THE UNIVERSITY OF SYDNEY

Sydney Analytical Protein Production Services

Extending the scope of your research with protein production solutions

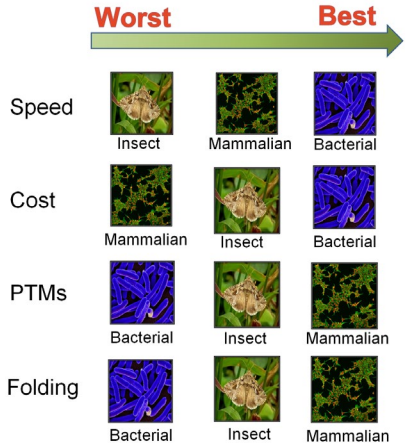
Our aim is to assist researchers from all backgrounds with the expression and purification of their proteins of interest. We work with three different expression hosts (bacterial, insect, and mammalian cells) and use highly specialised protein purification and characterisation equipment.

General workflow

Protein expression → Protein purification → Protein QC & characterisation

Protein expression

- We can express protein in bacterial, insect or mammalian expression systems. We can tailor these to suit each protein, dependent upon what the researcher requires
- The selection of expression host is crucial, and this table helps make an initial decision in the absence of published protocols
- We maintain all equipment required for protein expression, including incushakers for growing bacterial or insect cells, and static incubators for mammalian culture



Protein purification

- We employ a variety of initial purification techniques, including affinity or charge based methods. These include:
  - Ni-NTA, for polyhistidine tag.
  - Glutathione Sepharose, for GST tag.
  - Anti-FLAG affinity gel, for FLAG tag.
- Most purifications incorporate a second purification step by FPLC. We have several automated Cytiva ÄKTA systems – including an AKTA Pure M with sample pump – that we can use to carry out ion exchange (charge-based separation) and/or size exclusion (size-based separation) to ensure a highly pure final protein

Specialist Services:

- Membrane protein expression and purification
- Expertise in ultracentrifugation for complex sample separations – including density gradient separation followed by automatic gradient fractionation

Protein QC and additional characterisation services:

- All proteins made in the facility are assessed for final purity by SDS-PAGE, however, we can provide additional characterisation analysis on request
- **Please see our characterisation flyer for more information**

To request more information or for instrument training, please contact us:

sydney.analytical@sydney.edu.au

+61 2 8627 6903

Sydney Analytical
Biomolecular Characterisation Services

Extending the scope of your research with characterisation solutions

Sydney Analytical has a suite of equipment to assist in characterisation of your protein or macromolecule of choice. We can measure secondary structure composition, assess stability, aggregation propensity, and even determine solution state of proteins or complexes at a single molecular level.

Circular Dichroism (CD) Spectroscopy

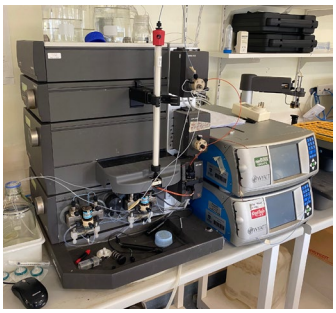
Measure the secondary structure composition and/or temperature stability of your sample
Can be used to assess proteins, peptides or other macromolecules, including polymers and even nanoparticles
Applications:
Assess protein fold and stability, measure D vs L-amino acid composition, compare secondary structure of a standard or WT vs a biosimilar or mutant(s), nanoparticle analysis

Nanotemper Prometheus Panta

Generates simultaneous measurements of Dynamic Light Scattering (DLS), Differential Scanning Fluorimetry (DSF) and sample turbidity. Measurements rely on intrinsic fluorescence and light scattering properties of the molecule, so no additional dyes or additives are needed
Applications:
Any protein stability and aggregation measurements e.g. T_m, T_{agg}, screening buffer conditions ahead of protein crystallography, assessing stability of engineered antibody variants or therapeutic formulations

Refeyn 2MP Mass Photometer

Provides a label-free determination of mass measurement for single protein molecules and/or complexes in solution using light.
Can be used with:
Single Proteins (> 30 kDa)
Multi-Protein or protein:nucleic acid complexes
Applications:
Assess solution state of individual proteins, confirm formation of multi-protein complexes ahead of structural determination studies by crystallography or CyroEM.



SEC MALS

Provides a shape-independent measurement of molecular weight using multi-angle light scattering (MALS) following size-exclusion chromatography (SEC)
Applications:
Assess solution state of individual proteins or complexes

To request more information or instrument training, please contact us:

sydney.analytical@sydney.edu.au
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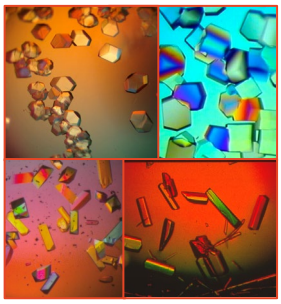
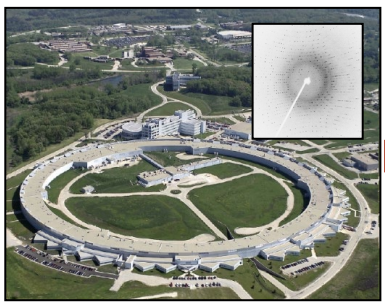
Sydney Analytical Macromolecular crystallography

Sydney Analytical provides expertise and access to advanced equipment to facilitate macromolecular crystallography. This includes a Perkin Elmer Janus liquid handler and crystallization robotics with our Mosquito® LCP (sptlabtech). These enable the automated setting of crystallization screens with hanging drop, sitting drop, additive, and seeding methods, as well as Lipidic Cubic Phase (LCP) experiments for membrane protein crystallisation.

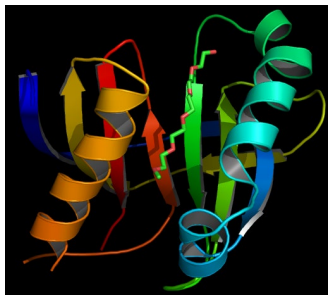
We have access to the Australian Synchrotron x-ray facility through the Collaborative Access Program (CAP) which allows for the screening and data collection for crystals. We also provide training to users on crystal screen set-up, crystal optimization as well as remote x-ray data collection at the Australian Synchrotron.



Crystallisation Screening



Data Collection at the Australian Synchrotron



Structure Solution

Janus Standard 8-Tip Varispan (Perkin Elmer)

Mosquito LCP (sptlabtech)

SERVICES PROVIDED

- ❖ Commercial crystallization screens
- ❖ Crystallization incubators
- ❖ Microscopes for crystal viewing
- ❖ Crystal cryo storage and shipping
- ❖ Remote x-ray data collection at the Australian Synchrotron
- ❖ Data collection and structure determination
- ❖ Crystallization and structure solution training

To request more information or for instrument training, please contact us:

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Sydney Analytical Macromolecular Interactions Analysis

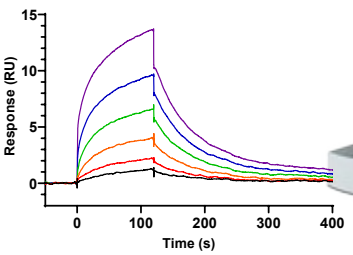
Sydney Analytical has the experience and instrumentation to characterize a wide variety of macromolecular interactions. We can assist to measure interactions between proteins, or with partners including AAV's, nucleic acids, peptides, small molecules or fragments. We can collect data or provide user training on a variety of instrumentation, with experiments best focused to your research needs.

Surface Plasmon Resonance (SPR)

Surface plasmon resonance (SPR) is an optical technique that can be used to measure interactions in real time.

A typical experiment involves a ligand immobilized to the surface of an SPR sensor chip, either directly or via an affinity tag. The analyte is then flown over the surface in increasing concentrations. If an interaction occurs, the change in mass on the sensor surface is detected and plotted as an output sensorgram. The facility has both a T200 and 8K+ system, covering both medium and high throughout applications

Measures: Affinity (KD) and kinetics (on & off rates). Range pM - mM



Biacore T200

Microscale Thermophoresis (MST)

Microscale Thermophoresis (MST) measures changes to the mobility of molecules in microscopic temperature gradients

The instrument can detect changes to the size, charge and hydration shell of molecules with high sensitivity

Performed in solution, and while requiring one partner to be fluorescently labelled, MST can measure a wide variety of interaction types, including molecules such as liposomes, nanodiscs or membrane proteins.

Measures: Affinity (KD). Range nM - mM

Nanotemper Monolith NT.115

CRICOS 00026A

BiLayer Interferometry

BiLayer interferometry is an optical analytical technique that assesses the interference pattern of white light reflected upon binding of a partner molecule across two surfaces: a layer of immobilized protein on the biosensor tip, and an internal reference layer.

Differences in response are used to determine interaction strength. While not as sensitive as SPR, it uses a smaller sample volume, and has an advantage in measuring interactions in more complex mixtures such as serum

Measures: Affinity (KD) and kinetics (on & off rates). Range nM - mM

Blitz

Isothermal Titration Calorimetry (ITC)

Isothermal Titration Calorimetry (ITC) measures in-solution, the binding affinity between any two molecules that either release or absorb heat when a binding interaction occurs.

The instrument measures the heat difference between a sample cell and a reference cell that occurs upon titration of the binding partner, and uses it to determine affinity, as well as additional parameters.

Measures: Affinity (KD), stoichiometry(n), enthalpy (DH) and entropy (DS). Range: nM - mM

PEAQ MicroCal ITC

To request more information or instrument training, please contact us:

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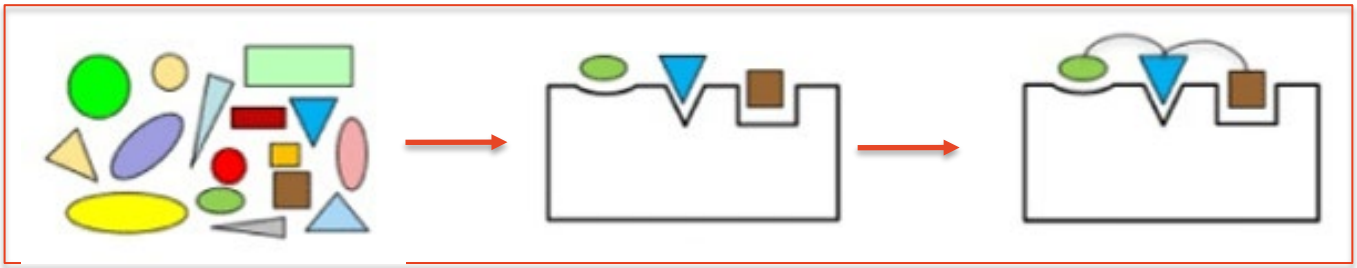
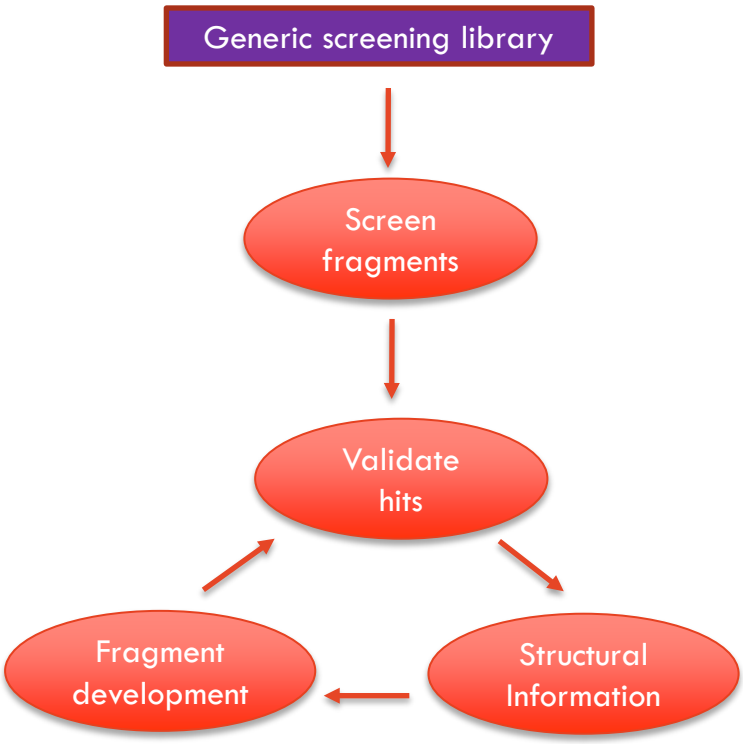
Sydney Analytical
Fragment Based Drug Discovery



Fragment Library | Drug Target | Fragment Development

Fragment Based Drug Discovery (FBDD) is an important and growing area of research. It provides a viable alternative to High Throughput Screening as a way of producing lead compounds for previously intractable biological targets. An FBDD approach is highly versatile, and can target bacterial, fungal and human proteins as well as difficult targets such as membrane proteins, with the aim to develop new inhibitory molecules.

FBDD Project Workflow



Our fragment library has been curated by medicinal chemists at Monash University. It contains 1100 fragments, covering significant portions of chemical space, and has been extensively QC'd.

The target protein is screened against the fragment library using NMR. This is carried out using cocktails of fragments. Data is processed using mNova software

Validation experiments are conducted using individual fragments. They are conducted using a variety of techniques, including NMR, SPR and crystallization.

Fragment development seeks to generate a "Structure Activity Relationship" (SAR). This is done using an "SAR by catalogue" approach, where fragment analogues are purchased and assayed using a variety of techniques including NMR or SPR

To request more information, please contact us:

Facility Manager: lorna.white@sydney.edu.au

sydney.analytical@sydney.edu.au

Sydney Analytical Cyclic Peptide Screening Platform

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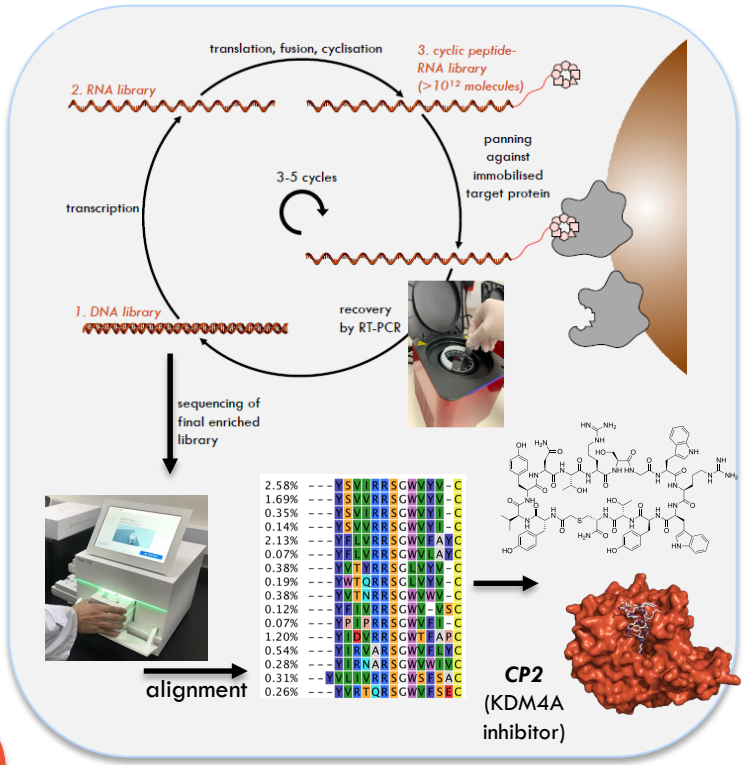
Explore new frontiers with our cyclic peptide screening technology

Cyclic peptides represent a cutting-edge approach in both drug discovery and research. Striking a delicate balance between size and efficacy, these molecules block problematic protein interactions and serve as invaluable tools in scientific exploration. Our screening platform can not only propel drug development by isolating potent cyclic peptide inhibitors but also provide researchers with crucial tools for studying disease-related proteins.

General workflow

- The process starts with the design of a DNA library, featuring common sequences at both the 5' and 3' ends, but with variability between
- When transcribed into RNA, it becomes the blueprint for diverse peptides.
- A Ribosome-based *in vitro* translation system is used to read the mRNA codons, adding corresponding amino acids and forming peptide bonds.
- The peptide library is panned against the immobilized target protein and RT-PCR utilised for easy recovery.
- Over 3-5 iterative screening rounds, the DNA library evolves, enriched for sequences encoding peptides with remarkable affinity.
- The final library is sequenced in-house, providing a set of peptide hits.



Affinity values of cyclic peptides:

Following identification, molecules of interest can be synthesised by solid phase peptide synthesis to obtain mg scale quantities for testing for affinity and activity. Some examples are shown.

	K_D (nM)	IC_{50} (nM)	
KDM4A lysine demethylase	30	42	Nature Commun. 2017. 8:14773.
Interleukin-6 receptor	44		J. Am. Chem. Soc. 2018. 140(37):11551-11555.
Hepatitis B virus receptor	4	< 1000	Cell Chem. Biol. 2018. 25(7):906-915.
Hepatocyte growth factor	0.4	8	Nature Chem. Biol. 2019. 15(6):598-606.
Zika virus protease	9	440	ACS Med. Chem. Lett. 2019. 10(2):168-174.
Acid-sensing ion channel 1A	< 0.1	0.4	unpublished
IMP-1 b-lactamase	1	14	unpublished
NDM b-lactamase	1	21	unpublished
VIM-2 b-lactamase	11	33	unpublished

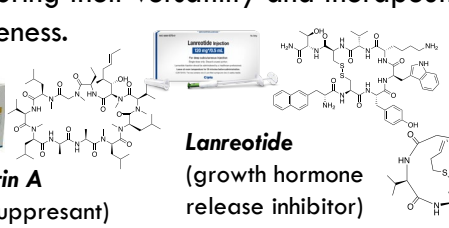
Specialised Screening

A customized solution of **aa-tRNAs containing canonical and non-canonical amino acids** can be used to give an extra level of complexity in the search for new drugs.

Cyclic Peptides show Translational Success

More than 40 cyclic peptide drugs are currently in use, underscoring their versatility and therapeutic effectiveness.

Cyclosporin A
(immunosuppresant)



Lanreotide
(growth hormone release inhibitor)

Romidespin
(HDAC inhibitor)

For more information please contact us:

Staff Scientist:
miguel.hernandez-prieto@sydney.edu.au
sydney.analytical@sydney.edu.au

RaPID Platform Screening Services

Random non-standard Peptides Integrated Discovery (RaPID)

What is RaPID?

The RaPID platform leverages vast libraries—comprising trillions of cyclic peptides—to identify **high-affinity binders** for your target proteins. We tailor every screen to match your specific application.

Workflow at a Glance

1. **Library Construction**
   - Peptides: 4–15 random amino acids
   - Methionine removed; N-chloroacetyl-tyrosine used as initiator (L- or D-enantiomer)
   - Spontaneous cyclisation with cysteine produces highly constrained cyclic peptides
2. **Binding & Selection**
   - Target protein immobilised (biotin, Fc, or His tags preferred)
   - Peptides that bind are retained, non-binders are washed away
3. **Enrichment Rounds**
   - Iterative binding increases specificity and affinity
4. **Decoding the Binders**
   - mRNA tags act as barcodes
   - Sequences are reverse-transcribed and identified via DNA sequencing
5. **Analysis**
   - Identify enriched motifs or consensus sequences
6. **Validation**
   - SPR-based binding assays available via our expert team
   - Peptide synthesis through trusted collaborators

Flexible Engagement Models

Training Option:

- Train your team to run screens independently (50% discount after initial screen)

Full-Service Option:

- We handle everything from setup to analysis

Pricing:

- Formal quote available upon request

Sample Requirements

Preferred Tags:

1. Biotin
2. Fc-tag
3. His-tag

Protein Amount:

- Minimum of 260 pmoles
- We accept user-supplied protein or can arrange procurement

Need help with production?

Our protein production team can assist. Contact Dr. Mario Torrado del Rey: *mario.torradodelrey@sydney.edu.au*

Why Work With Us?

- All screening conducted in-house by experienced scientists
- Transparent process with regular updates

Ready to discuss your project or request a quote? Contact Us

Dr. Miguel Hernandez-Prieto (*miguel.hernandez-prieto@sydney.edu.au*)
Senior Research Officer | Drug Discovery Node
Sydney Analytical, University of Sydney

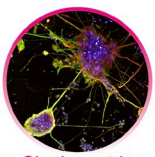
Sydney Analytical
3D Cell Biology Platform

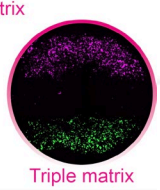
3D cell culture systems can mimic the complex microenvironment of tissues and organs more accurately than traditional 2D cell culture. Automation of 3D cell culture, assay and analysis pipelines deliver consistent, unbiased, and biologically relevant results at scale. At Sydney Analytical, we can assist with designing and developing automated 3D culture platforms for high-throughput drug discovery screening, personalized medicine screening, advanced therapeutics, and investigative biological studies.

Automated 3D Cell Culture and Analysis Platform

RASTRUM™ cell bioprinter

Prints primary and secondary cell lines and induced pluripotent stem cells (iPSCs) in 3D matrix (single or multiple) which closely mimics the complexity of in vivo microenvironments.

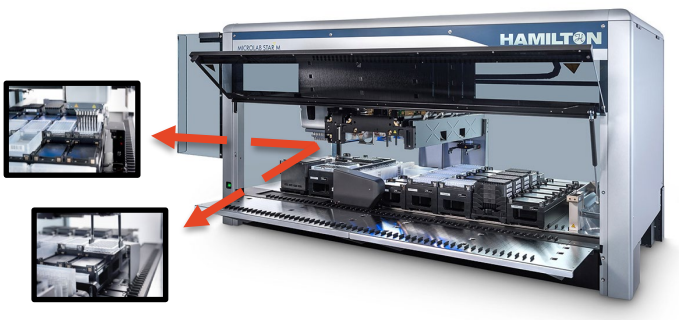




Rastrum Bioprinter (Inventia)

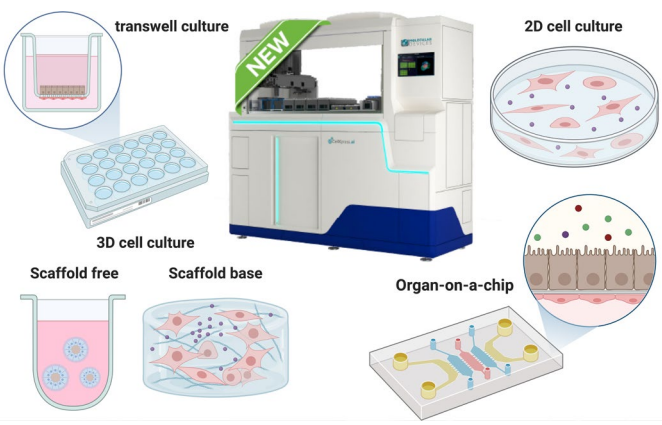
Hamilton Microlab® STAR™

Automates in-process and endpoint pipetting workflows such as transferring samples and preparing and changing reagents.



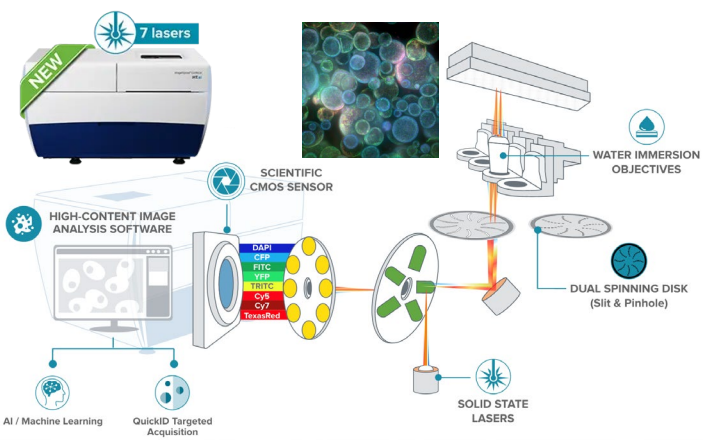
CellXpress. ai

Standardizes 2D and 3D biology development processes for iPSCs, spheroids, organoids, and organ-on-chip. Reduces hands-on time while maintaining a 24/7 schedule for seeding, feeding, monitoring, passaging, incubation, liquid handling, and imaging.



ImageXpress Confocal HT.ai

Enables high-content 3D screening assay with fast acquisitions, and 3D spatial and volumetric analysis with machine learning capabilities.



SpectraMax iD5

Measures absorbance, fluorescence, luminescence, time-resolved fluorescence and fluorescence polarization

For more information please contact us:

Staff Scientist:
ling.chen@sydney.edu.au
sydney.analytical@sydney.edu.au