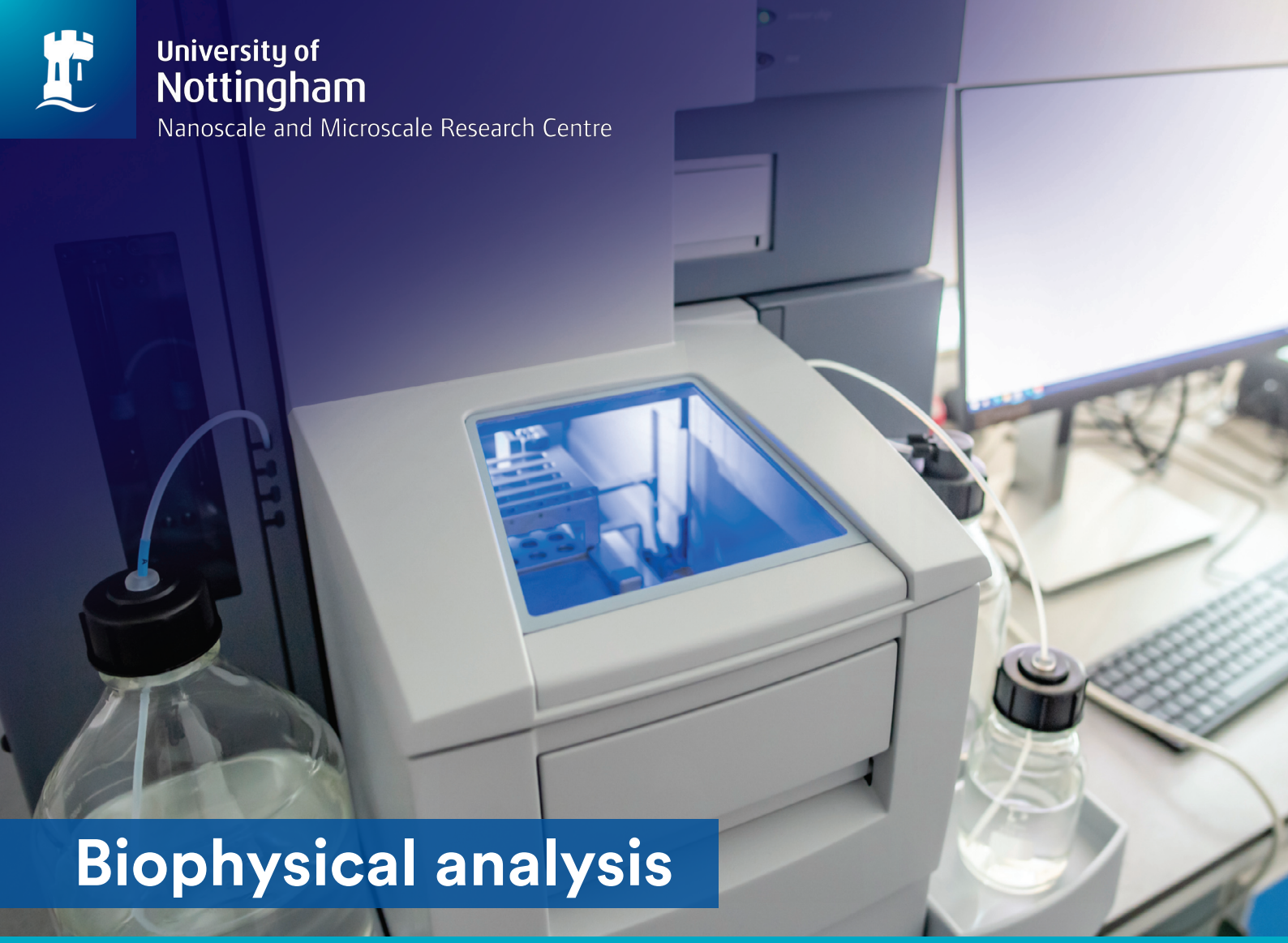
University of Nottingham
Nanoscale and Microscale Research Centre



Biophysical analysis

Biophysical analysis encompasses a range of techniques including Isothermal Titration Calorimetry (ITC) and Surface Plasmon Resonance (SPR). These techniques study the binding interactions of a range of biomolecules such as proteins, peptides, nucleic acids (DNA, RNA), carbohydrates, lipids as well as interactions with small molecules, polymers, ions, or drug compounds. They also obtain data on the affinity, kinetics, stoichiometry, and thermodynamics of such interactions.

Capabilities

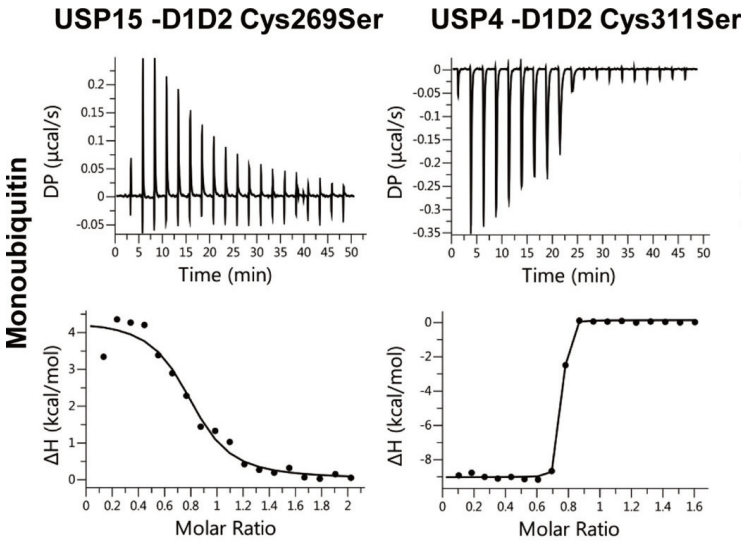
- Determination of thermodynamic parameters including binding affinity and stoichiometry
- Measurement of enzyme kinetics
- Molecular variant comparisons, e.g., mutant versus wild types
- Protein activity and stability analysis
- Epitope mapping

Typical applications

- Pharmaceutical drug discovery
- Identification of binding partners to targets
- Quality control for pharmaceuticals
- Detect and characterise molecular interactions

Enzyme-protein substrate and product interaction probed using MicroCal PEAQ ITC

ITC can enhance our understanding of the specificity of enzymes for substrates and thus was used to measure the interaction of USP15 and USP4 mutants with monoubiquitin (product) and linear diubiquitin (substrate) revealing insights on the interaction mechanism. Significant differences were observed in their thermodynamic isothermal profiles: an endothermic process was observed for the binding of USP15 mutant to both monoubiquitin and diubiquitin whereas an exothermic process was observed for USP4 mutant binding to the two proteins at 25 °C. The affinity of USP15 mutant for monoubiquitin was determined to be lower than that of USP4 mutant indicating that product inhibition plays a larger role for USP4 than USP15. Both mutant proteases had similar affinity for diubiquitin, but the interaction was associated with different enthalpy and entropy parameters.



Stephanie J. Ward, Hayley E. Gratton, Peni Indrayudha, Camille Michavila, Rishov Mukhopadhyay, Sigrun K. Maurer, Simon G. Caulton, Jonas Emsley, and Ingrid Dreveny. J. Biol. Chem. (2018) 293(45) 17362–17374.

SPR theory and setup

Surface Plasmon Resonance (SPR) uses optical biosensing for real-time monitoring of macromolecular interactions. In a standard SPR set up, one interacting partner (the ligand) is immobilized onto a gold sensor chip surface and an unbound interactant (the analyte) is then flowed over the surface. A change in the refraction index at the surface of the sensor (e.g., due to analyte binding or dissociation occurring near the surface) may be monitored as a shift in the resonance angle and is recorded as a sensorgram.

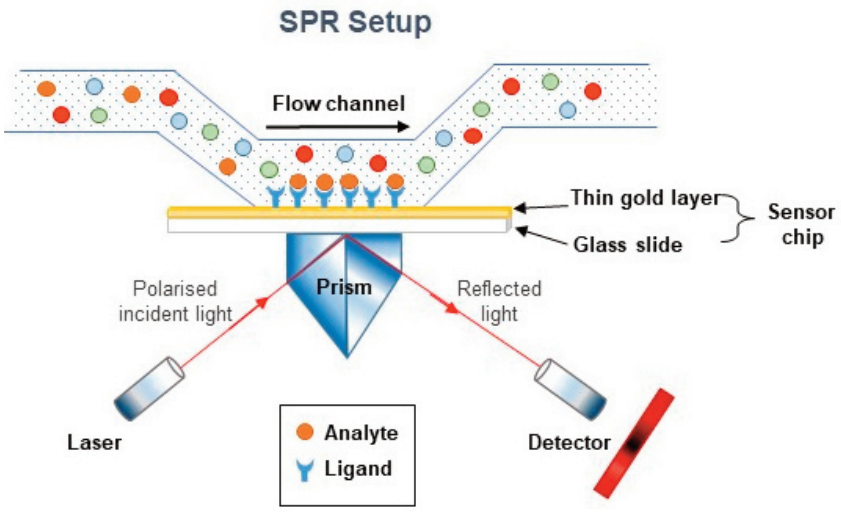


Figure courtesy of Marion J. Limo, nmRC, University of Nottingham

Our facilities

MicroCal PEAQ-ITC (Malvern)
A highly sensitive instrument designed for ease-of-use, requires as little as 10 µg of sample, and has a temperature range of 2 to 80 °C. It features user-friendly guided workflows with videos on running of experiments and performs automated cleaning of the sample cell and the titration syringe.

MicroCal PEAQ-ITC analysis software
Experimental design simulation software to guide users in selecting measurement parameters and simplify analyses with batch evaluation of large data sets and automated assessment of data quality. Final figures and graphs can also be generated quickly and easily.

Biacore T200 (Cytiva)
The Biacore T200 is a highly sensitive SPR instrument with high-throughput capabilities (up to 384 samples per run). Analysis temperature range from 4 to 45 °C, injection volume of 2 to 350 µL, affinity range from fM to mM, and concentration range > 1 pM.

Find out how biophysical analysis could help with your applications, designs or solutions:
nmrcenquiries@nottingham.ac.uk | +44 (0)115 951 5046
nottingham.ac.uk/nmrc-commercial