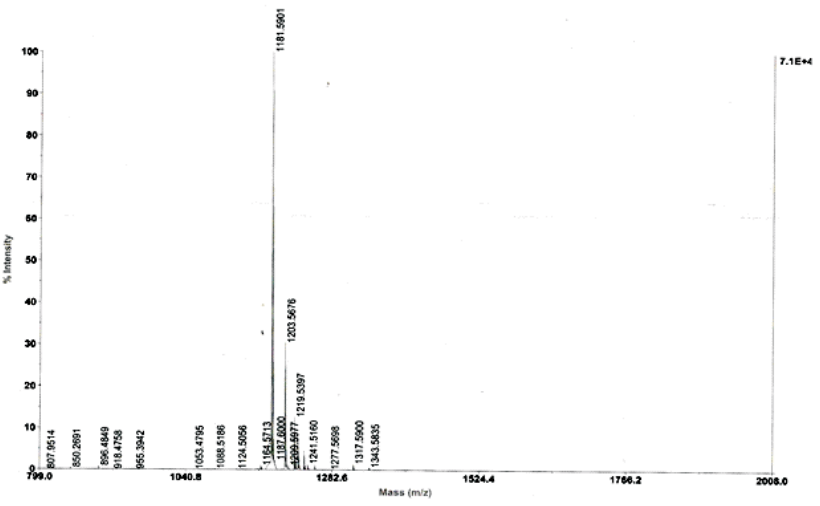


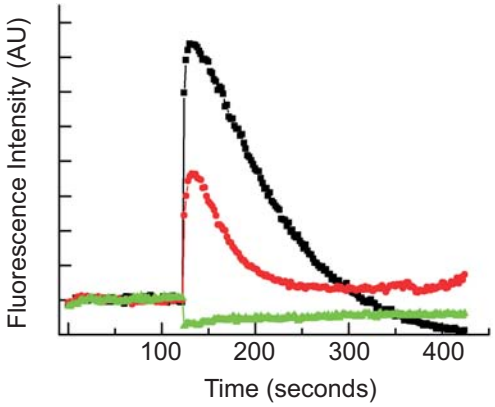
Figure 2. Coupling scheme to covalently link the molecule to peptide and typical characterization of probes



Functionality Assay

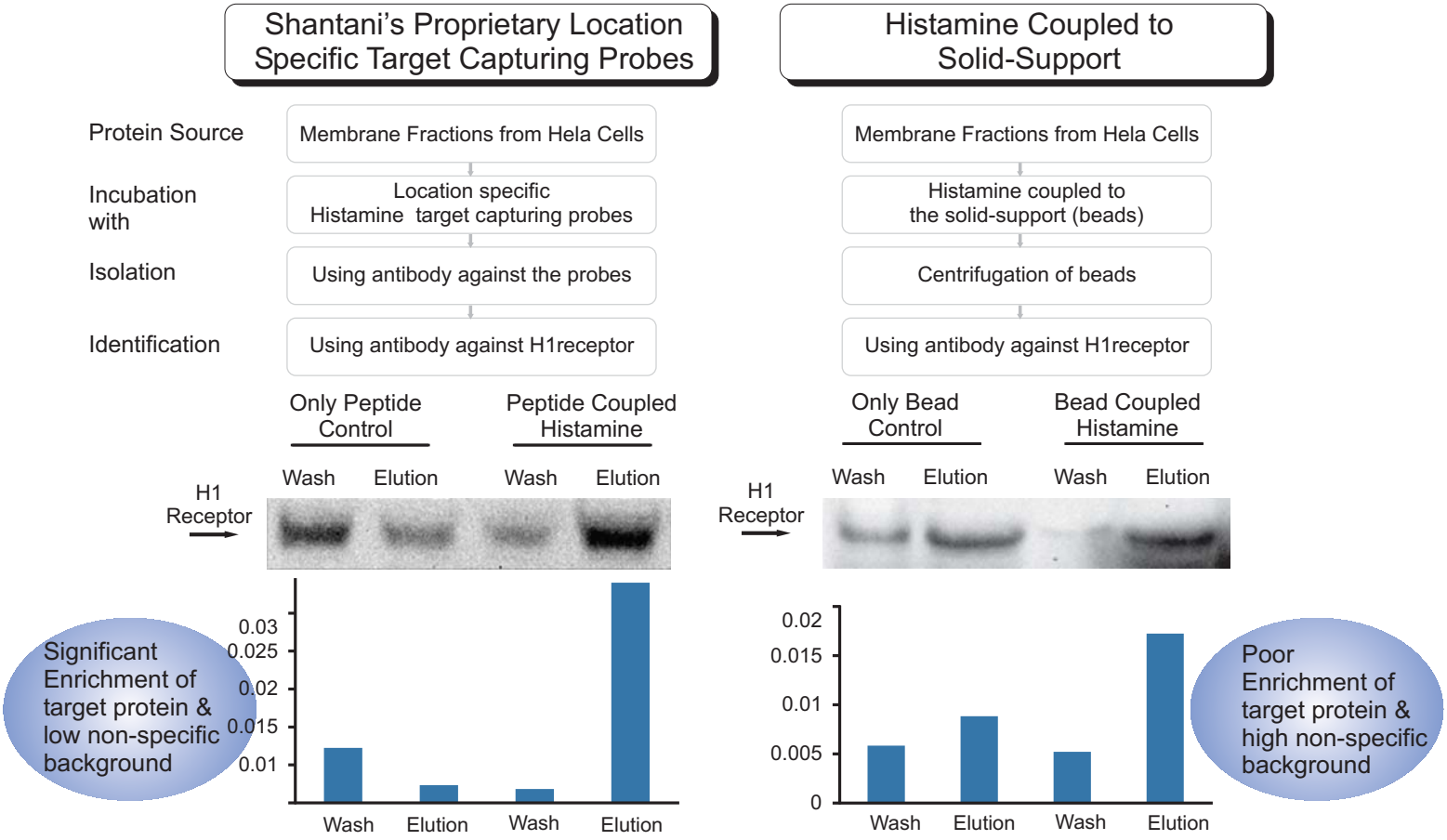
Following the Shantani's technology workflow Subcellular location specific target capturing probes were tested in cell based assays to (a) measure their functional activity, (b) locate the active proteome and (c) establish a concentration range of probes that is relevant for function (Figure 3).

Figure 3. Functional Assay - Increase in calcium in the Hela cells upon treatment with 200 μM Histamine (black), 200 μM Histamine probes (red) and vehicle (green) was monitored using the fluo-4-am dye to established the functional activity of the probes.



Target Capture

Following workflow was utilized to capture the target of Histamine using Shantani's proprietary probes and traditional solid support based chemical proteomics method.



Conclusions

Experiment above demonstrates that histamine retained its functional activity after coupling to the Shantani's proprietary probe. Utilization of the proprietary probe in target capture experiment allowed significant enrichment of membrane receptor proteins compared to bead-coupled histamine probes.