The Protein Crystallography and Biophysics Centre (BiophysX)

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https://biophysx.ismb.lon.ac.uk/

Introduction

The Protein Crystallography and Biophysics Centre is a core facility of the UCL/Birkbeck Institute of Structural and Molecular Biology (ISMB). The facility, located at Birkbeck, provides access to a wide range of instrumentation and expertise covering the characterization of protein stability, crystallization and biomolecular interactions. Amongst the Centre's instruments are state-of-the-art liquid handling equipment, automated imaging systems for monitoring crystal growth and aggregation, temperature-controlled CD and fluorescence spectrometers, isothermal and differential scanning calorimeters, biolayer interferometry, dynamic light scattering, and multi-angle light scattering attached to size exclusion chromatography for accurate sizing of molecular species.

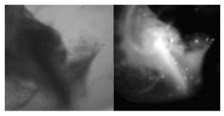
The Centre is experienced in working with a wide range of users. In addition to structural biologists, we have users engaged in protein engineering, ligand discovery, antibody therapeutic development, chemical kinetics in confined environments and the characterization of membrane proteins and protein/nucleic acid complexes.

Applications

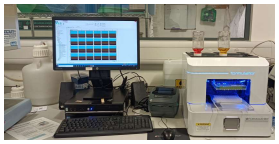
1. Protein crystallography and solubility/stability screening

The lab is equipped with a Mosquito LCP, a Formulator liquid handler and a JANSi UVExps 256 plate hotel. Combined with the auxiliary equipment, the lab fully supports protein crystallization experiments and long-term monitoring of protein stability in solution.

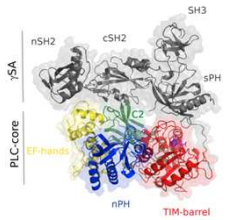
The Mosquito LCP is suitable for high throughput crystallization experiments in 96 well format. It is suitable for initial screening as well as for optimization and supports in addition LCP crystallization



The JANSi UVExps 256 plate hotel is a temperature controlled imaging system with lenses up to 20x, UV and polarizer. Combining brightfield and UV lenses, it can continuously monitor precipitation or crystal formation. The system supports several sitting drop, hanging drop and LCP plates and can be accessed remotely, making it suitable for monitoring and controlling experiments remotely.



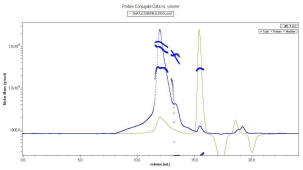
The Formulator is a highly versatile nine channel liquid mixer supporting volumes from few microliters to milliliters. Supports any plate with 96 or less wells, including Linbro plates.



Crystal structure of the rat phospholipase Cγ in complex with PIP2. (PDB ID 7Z3J). Crystals were grown and optimized at the BiophysX lab, data collected at the ESRF, Grenoble France (Huray et al, SciAdv 2022

2. Multi-angle laser scattering (SEC-MALS)

Multi-Angle Static Light Scattering provides direct information on the molecular weight of the sample, so it can identify aggregates or oligomerization, characterize large complexes and generally assess the homogeneity of a sample. Using in addition a UV detector, each species can be dissected into different components, distinguishing glycosylation, bound *detergents or protein/nucleic acid complexes.*

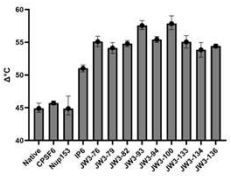


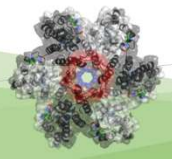
Left: Our SEC/MALS comprising an Agilent 1100 with autosampler injector and fraction collector linked to a DAWN 8+ and an Optilab T-rex (Wyatt).
Right: Example of a membrane protein detergent optimization. The ratio protein/detergent of the main peak is 0.75

3. Thermal Shift Assays (MyIQ, Biorad)

Temperature denaturation of a protein gradually exposes the hydrophobic patches buried in the native fold. A dye (usually SYPRO orange) allows denaturation to be monitored as it binds to the exposed hydrophobic patches and becomes more fluorescent.

This assay can conveniently be performed in 96-well format using a real-time PCR machine to screen the influence of buffer conditions, concentration or ligand binding on protein stability.



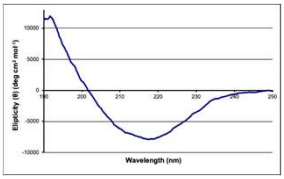


Thermal shift assays of various inhibitors of HIV-1(M) capsids. The specific batch of inhibitors stabilize the hexameric assembly of the capsid protein, thus inhibiting its infection mechanism in the host cell (M. Larsen, G. Towers, UCL)

4. Circular Dichroism (CD, Jasco-720)

Chiral molecules absorb the right and left components of circularly polarised light to a different extent. CD spectroscopy measures this differential absorbance. Nearly all biological macromolecules are chiral, and they have characteristic spectra which can provide an estimation of the secondary structure content of a protein can be analysed as alpha helices, beta strands and random coil. Furthermore, the temperature dependence of the CD signal gives information about protein stability and folding.

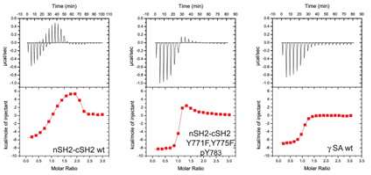
Our Jasco spectrometer gives publication quality scans down to 190 nm wavelength enabling quantitative secondary structure analysis. It is equipped with a thermostated sample chamber for melting experiments.



Spectrum of the domains 9-13 of myomesin-1 a modular protein of B-barrels linked by single a-helices. The secondary structure estimation provided 10% a-helix, 60% B-sheet, consistent with the crystal structures (Pinotsis et al PloS Biol 2012)

5. Isothermal Titration Calorimetry (VP-ITC)

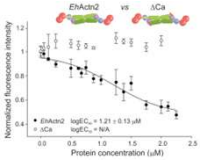
Isothermal titration calorimetry measures the heat that is released (or required) when molecules interact. In a typical ITC experiment, a single titration of ligand into a solution of its interaction partner results in a series of peaks representing the heat of each addition. These can be analysed to give a complete characterisation of binding thermodynamics, i.e. the binding affinity, stoichiometry, enthalpy and entropy.



Interaction of FGFR1-1p kinase domain with various fragments of the phsopholipase PLCγ1 measured at our VP-ITC. The data were corrected for the heat of dilution of the titrant and subsequently, fit to either a two-site model (left and middle panel) or a one-site model (right panel).
Bunney et al, Structure 2012

6. Fluorescence spectroscopy (Fluoromax3 & 4)

The two fluorescence spectrophotometers at the facility are highly accurate and versatile with excitation and emission monochromators and polarizers as well as a Micromax-384 plate reader.



Competition assays using Oregon Green 488 BAPTA-5N dye for the a-actinin-2 from Entamoeba histolytica. The calcium insensitive mutant (ΔCa) was used as negative control. For calculation of logEC50 values the protein was considered in its dimeric state.
(Pinotsis et al, PNAS 2021)

7. Additional equipment

The facility is also equipped with additional equipment:

- Bio-Layer Interferometry (BLItz) for measuring biomolecular interactions using immobilized protein on a biosensor tip.
- Analytical Ultracentrifugation (Beckman XL-I) for the determination of molecular weight of macromolecular complexes.
- Differential Scanning Calorimeter (VP-DSC) for the accurate measurement of temperature dependent thermodynamic properties of a sample

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