

Protein Quantification and Comparability of Measurements in Proteomics

A project funded by the
UK's National Measurement System
Measurements for Biotechnology programme

Background:

The National Measurement System (NMS) is the UK's national infrastructure of laboratories, which delivers world-class measurement science & technology, providing traceable and increasingly accurate standards of measurement for use in trade, industry, academia and government. The NMS has initiated the Measurements for Biotechnology programme to enhance comparable measurements within this rapidly expanding sector. The programme has five themes (Microarrays, Proteomics and Genomics, Cells, Physical-Chemistry and Trace measurement), connected via a core "hub" activity involving LGC Limited, the National Physical Laboratory (NPL) and the Bio-Industry Organisation (BIA).

Three complimentary projects make up Theme 2: Proteomics and Genomics:

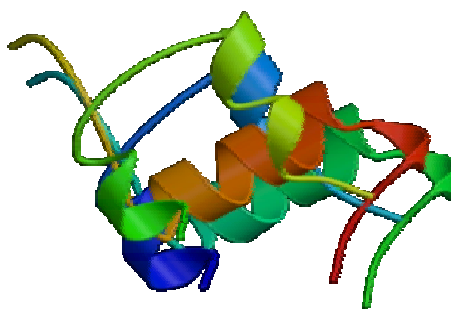
- 1) To develop optimised conditions to improve sample preparation in protein analysis
- 2) Metrology of the Proteome: Correlation of the identity and structure of post-translationally modified proteins to their function
- 3) Protein quantification and comparability of measurements in Proteomics

Quantitative Proteomics Project:

- Explore and improve on the best known and most successful options for quantification using Mass Spectrometry and produce useful protocols for quantitative analysis
- Develop industry relevant reference standards
- Transfer protocols by multi-centre comparative studies, by dissemination through guide notes distributed via the web and at relevant meetings and through the publication of a Good Practice Guide.

The proposed project seeks to achieve these goals via a logical sequence of experiments for defining protein quantitation methodologies utilising Mass Spectrometry leading to the development of protein reference mixtures.

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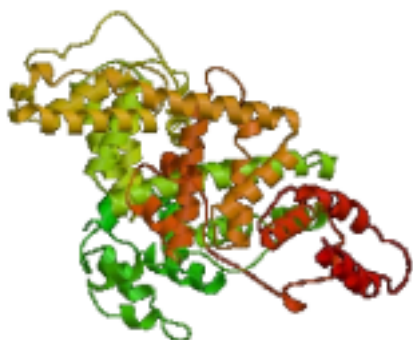
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Key project features:

- To concentrate on improving the quality, comparability and applicability of relative quantitation methods for the proteome using Mass Spectrometry
- To introduce good measurement practice through the development of robust mass spectroscopic methods supported by reference standards
- To examine the potential for a primary method of analysis in order to put the measurements on a sound metrological footing.
- To carry out research aimed at developing and evaluating methods for the relative and absolute quantification of post-translational modifications
- To interact and directly involve key users of quantitative protein measurements to achieve broad dissemination and high participation

Experimental Approach:

- Select and use a three-protein model system on which a robust proteolysis method will be established
- Investigate several mass tagging techniques for mass spectrometry quantification
- Assess the performance of mass spectrometers used for protein analysis for making ratio measurements
- Examine the potential of Isotope Dilution Mass Spectrometry as a potential primary absolute method for protein quantitation
- Produce reference standards protein mixtures to be tested by round-robin exercises by interested laboratories using mass spectrometry
- The relative quantification of phosphorylated proteins will be assessed using differential isotope labelling and different separation strategies



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Deliverables:

Several major and measurable deliverables will be produced by this project with the aim of improving the quality, comparability and applicability of relative quantitation methods for the proteome.

- The publication of a Good Practice Guide for robust and traceable absolute or generic relative methods for protein quantitation using mass spectrometry
- A group of interested laboratories will be recruited to robust test methodologies and evaluate prototype reference materials
- Prototype reference standards for relative measurements will be produced
- The development of a primary method of analysis using isotope dilution mass spectrometry to place proteomics on a metrological footing
- To carry out fundamental research aimed at developing and evaluating methods for the relative quantification of post-translational modifications (PTMs), specifically for phosphorylated proteins
- To interact and directly involve key users of quantitative protein measurements throughout the project in order to pool expertise in a key area of pre-competitive development.



*Setting standards
in analytical science*

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