This note explains various steps involved in de-convoluting the right target(s) of small molecule using Shantani's proprietor workflow.

Step-1) Preparation and Characterization of 'bait-molecule' Affinity Matrix

1. Bait-molecule is dissolved in matrix compatible solvents and quoted over the polymer surface. The molecule forms complementary weak interactions with the matrix.
2. Molecule-quoted surfaces are then dried and washed extensively. Molecule retention over the polymeric surface is confirmed through LC-MS/LC-UV analysis of the washes.
3. Once it is confirmed that 1-4 µmoles of molecule can be retained on the polymer the matrix is qualified for affinity chromatography experiments.

Step-2) Interaction with biological sample, elution and precipitation of target proteins

1. Bait-molecule coated matrix is then incubated with cell lysate for appropriate time at room temperature.
2. After incubation excess lysate is removed and matrix is washed.
3. The bound proteins are eluted using elution buffer (1 mM of test-molecule in TBST).
4. Eluted proteins are acetone precipitated.

Step-3) Target Capture and Identification Experiments

1. Protein concentration from both control and test experiments is measured.
2. Protein, normalized based on concentration, are separated using SDS-PAGE and stained with coomassie blue stain.
3. Protein bands are excised and digested with trypsin and identified using mass-spec analysis.

Step-4) Target Deconvolution

Target capture experiments are performed in triplicates. Specific target proteins are de-convoluted by comparing the protein profile obtained from bait-molecule specific affinity matrix and control experiments. Proteins that are significantly enriched in test experiments compared to control experiments are considered as specific binding partners of the test molecule.

Typical Target Deconvolution Approach in 'Target Enrichment' Based Chemical-Proteomics