

**Department of Chemistry & Biomolecular Sciences**  
**Seminar**  
**Semester 1 2008**  
**Macquarie University, Sydney, Australia**

**Thursday,**  
**August 7**  
**12 Noon**  
**F7B Room 322**

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*“Fractionation for proteomics”*

**Abstract**

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If you can't solubilise, you can't analyse. This simple statement presents a major challenge on two levels, firstly the chemical diversity within even the simplest proteome cannot be captured by any single extraction and separation step, and consequently, the wealth of literature on sample preparation is equally diverse. Secondly, a relatively small number of proteins are present in high concentration, whilst the majority are expressed at less than 50,000 copies per cell. This dynamic range issue, coupled with the chemical diversity has made sample preparation and fractionation key technology development areas of proteomics. In this presentation I will briefly focus on two of the sample preparation and fractionation techniques used in our lab.

1. Enriching low abundance proteins by proteome-wide affinity using a combinatorial hexapeptide library. A small number of abundant proteins often dominate proteomes, and obscure the signal of many others. One strategy which has been applied is immunodepletion, especially in sera or plasma, of up to 20 of the most abundant species. However, this strategy requires antibodies which are expensive and time consuming to produce. An alternative strategy consists of a solid-phase combinatorial library of hexapeptides, synthesised via a short spacer on porous poly(hydroxymethacrylate) beads. Proteins bind to the beads under mild conditions, which initially limited the beads to soluble protein samples such as plasma and serum. We have worked with derivatisation of proteins to enable a wider range of insoluble or hydrophobic proteins to be solubilised and applied to the hexapeptide beads. Examples of both soluble and insoluble derivatised samples will be shown.

2. Method development towards a new approach for the large scale study of protein-protein interaction (PPI) networks. The broad goal is large scale analysis which does not rely on genetically modified strains like yeast-two hybrid or the TAP-tag system. After a successful technology development using yeast, it should be possible to do global comparisons of PPI networks, including human PPI networks. Our approach is to use Blue Native PAGE in the Bio-Rad PrepCell to separate and purify protein complexes from crude cell extracts and then directly analyze the collected fractions with MS to get a global snapshot of the PPI network. In differential display mode, global PPI networks will be analysed to detect the differences in protein complexes between disease and normal tissue. The data presented has been obtained on yeast cells. To date we have optimised BN-PAGE separations in the PrepCell and analysed a number of the complexes by MS. We have been able to separate complexes on a global scale and demonstrate that we can detect interactions that have been documented by other techniques (yeast two hybrid or TAP-tag).

**To meet the speaker, please contact**

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