THE UNIVERSITY OF SYDNEY

Sydney Analytical
Protein Production and Characterisation

Extending the scope of your research with protein production solutions

Our aim is to assist researchers from all backgrounds with the expression, purification, and analysis 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.

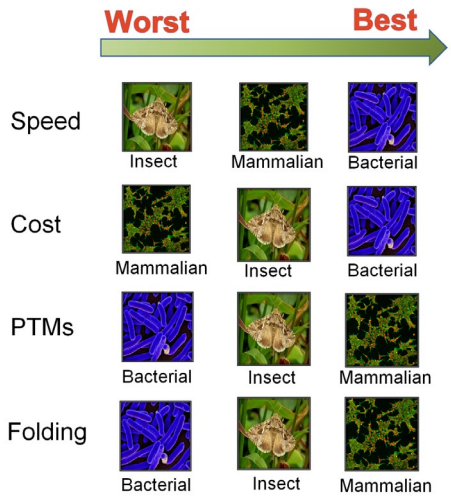
General workflow

Protein expression → Protein purification → Protein characterisation

Equipment and expertise

Protein expression

- We have several different expression strategies that we can tailor 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:



- We have specialised equipment to optimise expression and lysis conditions for your proteins.

Shaking incubators for bacterial, insect or mammalian cells culture

Cell culture cabinet (left) for sterile work with mammalian and insect cells, and microscope (right)

Homogeniser (left) and sonicator (right), for cell lysis

Centrifuges for a variety of uses including cell extract clarification

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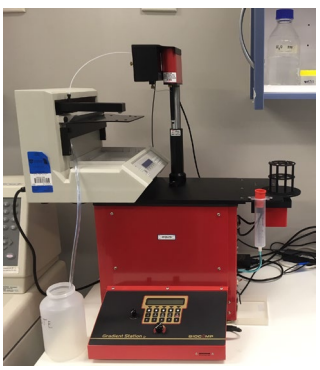
Protein purification

- We can 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.
- We also normally recommend a second purification step by FPLC. We have several automated GE ÄKTA systems that we can use with different columns:
  - Size exclusion, for separation by size.
  - Ion exchange, for separation by charge.
  - Different types of affinity chromatography.

ÄKTA Pure with a size exclusion column and two different affinity columns

- We also have expertise in ultracentrifugation for membrane protein preparations, and for density gradient separation followed by automatic gradient fractionation.



Ultracentrifuge (left) and Gradient Station (right)

Protein characterisation and quality control

- Sydney Analytical has the latest equipment to facilitate the biochemical, biophysical and structural characterisation of proteins, to better understand their function, and to ensure sample quality:
  - **Uncle** and **Prometheus Panta** instruments; measure protein stability and aggregation of multiple samples simultaneously, using a combination of DSF and DLS functionalities.
  - **Refeyn Two MP Mass Photometer**; label free determination of mass measurement for single protein molecules and complexes in solution using light.
  - **Circular Dichroism**, for secondary structure charaterisation and protein stability studies.
  - **SEC-MALLS**, for molecular weight and monodispersity measurements.
  - X-ray crystallography, NMR and molecular interactions facilities (see our other brochures).
  - Other techniques like SDS-PAGE, Western blot, endotoxin check and endotoxin removal, UV-vis spectrophotometry and chromatography profiling.

Prometheus Panta (left) and Refeyn Mass Photometer (right)

Circular dichroism (left) and SEC-MALLS (right)

For more information

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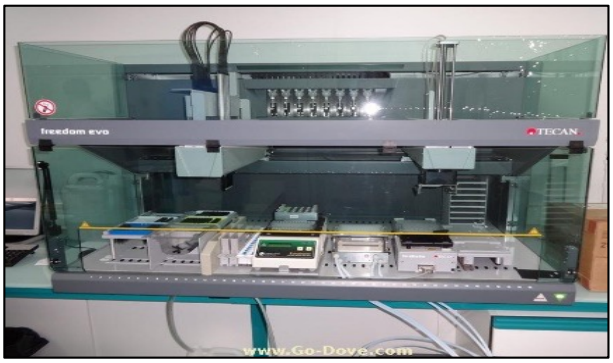
Sydney Analytical
Macromolecular crystallography

Sydney Analytical provides expertise and access to advanced equipment to facilitate macromolecular crystallography. This includes a Tecan liquid handler and crystallization robotics Mosquito® LV (sptlabtech) and NT8® (Formulatrix). These enable the automated setting of crystallization screens with hanging drop, sitting drop, additive, and seeding methods. Additionally, the NT8® can also facilitate Lipidic Cubic Phase (LCP) experiments for membrane protein crystallisation.

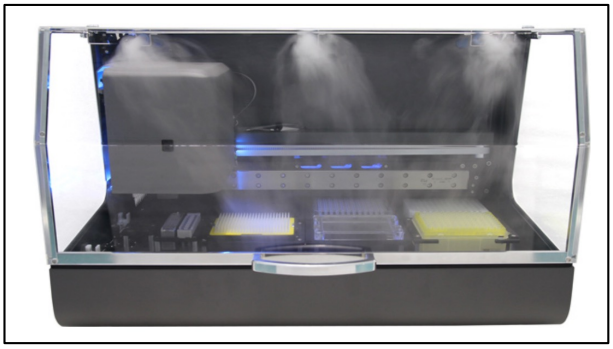
We have access to the Australian Synchrotron x-ray facility through the Collaborative Access Program (CAP) which allows for the screening and data collection for crystals. We also provide training to users on crystal screen set-up, crystal optimization as well as remote x-ray data collection at the Australian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



Freedom Evo liquid handler (Tecan)

Mosquito LV (sptlabtech)

Stereo Microscopes (Leica)



NT8 (Formulatrix)

Data collection at Australian Synchrotron

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

FOR MORE INFORMATION

Sydney Analytical – Protein crystallography

https://www.sydney.edu.au/research/facilities/sydney-analytical/drug-discovery.html

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Sydney Analytical
Macromolecular Interactions Analysis

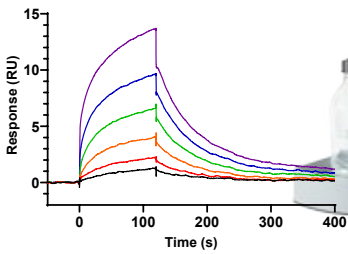
We have the experience and instrumentation to characterize a wide variety of macromolecular interactions. Most commonly, protein:protein, protein:peptide, protein:nucleic acid – including DNA, RNA and PNA, as well as protein:small molecule and protein:fragment interactions. We can collect data or provide 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.

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

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. The Blitz instrument is not as sensitive as SPR, but uses a smaller sample volume.

Measures: Affinity (KD) and kinetics (on + off rates). Range pM - 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 increasing amount of a 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

For more information

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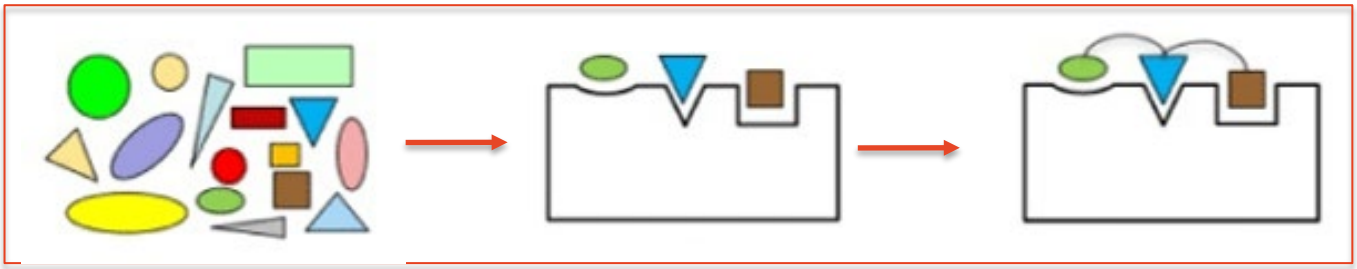
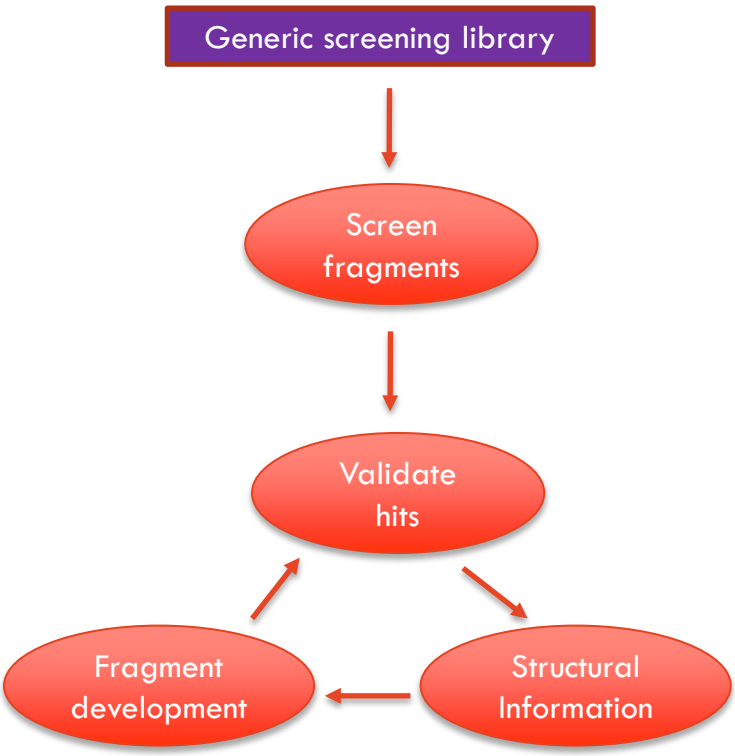
Sydney Analytical
Fragment Based Drug Discovery



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

For more information:

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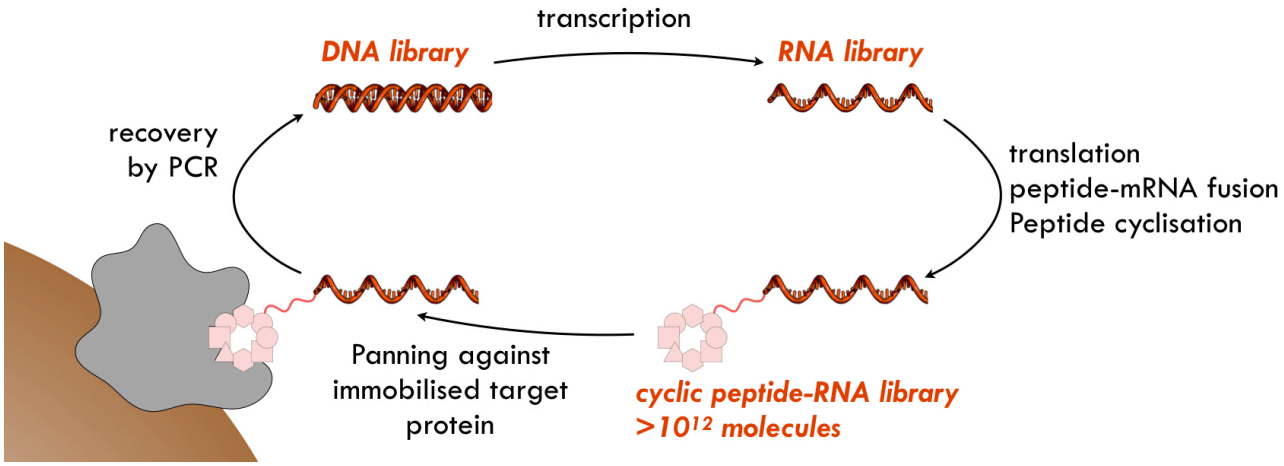
Sydney Analytical
Cyclic Peptide Display Screening

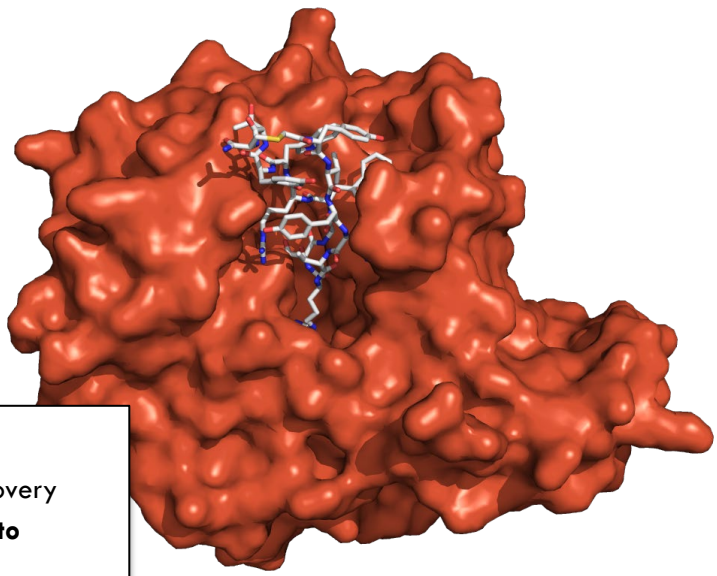


High affinity ligands to any expressible target

Small cyclic peptides are small enough to display drug-like properties, but large enough to block protein-protein interactions, making them applicable to the targeting of virtually any protein. Recently developed techniques allow for the parallel synthesis of trillions of cyclic peptides each linked to their cognate mRNA, which can easily be screened for target affinity (through iterative rounds of target binding and recovery by PCR) leading to the isolation of ligands with exceptional target affinity (low nM K_D) and selectivity (typically, at least an order of magnitude, even for closely related homologues). The molecules identified are amenable to chemical synthesis and can act as either agonists or antagonists. Using techniques that are currently restricted to a handful of academic institutions worldwide, our cyclic peptide display screening capability enables users to rapidly identify high affinity ligands to any expressible protein of interest.

Summary

- Applicable to any expressible protein.
- Leads to identification of multiple ligands.
- Affinities typically nM to pM range.
- Highly selective binders.
- “Hits” are amenable to chemical synthesis.
- Can identify agonists and antagonists



For more information:

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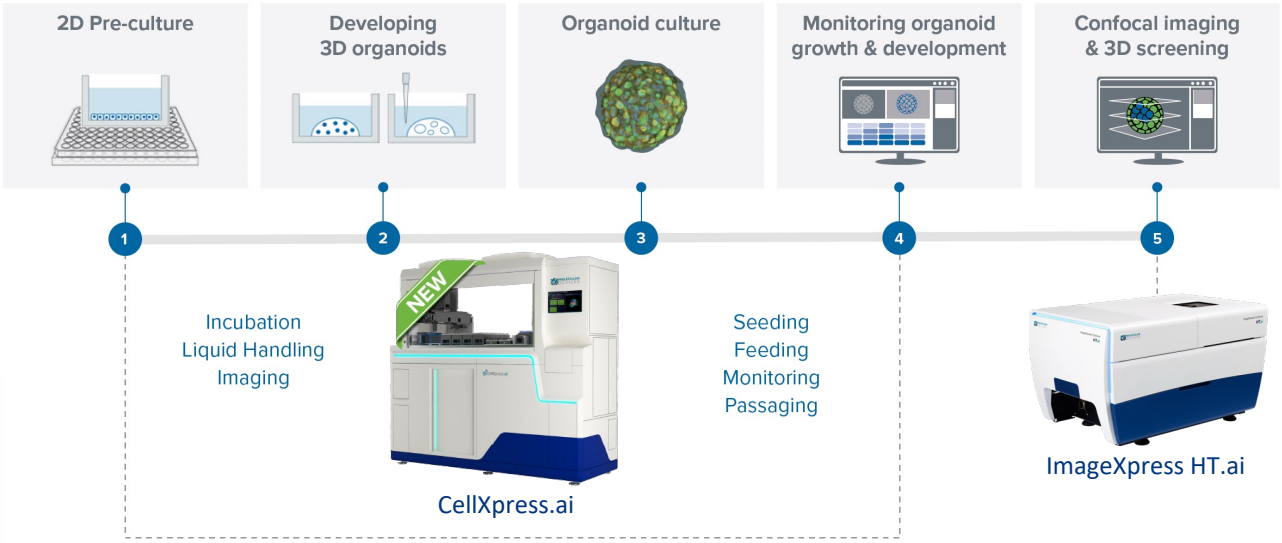
Sydney Analytical 3D Cell Biology Platform

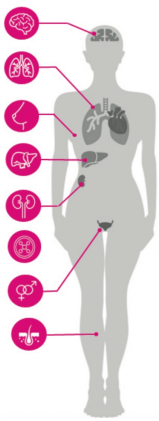
Automated 3D Cell Culture and Analysis Platform

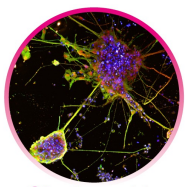


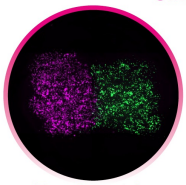
Design and develop your desired automated 3D culture platforms as tools for high-throughput drug discovery screening, personalized medicine screening, and investigative biological studies. The automated 3D biology platform improves workflows and makes assays more reliable and reproducible.

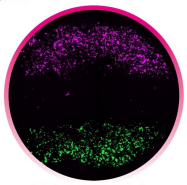
Key features

- RASTRUM™ 3D cell bioprinter prints cell lines, primary cells, and iPSCs in the 3D matrix (single or multiple) which closely mimics the complex of in vivo microenvironments and allows you to study a range of tissue types and diseases.
- CellXpress.ai standardizes the 3D biology development process (iPSCs, spheroids and organoids) with cell culture, treatment, and incubation, through to imaging, analysis, and data processing, delivering consistent, unbiased, and biological-relevant results.
- ImageXpress Confocal HT.ai enables high-sensitivity detection and fast acquisitions of 3D samples, and advanced phenotypic classification and 3D image analysis with machine learning capabilities.




Single matrix


Dual matrix


Triple matrix


RASTRUM

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