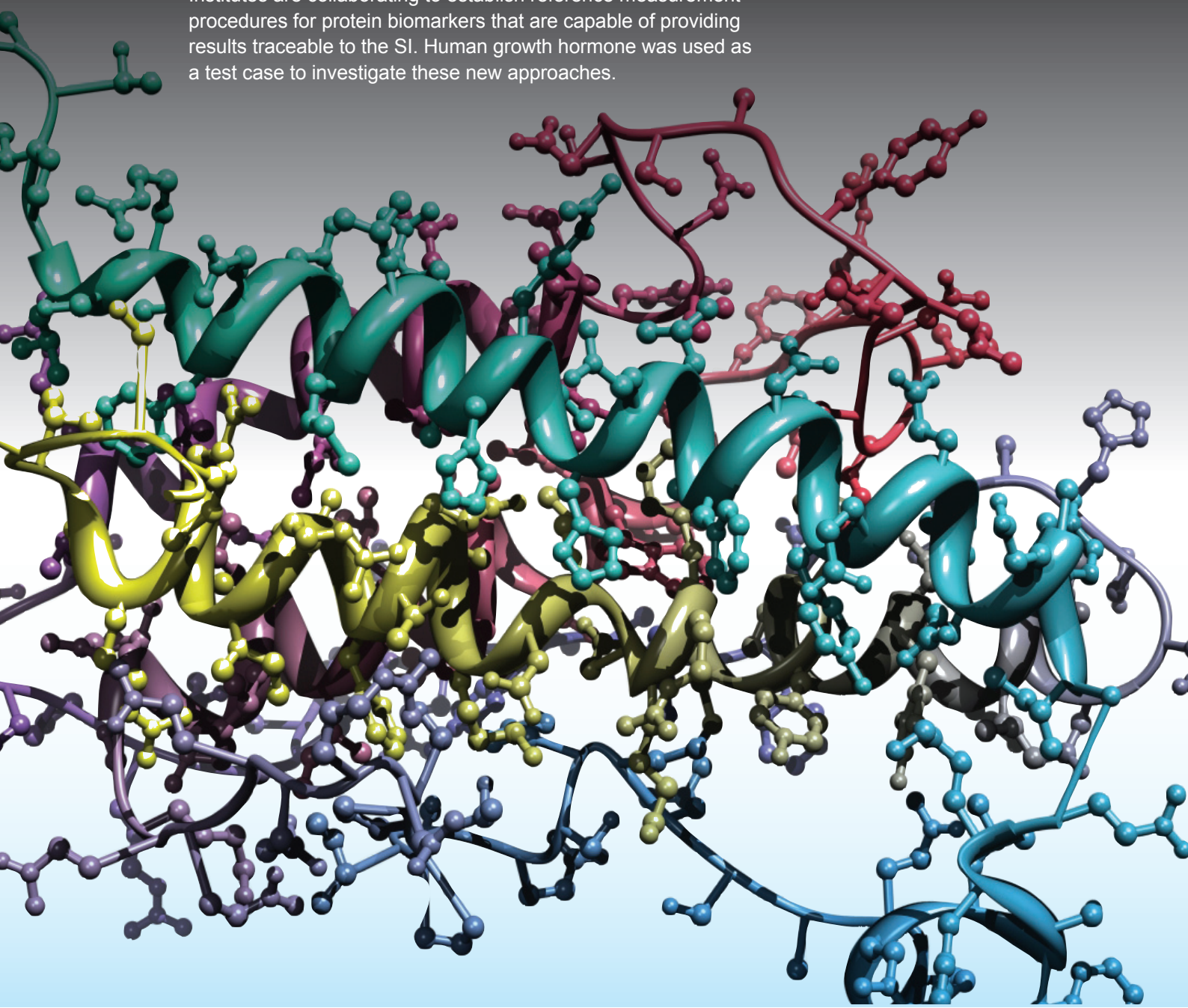
National Measurement System

From peptides to proteins – establishing traceability to the SI

In order to improve clinical measurement robustness for enhanced patient care, LGC and several European National Measurement Institutes are collaborating to establish reference measurement procedures for protein biomarkers that are capable of providing results traceable to the SI. Human growth hormone was used as a test case to investigate these new approaches.



LGC *Setting standards in analytical science*

The Requirement

Human growth hormone (hGH) is widely used in the diagnosis of disorders of children with short stature, management of disorders that lead to nutritional deficiency, and to monitor growth hormone replacement therapy. Therefore, there is a need for reliable and comparable measurements. To achieve this, routine measurement results need to be made traceable to a stable reference.

Whilst traceability in chemical and physical measurement is well established, the biological measurement field is at a much earlier stage in its development, and clinical measurements in particular present their own set of unique problems. Poor quality clinical measurements can lead to misdiagnosis or incorrect prescription of medicine for patients. It is therefore essential that certified reference materials are available for manufacturers to establish the traceability of values assigned to calibrators supplied with diagnostic equipment, and for medical and clinical laboratories to validate their methods. The ability to establish the traceability of biological measurements, would be a major step forward in the clinical sector.

The Solution

To determine the concentration of hGH in serum, hGH is first 'broken down' into peptides. By choosing a number of peptide sequences unique to hGH, and by employing isotope dilution mass spectrometry approaches, the concentration of the protein can be accurately determined. In this case the standards used were peptides and the concentration of the standard peptide solutions was determined by amino acid analysis. This establishes a firm anchor to the amount of substance of the amino acids whilst maintaining specificity to the hGH molecule, hence providing an unbroken link for the measurement results to the SI.

However small differences in the primary structure of hGH may be expected due to differences in the genetic makeup of the person in which the protein resides. These differences may give rise to a different chemical molecule due to the different sequence of amino acids, but since many proteins are identified by function alone, the generic identity of the protein may remain unaltered. In addition, changes to a protein's secondary, tertiary or quaternary structure may be transient, affecting the protein function, and be in itself indicative of a disease state. This means that it is not necessarily the total amount of the protein's primary sequence that is important, but the amount of a protein in a particular structure or folding state. Many of the routine measurement methods used for protein biomarkers are immunoassay based. The specific binding of proteins can be structurally dependent. Therefore, the key challenge for comparability of protein measurement results is not just the "amount of substance", but the "function/activity" of the protein. Both these requirements may need consideration in the future production of commutable reference materials. There is therefore a need to develop an understanding of the effects that protein structure may have on the traceability of measurement results.

Advanced mass spectrometry-based techniques such as hydrogen deuterium exchange experiments and ion mobility coupled to mass spectrometry enable interrogation of protein structure under physiological conditions, thereby providing a measure of the different structural forms of the protein present. During this research a number of different standard protein preparations of recombinant hGH were investigated and using these methods it was possible to identify different hGH structures. Knowing the difference in protein structures, the relative amounts of these structures, and the interaction of these structural forms with the detection antibodies will be an important factor in establishing the suitability of reference materials to standardise measurements.

Impact

The ultimate aim of standardisation in clinical chemistry is to ensure comparability of routine measurements in order to achieve equivalence between laboratories. This research demonstrates the first steps towards developing a biometrology framework that aims to encompass both metrological rigour in developing SI traceability, and the real concerns of the clinical community that measurements must be relevant and independent of the analytical platform used.

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National Measurement System

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