EMPIR Call 2020 – Fundamental, Industry and Normative

Selected Research Topic number: **SRT-f06**
Version: 1.0

Title: Fundamental metrology for proteins

Abstract

The accurate quantification of proteins is important in food, biopharma and health sectors but protein characterisation is hampered due to factors such as protein heterogeneity, instability and dependency on the biologically relevant environment. A lack of certified reference materials along with a lack of clarity regarding the appropriate measurand also complicates such measurements. A coordinated, interdisciplinary approach across physics, chemistry and biology is needed to provide the SI traceable measurement methods for purity determination of heterogenous proteins and protein 3D structure. This is required to boost emerging areas such as biotechnology, therapeutics and regenerative medicines and ultimately accelerate their implementation, which is the purpose of this proposal.

Keywords

Identifying the measurand, protein heterogeneity, purity, protein higher order structural analysis, International System of Units, measurement uncertainty

Background to the Metrological Challenges

Protein measurements underpin many fast-growing sectors including healthcare, biotechnology, and food. 75 % of clinical biomarkers are complex biological molecules of which the majority are proteins; most food allergens are proteins; 50 % of pharmaceuticals currently under development are proteins. The pace of discovery via macromolecular measurements vastly exceeds the ability of NMIs to provide a route to traceability in supporting these important biological discoveries. The workings of molecular machines and advances in synthetic biology require dimensional, counting, amount-of-substance and time (rate of interaction & protein turnover) measurements to be made at the molecular level and ideally in biologically relevant environments. Overall, the lack of higher order reference methods and materials that cover the challenges of protein measurements is the major hindrance for deriving traceability and comparability in bio-measurements. This has a major impact upon accreditation (e.g. ISO 17025) and regulatory compliance including, for example: Regulation (EU) 2017/746r on *in vitro* diagnostic medical devices and their traceability requirements; and Regulation (EU) No 1169/2011 on the provision of food information to consumers.

Traceability to the SI is a recognised way to achieve global comparability of measurements but different SI units can be used to describe proteins and other International Units (IU, designated by WHO) are broadly utilised to describe biological materials. However, dimensional, stoichiometry and time (interaction) are often as critical in defining the appropriate protein measurand when addressing diverse analytical and biological questions. Those span from quantification of the primary structure of protein calibrants, to detection and quantification of aggregates in bio-products, to monitoring progression of important diseases such as neurodegeneration. In addition, a single unit is often given for a biological measurement when in fact it may require more than one measurement parameter and, therefore, a more holistic approach is required

Several requirements for protein measurements were identified in 2011 that include (i) workflows to assure the traceability of quantitative measurements of proteins to the SI; (ii) approaches involving metrological principles where traceability to the SI is unlikely to be feasible due to protein complexity; (iii) traceability chains for inherently unstable proteins.

Little work has been carried out to date by the international measurement community to build the capabilities required to monitor the higher order structure of proteins, workflows to discriminate measurement variability *vs* structural changes and to develop reference materials in this space. Fundamental research and the development of conventional and innovative metrological approaches is required to better describe proteins and their complexity. Those include a better definition of the measurand, the use of appropriate SI units and

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description measurement uncertainty. The current metrological approaches cannot provide traceability to the SI for complex molecules.

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 the traceable measurement and characterisation of purity and 3D structure of heterogenous proteins along with associated measurement uncertainties.

The specific objectives are

1. To determine the relevant physio-chemical characteristics of at least three modified, heterogeneous proteins of increasing complexity to define their purity. This will be done by developing and applying multidisciplinary and complementary analytical approaches alongside conventional mass balances.
2. To define the measurement uncertainty and appropriate measurand for the higher order structure (HOS) of proteins from objective 1 using organic and physical methods. In addition, reference protocols will be developed to determine structural changes *vs* measurement variability allowing the calculation of the sensitivity of HOS methods to structural changes. Guidelines for method validation and enable measurement comparability will also be produced.
3. To quantify protein HOS using the most promising organic and physical methods from objective 2. This data will be combined with results from objectives 1 and 2 to determine the correlation between measurement results in SI (System of International Units) and IU (International Units).
4. To define the key parameters affecting protein function using computational methods to predict the HOS of proteins such as biopharmaceuticals or clinical biomarkers with and without post-translational modifications and in different suitable solvents. This data will be compared with data from objectives 1, 2 and 3 and used to develop validated mathematical models to predict stability, functionality and potentially also commutability of reference materials.
5. To facilitate the take up of the technology and measurement infrastructure as well as the mathematical model developed in the project by the measurement supply chain (NMIs, DIs, European Metrology Networks), standards developing organisations (e.g. ISO, IFCC - Scientific Division, Eurachem) and end users (e.g. pharmaceutical sector, regenerative medicines and neurodegeneration research).

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, 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 EMRP project SIB54 BioSItrace and how their proposal will build on those.

EURAMET expects the average EU Contribution for the selected JRPs in this TP to be 1.5 M€, and has defined an upper limit of 1.8 M€ for this project.

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

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,
- Transfer knowledge to the healthcare, biotechnology, and food sectors.

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 EMPIR 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.

Additional information

The references were provided by PRT submitters; proposers should therefore establish the relevance of any references.

[1] The Strategic Research Agenda for Metrology in Europe (Version 1.0 (03/2016))