Publishable Summary for 23FUN06 ProMET
Fundamental protein metrology to support the definition of measurands, analytical targets, and their associated measurement uncertainty

Overview

Proteins are dynamic and heterogeneous biomolecules whose accurate quantification is key in sectors such as food, health and biopharma. Unravelling protein structures, their interactions, and the relationship to function are critical for accurate and comparable protein measurements. However, the metrology community has only recently started to address the developments for measurements of the higher-order structures of proteins. In addition to this, the definition of the measurand is complex for heterogeneous molecules such as proteins which are measured by different techniques. This project will investigate overall protein structures and their influence on measurements and protein function. The project will also develop a metrology framework and guidelines to better define the measurands, analytical targets and estimate measurement uncertainty for proteins.

Need

Proteins are complex macromolecules that perform vital biological functions in all living cell processes. Proteins consist of a sequence of building blocks called amino acids. The overall structure can be constituted of a sequence of amino acids (primary level), its folding in alpha helices or beta sheets in space (secondary level), the arrangement of the alpha helices and beta sheets in space (tertiary level) and the aggregation of several sequences (quaternary level). The protein's function is strongly associated with its overall structure and can be affected by structural changes that may result from (i) genetic variations, (ii) alternatively spliced RNA transcripts or (iii) post-translational modifications (PTMs). Thus, any given protein may result (due to structural changes) in a complex mixture of different protein versions named proteoforms.

Protein measurement can be affected by the presence of different proteoforms of the protein and the measurement principles used, which in turn can lead to discrepancies in measurement results. Therefore, reliable and comparable measurements are needed as well as improved confidence in the data for proteins. This information is crucial for life science sectors such as food industry, food safety, health diagnosis, biopharma, and doping control. Given the complexity and heterogeneity of proteins and their structures, one of the main challenges is defining the measurand. This is further complicated by the different techniques used for protein measurement, as these rely on analytical targets that usually differ from the measurand.

The following are needed to address these challenges:

- development of accurate methods for the characterisation of the whole protein structure (primary to quaternary),
- robust evaluation of the impact of protein structure on measurement results,
- improved metrological developments focused on protein primary structure to higher order structure (HOS) (i.e. secondary, tertiary and quaternary structure is often collectively termed as HOS) protein analysis,
- tools to support better definition of the measurand(s) associated to specific proteoform(s).

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METROLOGY PARTNERSHIP EURAMET

Addressing these needs will require a multidisciplinary approach and collaboration between researchers, metrologists, and key stakeholders in the life science sectors in order to improve the accuracy and reliability of protein measurements.

Objectives

The project's overall goal is to develop strategies to better understand the influence of protein structure on protein measurement and function and to establish a metrology framework with a better description of the protein measurand, the analytical targets and their influence on the measurement uncertainty.

The specific objectives are to:

1. To investigate the influence of primary sequence variants and PTMs of proteins, such as glycosylation, phosphorylation, deamidation, oxidation, or glycation on quantitative measurement results. Protein materials of increasing complexity will be characterised by expanding conventional metrological approaches based on mass spectrometry (MS) and nuclear magnetic resonance (NMR).
2. To investigate the influence of the primary sequence and PTMs variants considered in the Objective 1 on the secondary and tertiary structure of the protein and the influence of the presence of different structure variants (from primary to tertiary) on protein-protein interactions. This will be achieved by developing fit-for-purpose strategies using native and structural MS (e.g. ion mobility spectrometry-MS (IM-MS), hydrogen deuterium exchange MS (HDX-MS), chemical cross-linking MS (XL-MS)) combined with other biophysical approaches (e.g. dynamic light scattering (DLS), cryoEM, and NMR) and existing computational techniques to characterise the higher-order structure (HOS) of the proteins to distinguish and quantify different structures and to explore the protein-protein interactions observed in biological systems (e.g. antibody-antigen interactions). Protocols to ensure traceability and estimation of the measurement uncertainties of the results will be developed.
3. To study the impact of structure heterogeneities from objective 1 and 2 on the measurement procedures using either purified peptides, recombinant proteins, extracted proteins or endogenous proteins in simplified buffer or matrix-matched solutions as calibrators. The influence of structure and interactions on isotope dilution-based reference measurement procedures (RMPs), and routine methods using proteomics approaches and immunoassays including sample preparation, calibration and incorporation of isotopically labelled analogues will be investigated.
4. To determine mathematical models based on the results of Objectives 1 to 3 to understand the influence of interferences, protein structure and protein-protein interactions on the different measurement procedures and evaluate their influence on protein function. Approaches to estimate the appropriate measurement uncertainty associated with procedures targeting entities that are not the intended measurand will be developed. Based on the measurement results and the model outcomes, guidelines will be developed for the definition of protein measurands (including appropriate units), of the analytical targets and the estimation of measurement uncertainty for the routine methods and the method developed within the project.
5. To facilitate the take up of the technology and measurement infrastructure developed in the project by the measurement supply chain (NMIs, DIs, research laboratories), research organisations (European Metrology Networks (EMNs) on Traceability in Laboratory Medicine and Safe and Sustainable Food), standards developing organisations (International Federation of Clinical Chemistry and Laboratory Medicine (IFCC), ISO and Codex Alimentarius.) and end users (biopharma, biomedicine, food producers, academic laboratories and clinical diagnostic laboratories).

Progress beyond the state of the art and results

A proven way towards reliable and comparable results is to establish the results' metrological traceability to the international system of units (SI). The metrology community is currently developing tools, such as RMPs for the purity assessment of peptide and protein materials in order to produce reference materials (RMs). However, to date, most of these RMPs determine the protein content based only on the proteins primary structure and protein modifications are not addressed.

This project will advance the state-of-the-art by:

- developing cutting-edge analytical approaches, mainly using MS, and a new metrological framework for the whole structure characterisation of proteins (i.e. primary, secondary, tertiary and quaternary) in a pure solution material,
- extending the knowledge on the influence of the primary structure and HOS on protein measurements.

Characterisation of the primary structure: sequence variants and PTMs (Objective 1)

Metrological purity assessment has been applied to peptide materials with ≤ 30 amino acids. Quantifying the target peptide is currently well established, however challenges arise in identifying and quantifying impurities. Further to this, the move from peptides assessments to proteins represents additional challenges due to protein size, complexity, and the potential number, size and structure of the impurity present in pure samples.

This project will go beyond the state of the art by developing alternative methods to the widely used but costly and time-consuming isotopic dilution MS (IDMS) method for impurity quantification. Alternative methods that will be investigated include label-free, relative quantification, and standard addition. The project will also evaluate new technologies such as mass photometry, as well as addressing the lack of consensus and guidelines for modified protein measurement (glycoproteins).

Characterisation of the HOS and influence from the primary structure (Objective 2)

MS has emerged as a complementary technique to the traditional protein 3D structure analysis. However, analytical performances of MS for protein structural analysis still need to be documented. This project will go beyond the state of the art by establishing reference protocols for protein measurements that have an estimation of measurement uncertainty. This project will do this to improve the reproducibility and bias of cutting-edge IM-MS and HDX-MS techniques. In addition, where existing software contributes to measurement variability, the project will develop machine learning methods that can detect conformational changes in proteins, in order to overcome this measurement variability.

Characterisation of the protein-protein interactions and influence from the protein structure (Objective 3)

The project will establish a metrological framework for HDX-MS and promote its use for characterising protein-protein interaction. In addition, the project will progress XL-MS measurements beyond the current state of the art towards quantitative methods for differentiating protein conformational states. These advances will then be validated through an interlaboratory comparison.

Influence of protein structure, and interactions on protein measurement and protein function and modelling for measurand definition and uncertainty estimation (Objective 4)

Using input from Objectives 1 to 3 and the development of innovative in vitro assays for determining protein function, this project will advance the state-of-the-art by developing mathematical correlations between protein structure and protein function. This integrative and holistic approach will combine multiple techniques with a metrology framework to enable estimation of the measurement uncertainty. These results can then be used to address the fundamental question of how the quantity of a protein can be best described. The results will also be used to develop guidelines to better define protein measurands, analytical targets and how to estimate measurement uncertainty.

Outcomes and impact

Outcomes for industrial and other user communities

This project will support the industrial end-users to:

- refine and target relevant measurands and analytical entities for protein analysis,
- implement metrological traceability for protein measurements and derive their measurement uncertainty,

- improve the quality of their products (e.g. new measurement technologies or instruments, highly characterised materials).

The project will develop analytical and metrological tools for protein analysis, particularly new RMPs for protein characterisation, and will produce guidelines to support the definition of protein measurands, analytical targets, and their associated measurement uncertainty.

The manufacturers of quality control (QC) / RMs or new protein drugs will particularly benefit from this project's SI traceable metrology framework for the purity assessment of proteins. The project's accurate and improved methods will also be a significant step forward for biopharmaceutical industries as they need the ability to control and validate bioproducts throughout development and manufacturing. Furthermore, through the biopharma industry the project will provide input and support to the currently used guidelines e.g. the International Council for Harmonisation (ICH) Q6B Specifications: test procedures and acceptance criteria for biotechnological/biological products - Scientific guideline.

The lack of suitable primary calibrators traceable to the SI is one of the main obstacles for the food and clinical industries to achieve comparable and reliable results. Providing full SI traceability for protein analysis, including a better understanding of the influence of biological and structural variations on protein measurements, will enable industries to improve their methods and provide clearly defined calibration materials to the end-users.

The project will engage with stakeholders from such industrial and biopharma communities via the setup of a stakeholder network. The stakeholder network will be used to gain feedback on the project and to help maximise impact (project promotion) and to facilitate the uptake of the project's outputs.

The results of the project will also be provided to EURAMET's EMNs on Traceability in Laboratory Medicine and on Safe and Sustainable Food, so that they can be promoted widely to their stakeholders in the Health and Food industries.

Outcomes for the metrology and scientific communities

The expected developments from this project (e.g. the characterisation of the protein structure in biological systems, purity assessment of large and modified proteins, and the evaluation of new calibration strategies for RMPs) are align with the current work of the Protein Analysis Working Group (PAWG) of the Consultative Committee for Amount of Substance (CCQM). Thus, the project outputs will be disseminated to the PAWG in order to support the expansion of NMIs/DIs capabilities, particularly for protein HOS analysis, and paving the way towards new CCQM comparisons on protein structural analysis.

The project's knowledge of the influence of the protein structures on the protein measurement results, will be used to develop guidelines including recommendations to support the definition of protein measurands, analytical targets, and their associated measurement uncertainty based on verified models. These good practice guidelines will be made available to the scientific community via the project website, the participant's websites and a copy of them will be available in an open access repository. The good practice guidelines will enable the better definition of protein measurands and help to improve RMPs targeting the relevant form(s) of the protein.

During the project, close ties to the EMN on Traceability in Laboratory Medicine and the EMN on Safe and Sustainable Food will be sought so that the outcome of this project can be used in outlining roadmaps and updating the EMN's existing strategic research agendas documents.

Further to this, the project will engage with the metrology and scientific communities via the publication of joint research papers in high impact peer-reviewed journals, through the presentation of the project at conferences and as part of knowledge transfer the project will host three educational workshops, secondments for the consortia and at least two training courses for postgraduates.

Outcomes for relevant standards

By developing a metrology framework with a better description of the protein measurand and strategies to better understand the influence of protein structure on measurement and activity, this project will support end-users to meet regulations and standards (e.g. European Medicines Agency (EMA), U.S. Food and Drug Administration (FDA), French National Agency for the Safety of Medicines and Health Products (ANSM), ISO 17511:2020, European Regulation (EU) 2017/746, European Regulation (EU) No 1169/2011 or ICH) Q6B specifications).

This project will also provide input to ISO standards under revision via ISO/TC 212 WG2 Clinical laboratory testing and in vitro diagnostic test systems - reference systems, which is responsible for ISO 15193 (RMPs) and 15194 (RMs) which are both currently under revision. The project will also disseminate it's outputs via technical committees ISO/TC 212 WG3 Clinical laboratory testing and in vitro diagnostic test systems - In vitro diagnostic products that is responsible for ISO 17511, ISO/TC 276 Biotechnology, ISO/TC 229 Nanotechnologies, ISO/TC 334 Reference Materials, ISO/TC 194 Biological and clinical evaluation of medical devices, AFNOR S94C Clinical Laboratory Testing and In Vitro Diagnostic (IVDs), EURAMET Technical Committee of Metrology in Chemistry (TC-MC), International Federation of Clinical Chemistry and Laboratory Medicine (IFCC) Scientific Division (SD), IFCC WG-APOMS (Apolipoproteins by Mass Spectrometry), IFCC WG-BND (Biomarkers of Neurodegenerative Diseases), IFCC WG-PCT (Procalcitonin), IFCC WG-hGH (human Growth Hormone), IFCC WG-HbA2 (Haemoglobin A2), IFCC WG-TNI (cardiac troponin I), IFCC WG-NP (Natriuretic Peptides), IFCC C-BM (Bone markers), Joint Committee for Traceability in Laboratory Medicine (JCTLM) Working Group for Traceability: Education and Promotion (WG-TEP), JCTLM Task Force on Reference Measurement System Implementation (JCTLM-TF-RMSI), JCTLM Task Force on Nomenclature, Clinical and Laboratory Standards Institute (CLSI) CLSI EP32 on Metrological Traceability and Its Implementation, Versailles Project on Advanced Materials and Standards (VAMAS) TWA-40 Synthetic Biomaterials.

Longer-term economic, social and environmental impacts

In the longer-term the uptake of the methodologies, protocols and guidelines from this project should impact stakeholders from the health, food, biopharma, diagnostic or environmental sectors, and will further improve the health and safety of consumers and citizen, in Europe and beyond. This project will impact European citizens via improved patient health. Although proteoforms can provide valuable information about patient health, their clinical analysis has not yet received much attention, as methods to identify and quantify them accurately are lacking. This project will expand the knowledge of proteoforms and, thus, support manufacturers to refine existing diagnostic or prognostic tests. In addition, this project should contribute to developing new specific tests (precision medicine), to complementing existing tests or to helping to fill in the gaps at an early and curable stage. An improvement in measurement quality should also minimise false negative or false positive results and, thus, help to prevent the repetition of analyses. Which in turn should reduce costs for patients and the healthcare system.

The long-term uptake of the project's analytical and metrological tools for the structural analysis of proteins and their purity assessment will support better reliability and comparability of protein measurements in biopharma. The implementation of characterisation methods in biopharma should also enable quicker and more reliable identification of issues in biomanufacturing (e.g. the presence of impurities causing adverse effects), thus saving time, resources and costs. This will support better access to therapies at lower costs.

This project will help fill the current gap in the availability of primary calibrators traceable to the SI for accurate allergen detection and biotoxin quantification in the food sector. Furthermore, the availability of reliable methods will help to establish higher quality, robust data in both industrial and fraud control laboratories and support well-informed choices for establishing safety thresholds. All this will help to improve the safety of the food chain and increase consumer protection.

In the fight against doping, this project's outputs will benefit the development of new materials for calibrating doping assays and will support the structural characterisation of different sources of the same doping substance. The advanced characterisation tools that this project will provide will support doping laboratories to demonstrate their ability to meet criteria validated by the Anti-Doping Agencies.

Finally, in the environment, protein analysis is mainly done for total protein content or identification. In line with the One-Health concept supported by Europe, this project will support long-term environmental applications with procedures to identify and quantify proteins reliably.

List of publications

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Project start date and duration:	1 September 2024, 36 months	
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