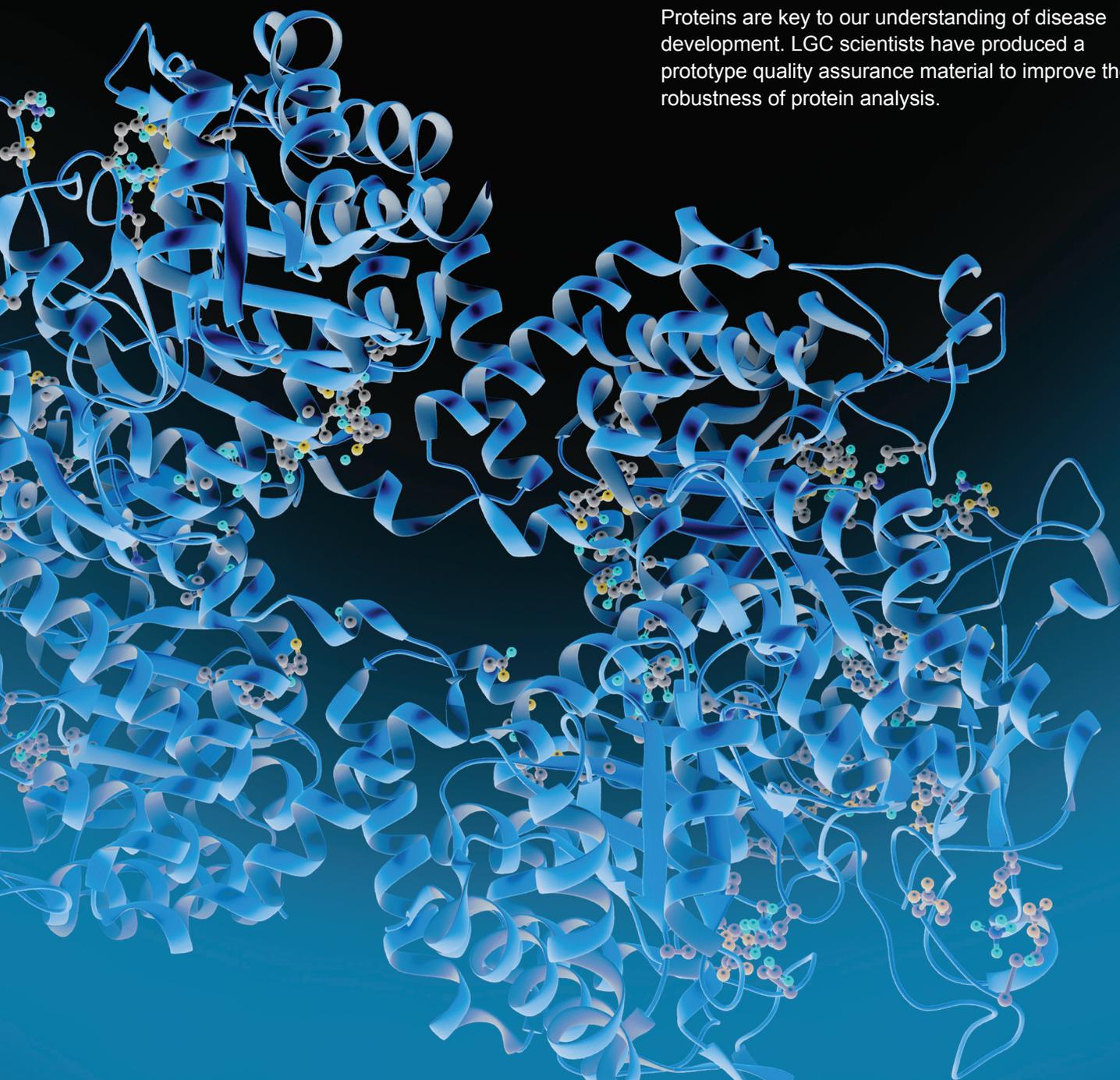




A new tool for proteomic analysis

Proteins are key to our understanding of disease development. LGC scientists have produced a prototype quality assurance material to improve the robustness of protein analysis.



The Requirement

Proteins are large biological molecules that are made up of linear sequences of amino acids held together by peptide bonds. Proteins are essential in any living organism and perform a wide range of functions; they act as enzymes in catalysing biochemical reactions, as antibodies in immune response and have a key role in the replication of DNA.

Proteins play a critical role in our understanding of the development and state of disease and are becoming increasingly important as clinical biomarkers to inform diagnostic and therapeutic decisions.

The analysis of proteins, called “proteomics”, is the large-scale identification, characterisation and quantification of proteins in a biological system. However, optimisation and validation of proteomic methods remains highly challenging due to the natural biological variability of cells (protein composition varies from cell to cell and from time to time), the complexity of the samples and the large dynamic range of the protein concentrations.

One technique used widely for proteomic analysis is liquid chromatography-mass spectrometry (LC-MS). In proteomics studies of this kind, the proteins of interest are not analysed intact. Instead they are chopped up into pieces – known as peptides – by an enzyme (trypsin) prior to LC-MS analysis. As a result the actual analytes being measured are the peptides, which are surrogates for the real analytes of interest, the proteins.

Consequently, having a complete understanding of the analytical variability associated with the enzymatic digestion step is critically important in determining whether any observed difference in protein composition is due to true physiological variations in the biological sample or as a result of analytical variability.

There is therefore a requirement for standards and quality control materials to support optimisation and validation of proteomic experiments in order to enable the discovery and further understanding of appropriate biomarkers.

The solution

Following a review of commercially available standards to support proteomic analysis, and consultation with scientists from a variety of academic and industrial organisations, LGC’s metrology experts have developed a prototype quality control

material (QCM) to help monitor the reproducibility of the tryptic enzymatic digestion step used in proteomic experiments.

The QCM comprises a mixture of proteins and isotopically labelled peptides which have been carefully selected and optimised for proteomic analysis of human samples. The QCM functions as an internal standard for use during tryptic digestion and provides analysts with a tool to optimise and validate this stage of their experimental design to ensure accurate and reproducible protein digestion.

The QCM has been evaluated by six independent laboratories that carry out proteomic analysis. Each laboratory analysed the QCM according to their normal enzymatic digestion protocols and on the analytical platforms routinely used in their laboratories. By spiking the QCM into real samples, the laboratories were able to assess the reproducibility of their digestion procedures and identify sources of variability within the methods used.

Impact

The studies have demonstrated how such a QCM can be used to better understand sources of variability associated with the enzymatic digestion step in proteomic analysis and to assist the development and validation of these measurements.

Its larger scale use requires investigation of factors such as stability and homogeneity of the QCM, however this work has shown that it has the potential to help provide confidence in the reliability of proteomic measurements, and their role in biomarker diagnostics and the development of novel drug therapies.

Dr David Knight from the Faculty of Life Sciences at the University of Manchester, comments:

“Participation in the trial has really helped us to focus our effort in developing protein quantification methodologies, both in highlighting issues in our internal processing and also in the comparison of our methodologies with those of other highly experienced groups across the UK.”

The work described here was delivered by LGC as part of its role as a designated National Measurement Institute and was funded by the UK National Measurement System.

For further information, contact:

LGC, Queens Road, Teddington, Middlesex TW11 0LY, UK

Tel: **+44 20 8943 7000** Email: info@lgcgroup.com Web: www.lgcgroup.com

NMS Helpdesk

Tel: **+44 20 8943 7393** Email: nmshelp@lgcgroup.com Web: www.nmschembio.org.uk

**National
Measurement
System**



The National Measurement System delivers world-class measurement for science and technology through these organisations

