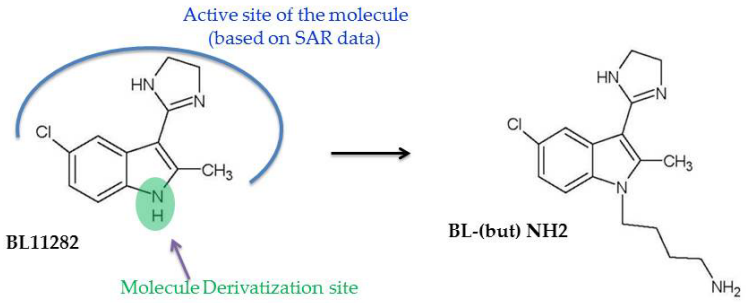
This note explains various steps involved in de-convoluting the right target(s) of small molecule by immobilizing the bait-molecule on solid support.

Step-1) Derivatization of 'bait-molecule'

1) Analyze SAR (Structure-Activity Relationship) of the bait molecule.
2) Define a point of dervization for minimal activity loss
3) Synthesis a primary amine (or other reactive functional group) derivative of the compound



Step-2) Immobilization of 'bait-molecule' on Agarose Beads

'Bait-molecule' specific affinity matrix is prepared by immobilizing primary amine derivative of the compounds on epoxy activated agarose beads. Epoxy group on the beads are placed on a 12 atom long hydrophilic spacer for efficient coupling of small-molecule ligands. 'Control' affinity matrix is prepared by blocking the activated epoxy group through amine-based buffers.

Step-3) Affinity Chromatography and Isolation of Target Proteins

1) Well washed 'Bait-molecule' specific and control affinity matrix are incubated with cell-lysate (protein source) for 3 hours in high salt buffer (1M NaCl in PBS) at 4 degree centigrade.
2) fter incubation beads are brief centrifuged and unbound protein fraction (supernatant) is separated.
3) Matrix is then washed 2X times with high salt buffer and 2X times with low salt buffer (150 mM NaCl in PBS).
4) Proteins bound to beads are then eluted 2X with elution buffer (1mM bait-molecule in PBS).
5) Proteins from the elution are the precipitated by chloroform-methanol based method.

Step-4) Protein Identification

1) Precipitated proteins are separated over SDS-PAGE gel.
2) Separated proteins are then subjected to *'in-gel'* trypsin digestion protocol and proteins are identified using mass-spectrometry based workflow.

Step-5) Deconvolution of Targets and Conclusion

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.