European Partnership on Metrology
Call 2023 –Fundamental Metrology, Industry, Normative and Research Potential

Selected Research Topic number: **SRT-f11**
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Title: Fundamental protein metrology to support the definition of measurands, analytical targets, and their associated measurement uncertainty

Abstract

Defining the measurands for a protein is not trivial due to their complexity, heterogeneity and biological variations. What biologists, clinicians or analysts refer to as the same protein may vary depending upon its primary to quaternary structure. The NMI community have only recently started to address issues related to higher-order protein structures. However, unravelling the structure and its relationship to its function is key to improved protein measurements. This project aims at investigating the protein structure and its influence on measurements and activity. It will provide guidelines to support a better definition of the measurands and analytical targets and an estimation of measurement uncertainty related to measurement procedures.

Keywords

Protein measurand definition, protein quantification, protein homogeneity, protein dynamics, structural

Background to the Metrological Challenges

Proteins are dynamic, complex macromolecules comprised of a sequence of building blocks called amino acids. This sequence constitutes the primary structure of the protein (polypeptide) and drives the three-dimensional folding into alpha helices and beta sheets composing the secondary structure, and how these are arranged determines the tertiary structure. Finally, if composed of two or more polypeptide chains this forms the quaternary structure of the protein.

The accurate detection and quantification of proteins is important in almost every aspect of our daily lives. In the food safety sector, 13 of the 14 priority food ingredients known to trigger allergic responses are related to their protein content. As the avoidance of the specific food ingredients is the only way to avoid an allergic reaction, the accurate identification, detection and quantification of allergens in final products is essential. In the health sector, clinical biomarkers for the prognosis, diagnosis, and monitoring of diseases are often proteins. Numerous assays, mainly immunoassays, with different measurement principles have been developed to detect or quantify proteins. To support healthcare and safety the accurate detection and quantification of proteins and the comparability of measurements in biological matrices are crucial. In biopharma protein measurements are essential for the development of new biopharmaceutical products such as monoclonal antibodies. The characterisation campaign of reference material (RM) for use as a test material for monoclonal antibodies in industry showed challenges remained relating to the thorough characterisation of complex molecules. For biomedicine identification, quantification and structural modifications of host cell proteins is important, as these can affect the activity and could negatively affect the health of the patients by causing unwanted side effects. The impact of a protein's structure on its activity is also important for enzymes that are widely used in industry to speed up production and processes due to their catalytic activity.

Several initiatives have been conducted by NMIs/DIs to develop metrological references for the quantification of proteins in complex matrices, such as higher-order reference measurement procedures (RMPs) or certified RMs. However, RMPs usually determine protein content based on the primary protein sequence and biological variations, such as post-translational modifications (PTMs) or secondary to quaternary structural changes are not considered. This can affect methods such as immunoassays as structural changes may lead to different binding to the antibodies or impact the protein activity. Therefore, improved methods are needed to distinguish and quantify the different protein structures.

EMPIR projects (18HLT03 SEPTIMET, 18HLT09 NeuroMET2, 18HLT10 CardioMet) and interlaboratory comparisons highlighted discrepancies between mass spectrometry (MS) based RMPs and immunoassay

methods, raising the question of whether they actually measure the same compound(s). A workshop organised by the JCTLM (Joint Committee for Traceability in Laboratory Medicine**)** and IFCC (International Federation of Clinical Chemistry and Laboratory Medicine), in December 2022 highlighted the metrological challenges associated with measuring the pentameric C-reactive protein. The question of how best to measure such protein complexes and molecular assemblies, and provide traceability routes to the SI, is of fundamental importance to gain the benefits associated with long-term, comparable measurement results.

Another fundamental question raised with the above examples is the definition of the measurand (namely, the quantity intended to be measured). For proteins, this definition depends on protein size, dynamics, heterogeneity and is complicated by post-translational and processing modifications that occur in vivo, during sampling and/or caused by the measurement procedure itself. There is a strong requirement for more accurate techniques to measure different protein structures and to identify the uncertainty contributions when converting from the measured target to the measurand.

Approaches have been developed to characterise proteins by NMIs/DIs. However, these have been mainly applied to peptides or small proteins and need to be extended to the characterisation of more complex proteins presenting sequence variants and PTMs. Recent advances in MS technologies and bioinformatics have improved the robustness of protein structural analyses, providing structural and dynamic information with high sensitivity for proteins and antibodies (large proteins) characterisation but these still lack measurement uncertainties. Up to now, calibration strategies based on synthetic peptides or recombinant proteins have been used for these methods, but the ability of the calibrant to be representative of the endogenous protein and to fit with the definition of the measurand needs to be evaluated.

Finally, the unit of the measured quantity used to describe heterogeneous and dynamic proteins has yet to be agreed. When dealing with the quantification of a protein, the results can be expressed in mass or as an amount of substance. When dealing with biological activity or effect, international unit (IU) is broadly utilised. In addition, dimension, stoichiometry, and time may be critical in defining the appropriate measurand when addressing diverse analytical and biological questions.

Objectives

Proposers should address the objectives stated below, which are based on the PRT submissions. Proposers may identify amendments to the objectives or choose to address a subset of them in order to maximise the overall impact, or address budgetary or scientific / technical constraints, but the reasons for this should be clearly stated in the protocol.

The JRP shall focus on fundamental protein metrology to support the definition of measurands, analytical targets, and their associated measurement uncertainty

The specific objectives are

1. To investigate the influence of primary sequence variants and post-translational modifications (PTMs), such as glycosylation, phosphorylation, deamidation, oxidation, or glycation on protein 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).. The impact of heterogeneities on the calibration of the measurement procedures using either purified peptides, recombinant proteins, extracted proteins or endogenous proteins in simplified buffer or matrix-matched solutions and the selection of the best ways to calibrate will be studied.
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 to develop methods to distinguish and quantify the different structures. This will be achieved by developing fit-for-purpose strategies using native and structural MS (e.g. ion mobility spectrometry-MS, hydrogen deuterium exchange, chemical crosslinking) combined with other biophysical approaches (e.g., dynamic light scattering, cryoEM, NMR) and existing computational techniques to characterise the higher-order structure of the proteins. Protocols to ensure traceability and estimation of the measurement uncertainties of the results will be developed.
3. To explore the protein-protein interactions observed in biological systems such as antibody-antigen interactions and the influence of output from Objectives 1 and 2 on these interactions. The influence of interactions on isotope dilution-based reference measurement procedures and routine methods using proteomics approaches and immunoassays will be investigated.
4. To develop models based on all 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 activity. 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 on Traceability in Laboratory Medicine and Safe and Sustainable Food), standards developing organisations (IFCC), CEN, and Codex Alimentarius.) and end users (biopharma, biomedicine, food producers, academic laboratories and clinical diagnostic laboratories).

These objectives will require large-scale approaches that are beyond the capabilities of single National Metrology Institutes and Designated Institutes. Proposers shall give priority to work that aims at excellent science exploring new techniques or methods for metrology and novel primary measurement standards and brings together the best scientists in Europe and beyond, including other European Partnerships, whilst exploiting the unique capabilities of the National Metrology Institutes and Designated Institutes.

Proposers should establish the current state of the art and explain how their proposed project goes beyond this. In particular, proposers should outline the achievements of the 18HLT03 SEPTIMET, 18HLT09 NeuroMET2, 18HLT10 CardioMet and how their proposal will build on those.

EURAMET expects the average EU Contribution for the selected JRPs in this TP to be 2.0 M€ and has defined an upper limit of 2.5 M€ for this project.

EURAMET also expects the EU Contribution to the external funded beneficiaries to not exceed 40 % of the total EU Contribution across all selected projects in this TP.

Any industrial beneficiaries that will receive significant benefit from the results of the proposed project are expected to be beneficiaries without receiving funding or associated partners.

Potential Impact

Proposals must demonstrate adequate and appropriate participation/links to the 'end user' community, describing how the project partners will engage with relevant communities during the project to facilitate knowledge transfer and accelerate the uptake of project outputs. Evidence of support from the "end user" community (e.g. letters of support) is also encouraged.

You should detail how your JRP results are going to:

- Address the SRT objectives and deliver solutions to the documented needs,
- Feed into the development of urgent documentary standards through appropriate standards bodies,
- Facilitate improved industrial capability or improved quality of life for European citizens in terms of personal health, protection of the environment and the climate, or energy security,
- Transfer knowledge to the protein characterisation sector.

You should detail other impacts of your proposed JRP as specified in the document "Guide 4: Writing Joint Research Projects (JRPs)"

You should also detail how your approach to realising the objectives will further the aim of the Partnership to develop a coherent approach at the European level in the field of metrology and include the best available contributions from across the metrology community. Specifically, the opportunities for:

- improvement of the efficiency of use of available resources to better meet metrological needs and to assure the traceability of national standards
- the metrology capacity of EURAMET Member States whose metrology programmes are at an early stage of development to be increased
- organisations other than NMIs and DIs to be involved in the work.

Time-scale

The project should be of up to 3 years duration.