

Government Chemist Conference
Safe food for tomorrow's world

23-24 June 2021
Virtual

@NML_ChemBioGC
#GCconf2021
www.gov.uk/governmentchemist



Government Chemist

safe
authentic
sustainable | food



Dr. Milena Quaglia

Principal Scientist Protein Metrology

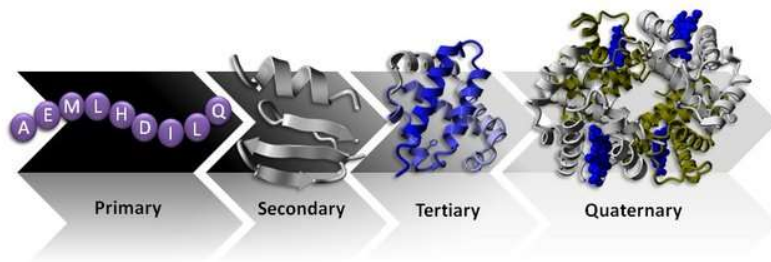
**Challenges in the
standardisation of protein
measurements for clinical
and food applications**

24 June 2021

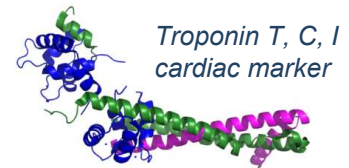


The obvious...

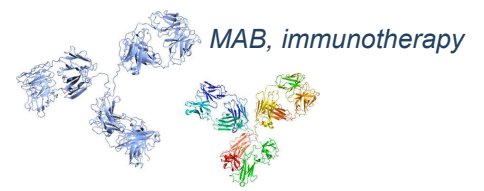
- What is a protein?



Clinical biomarkers



Bio-therapeutics



Meat authentication

Biomarkers_food health

Food

Food allergens

Pathogens and microbial contamination

Biomarkers_animal and plant health



Protein measurements



- **Total protein**

- Kjeldahl method
- Dumas
- UV-spectroscopy
- Biuret method
- Bradford Coomassie Blue
- Fluorescence Dye
- Amino acid analysis

- **Amount of target protein**

- Antibody based assays
(immunoassays)
- Mass spectrometry
followed proteolytic
digestion

*Mass as
mg/g;
mg/L;
mols/L.....*

- **Amount of target protein (activity)** *IU*

- **Analysis of higher order structure**

- Circular dichroism *mA*
- Fourier transform infrared *Hz*
- NMR *k*
- Thermal stability measurements (DSC, OtpimTM) *m*
- Raman *s* *v*
- Capillary electrophoresis
- Mass Spectrometry (HDX-MS, IMS-MS crosslinking-MS).....



Standardising of protein measurements to support legislation: challenges



- Why is important?
- How can be achieved?
- Where we are now and what can be achieved?
- What next: our program to expand protein measurement standardisation and describe a protein as a dynamic entity



Measurement standardisation



space...



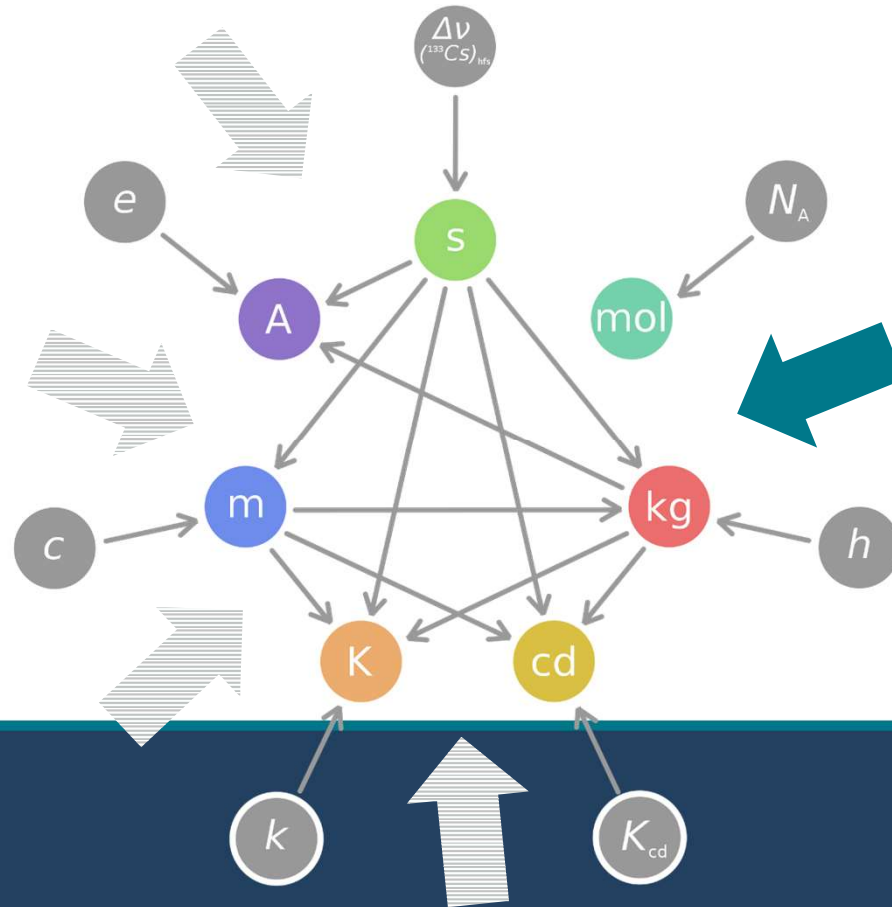
time...



Measurement standardisation through metrological traceability to the System of International Units



Measurement standardisation: the SI Units



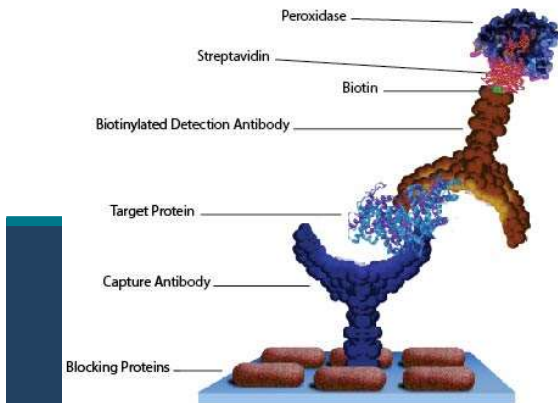
Metrological traceability



.....is defined as the 'property of a measurement result whereby the result can be related to a reference through a documented unbroken chain of calibrations, each contributing to the measurement uncertainty" VIM3, 27

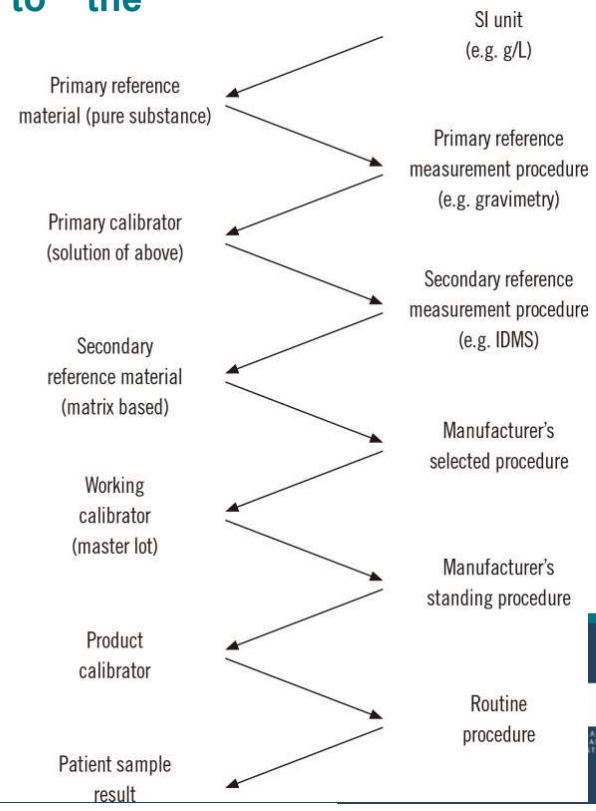
Primary reference material for proteins?
Source, properties, stability, purity.....

Routine measurements and clinical thresholds are often defined by immunoassays

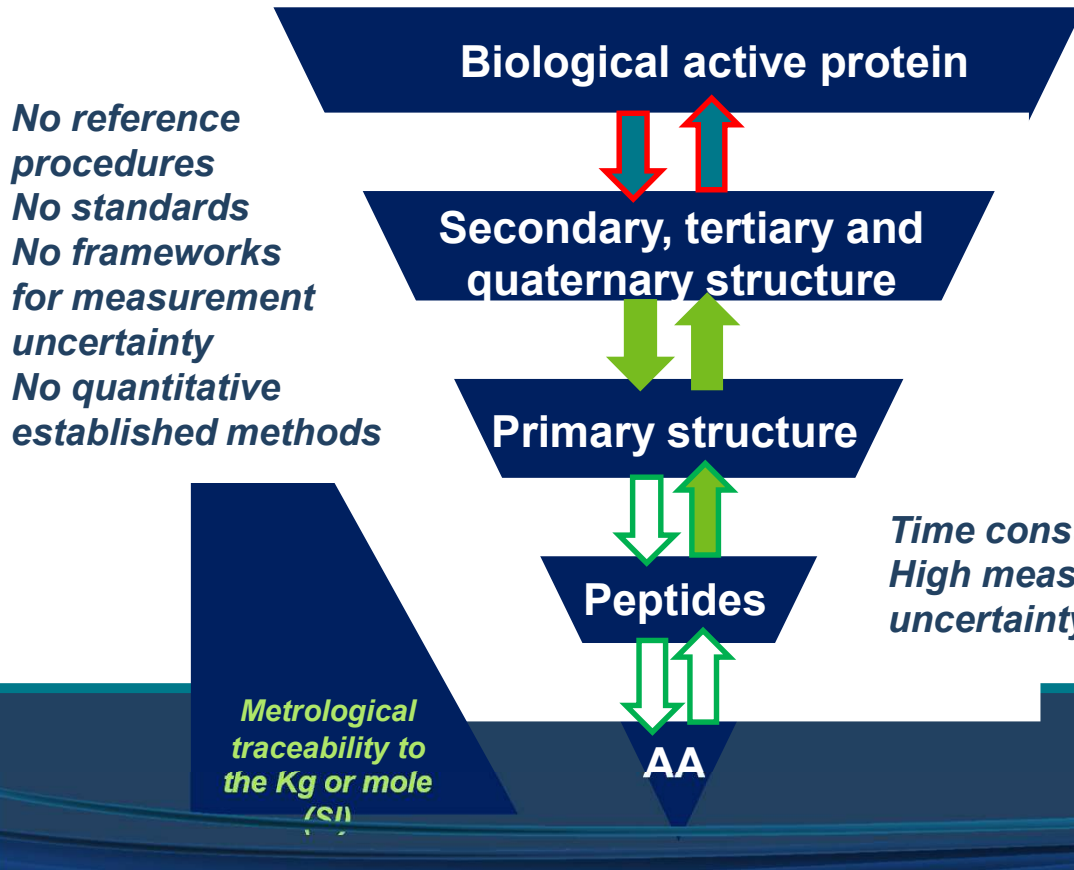


Proteins are not just chemical entities

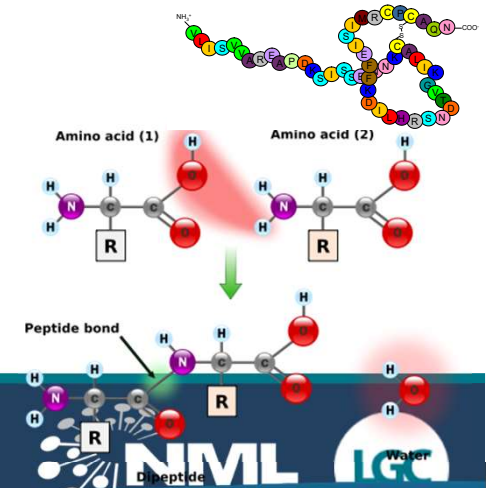
Patient result traceable to higher order reference components



Where are we now....



“What is the measurand?”
“Is one target analyte enough?”



Successful example from clinical community



DOI: 10.1002/alz.12145

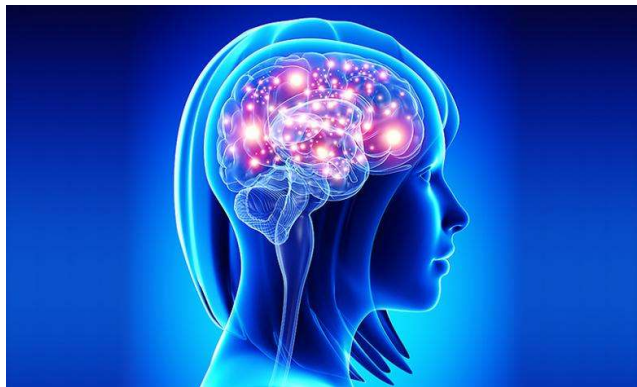
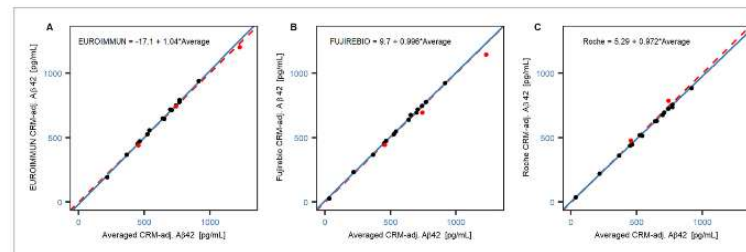
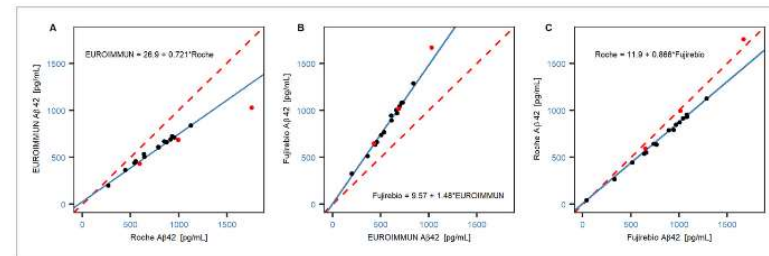
FEATURED ARTICLE

alzheimer's
association

Alzheimer's & Dementia®
THE JOURNAL OF THE ALZHEIMER'S ASSOCIATION

First amyloid β 1-42 certified reference material for re-calibrating commercial immunoassays

Sébastien Boulo¹ | Julia Kuhlmann¹ | Ulf Andreasson^{2,3} | Britta Brix⁴ |
Iswariya Venkataraman⁵ | Victor Herbst⁴ | Sandra Rutz⁶ | Ekaterina Manuilova⁶ |
Manu Vandijck⁷ | Filip Dekeyser⁷ | Maria Bjerke^{2,8} | Josef Pannee^{2,3} |
Jean Charoud-Got¹ | Guy Auclair¹ | Stéphane Mazoua¹ | Gregor Pinski¹ |
Stefanie Trapmann¹ | Heinz Schimmel¹ | Hendrik Emons¹ | Milena Quaglia⁹ |
Erik Portelius^{2,3} | Magdalena Korecka¹⁰ | Leslie M. Shaw¹⁰ | Mary Lane¹¹ |
Erin Chambers¹¹ | Hugo Vanderstichele^{12,13} | Erik Stoops¹² | Andreas Leinen
Tobias Bittner¹⁴ | Rand G. Jenkins¹⁵ | Vesna Kostanjevecki⁷ | Piotr Lewczuk¹
Johan Gobom^{2,3} | Henrik Zetterberg^{2,3,18,19} | Ingrid Zegers¹ | Kaj Blennow^{2,3}



Clear measurand
Standardisation carried out alongside
establishment of thresholds



There are example....



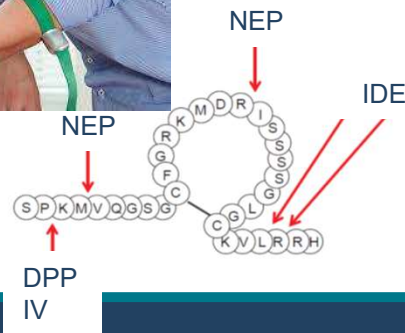
DE GRUYTER

Clin Chem Lab Med 2017; 55(9): 1397–1406

Open Access

Attila F. Torma, Kate Groves, Sabine Biesenbruch, Chris Mussell, Alan Reid, Steve Ellison, Rainer Cramer and Milena Quaglia*

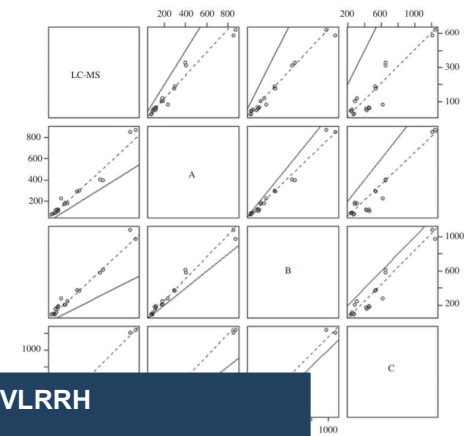
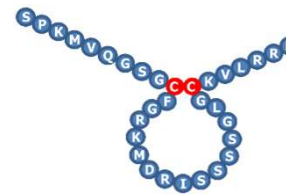
A candidate liquid chromatography mass spectrometry reference method for the quantification of the cardiac marker 1-32 B-type



32 amino acids

20min life time

Recombinant or synthetic BNP used to calibrate assays



BNP(1-32) SPKMQGSGCFGRKMDRISSSSGLGCKVLRH

BNP(3-32) KMVQGSGCFGRKMDRISSSSGLGCKVLRH

BNP(4-32) MVQGSGCFGRKMDRISSSSGLGCKVLRH

BNP(5-32) VQGSGCFGRKMDRISSSSGLGCKVLRH

BNP(5-31) VQGSGCFGRKMDRISSSSGLGCKVLR

BNP(5-27) VQGSGCFGRKMDRISSSSGLGCK

BNP(5-26) VQGSGCFGRKMDRISSSSGLGC

What is the measurand?
Is the target analyte appropriate?



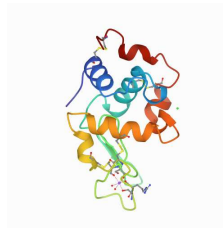
Food allergens RMP



1350 CRYAR ET AL.: JOURNAL OF AOAC INTERNATIONAL Vol. 96, No. 6, 2013

FOOD COMPOSITION AND ADDITIVES

Towards Absolute Quantification of Allergenic Proteins in Food—Lysozyme in Wine as a Model System for Metrologically Traceable Mass Spectrometric Methods and Certified Reference Materials



Food allergens RMP

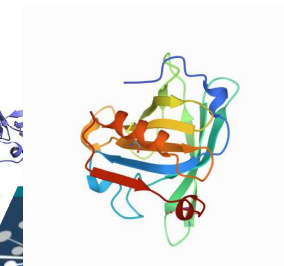
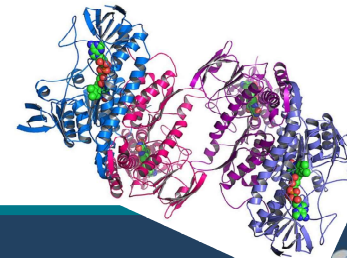
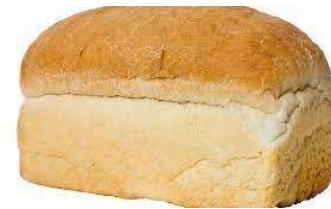


Measurand

Correlation target analyte to measurand

Extraction/recovery

Food processing effect on allergenicity



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HOSTED AT LGC

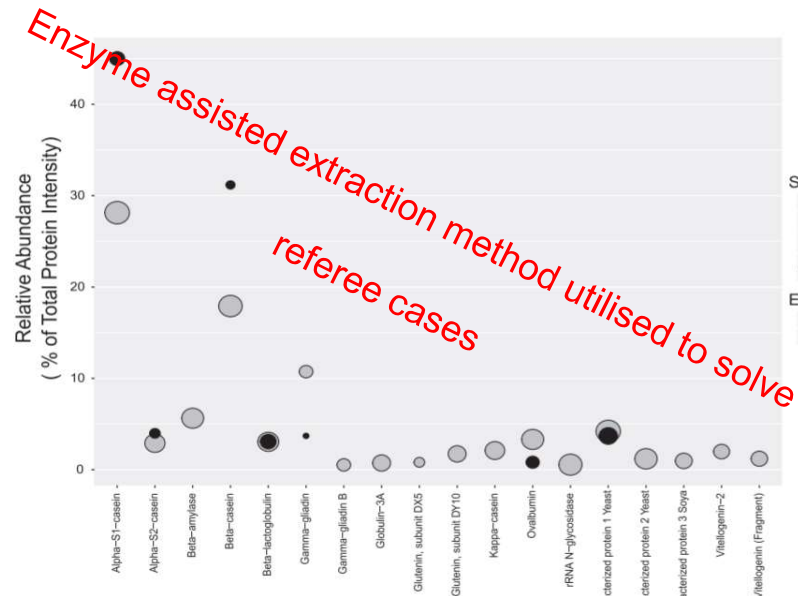


Progress.....

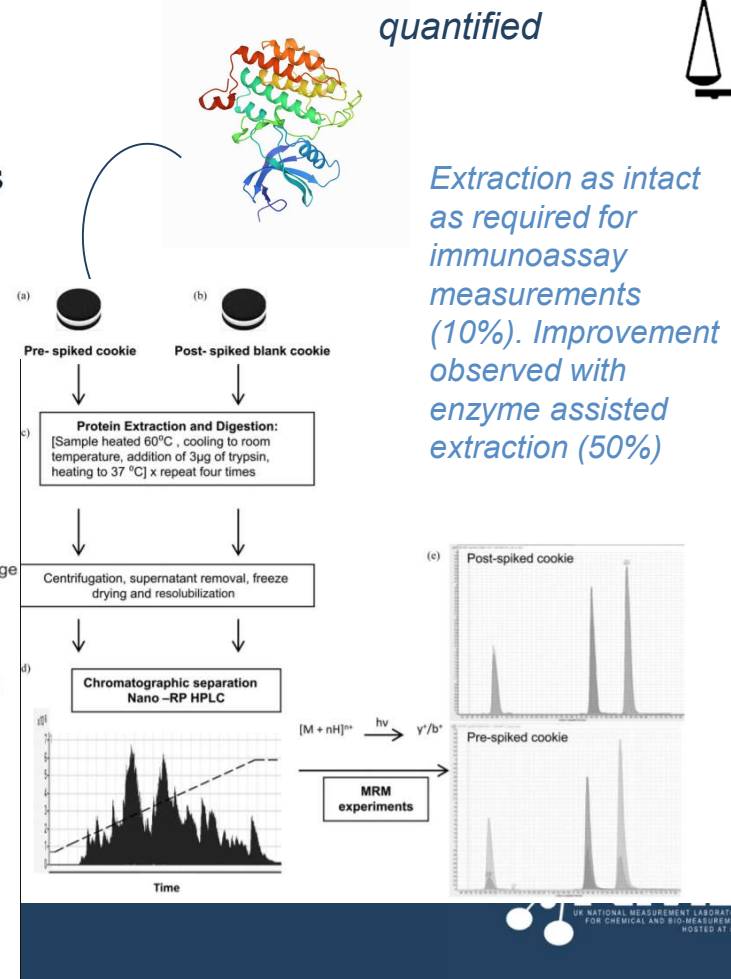
> J AOAC Int. 2018 Jan 1;101(1):152-161. doi: 10.5740/jaoacint.17-0214. Epub 2017 Dec 5.

Assessment of Recovery of Milk Protein Allergens from Processed Food for Mass Spectrometry Quantification

Kate Groves¹, Adam Cryar¹, Michael Walker¹, Milena Quaglia¹



SI traceable
quantified



Extraction protocol for Referee Cases



Almonds in cumin and paprika?



Cow milk in coconut sweets?



Progress.....



Paper in Forefront | [Open Access](#) | Published: 02 October 2020

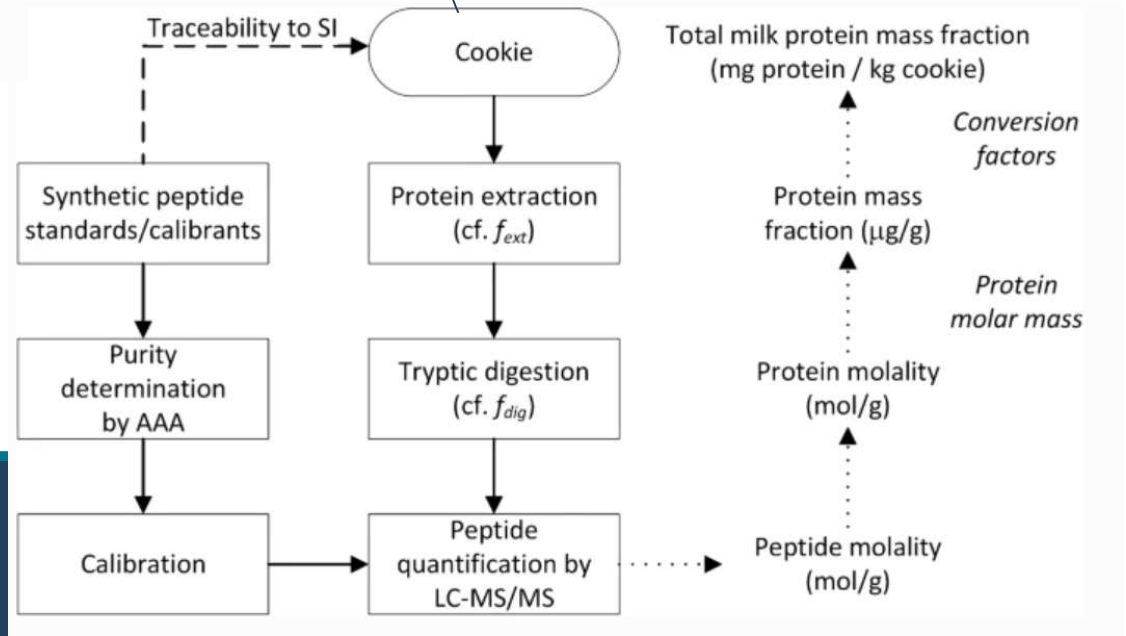
A reference method for determining the total allergenic protein content in a processed food: the case of milk in cookies as proof of concept

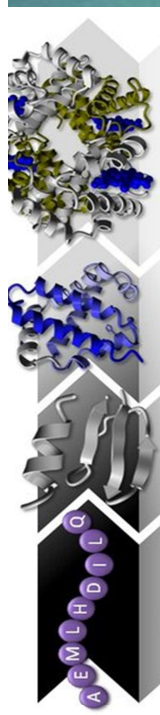
[Maria José Martínez-Esteso](#), [Gavin O'Connor](#), [Jørgen Nørgaard](#), [Andreas Breidbach](#), [Marcel Brohé](#), [Elena Cubero-Leon](#), [Chiara Nitride](#), [Piotr Robouch](#) & [Hendrik Emons](#) ✉

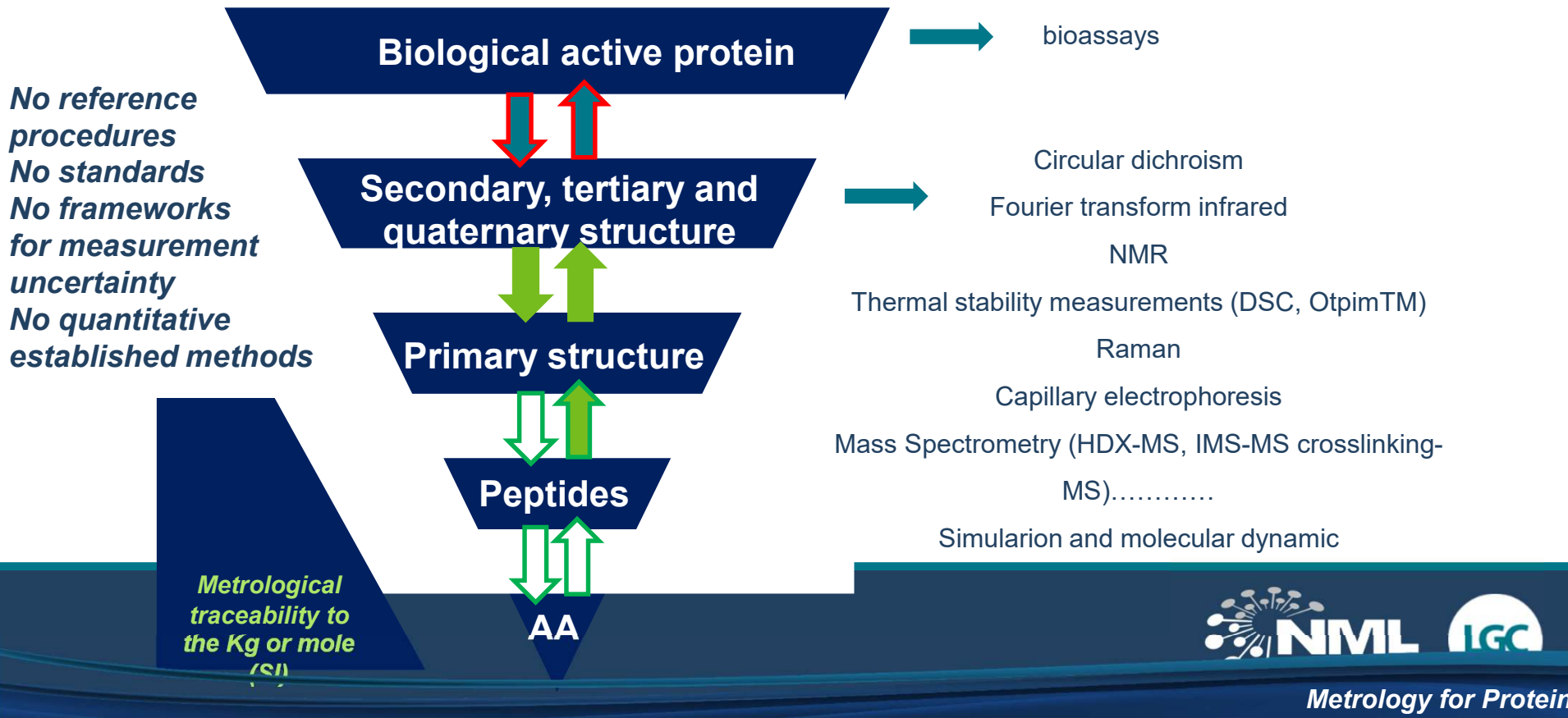
[Analytical and Bioanalytical Chemistry](#) **412**, 8249–8267 (2020) | [Cite this article](#)

1520 Accesses | 1 Citations | 4 Altmetric | [Metrics](#)

Can we apply those concepts and SI traceably quantify currently available CRM?









Protein Structure Plays a Critical Role in Peanut Allergen Stability and May Determine Immunodominant IgE-Binding Epitopes

Editorial: Protein Quality Controlling Systems Plant Responses to Environmental Stresses

 [Minghui Lu](#)^{1*},  [Hanjo A. Hellmann](#)^{2*},  [Yule Liu](#)^{3*} and  [Wei Wang](#)^{4*}

¹College of Horticulture, Northwest A&F University, Shaanxi, China

²School of Biological Sciences, Washington State University, Pullman, WA, United States

³MOE Key Laboratory of Bioinformatics, Center for Plant Biology, Tsinghua-Peking Joint Center for Life Sciences, School of Life Sciences, Tsinghua University, Beijing, China

⁴College of Life Sciences, State Key Lab of Wheat & Maize Crop Science, Henan Agricultural University, Zhengzhou, China

Concise Reviews and Hypotheses in Food Science | [Free Access](#)

Can food processing produce hypoallergenic egg?

MICROBIOLOGY

RESEARCH ARTICLE
Pinheiro *et al.*, *Microbiology* 2018;164:11–19
DOI 10.1099/mic.0.000592



Fusion proteins towards fungi and bacteria in plant protection

Ana Margarida Pinheiro,¹ Alexandra Carreira,² Ricardo B. Ferreira¹ and Sara Monteiro^{1,2,*}

Need



to be used to: **Define sensitivity to structural changes**

Assess instrument performance

Benchmark new platforms

Higher order structure progress in the metrological community



Development of a reference protocol to stress a WHO standard and interlaboratory comparison (NML)

sac00 | ACSJCA | JCA11.2.S208/W Library-x64 | manuscript.3f (R5.1.i2:5007 | 2.1) 2021/01/11 08:51:00 | PROD-WS-118 | rq_3382475 | 6/18/2021 11:03:03 | 8 | JCA-DEFAULT

analytical
chemistry

Analytical platform	Lab ID	rhGH:Zn 1:2	rhGH:Zn 1:5	rhGH:Zn 1:10	
HDX-MS *	1				
	2				
	3				
	4				
msHDX-MS *	12				
IMS-MS (inc. CIA-IMS-MS)	1				globa
	5				
IMS-MS	3				
	6				
SEC-UV	1				
Raman spectroscopy	7				
CZE	8				glc
IPF	9				
DSC	9				
UNit	13				
AUC	9				
Bio-assay	10				
CD	11				

pubs.acs.org/ac

Article

1 Reference Protocol to Assess Analytical Performance of Higher 2 Order Structural Analysis Measurements: Results from an 3 Interlaboratory Comparison

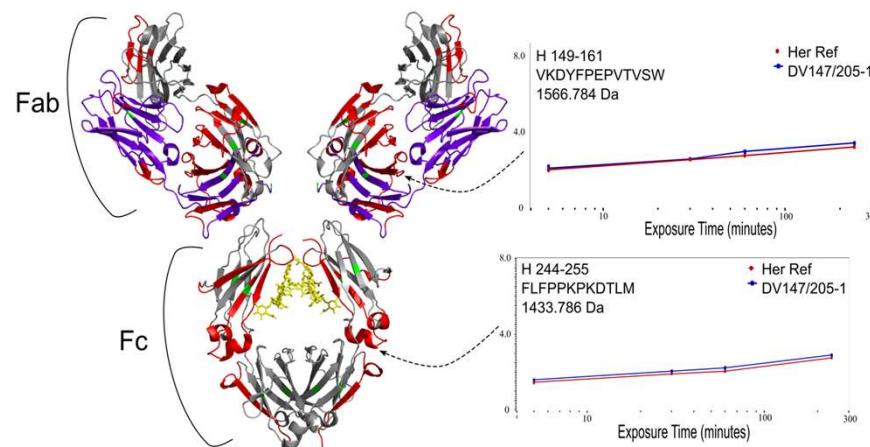
4 K. Groves,* A. E. Ashcroft, A. Cryar, A. Sula, B. A. Wallace, B. B. Stocks, C. Burns, D. Cooper-Shepherd,
5 E. De Lorenzi, E. Rodriguez, H. Zhang, J. R. Ault, J. Ferguson, J. J. Phillips, K. Pacholarz, K. Thalassinos,
6 L. Luckau, L. Ashton, O. Durrant, P. Barran, P. Dalby, P. Vicedo, R. Colombo, R. Davis, R. Parakra,
7 R. Upton, S. Hill, V. Wood, Z. Soloviev, and M. Quaglia*

biological activity	0.35 - 0.45 nM	N
secondary structure	36 µM	N

Epitope mapping



- Use our MS capability to better characterise binding assay, including the effects on immobilisation of the antibody on the epitope and epitope mapping



Luckau L. et al. paper draft

A4I Analysis for Innovators

MAKING SENSE OF OUTSOURCING
FleetBioprocessing

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HOSTED AT LGC

LGC

Summary



Standardisation of proteins is possible

Understanding what we are measuring (target analyte) and what we intend to measure (measurand) is very important

Progress in protein measurements will potentially lead to new markers



....there is not just MS and ELISA....

Acknowledgments

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Caroline Pritchard
Valentina Faustinelli
Salome,' Coppens
Matthew Harris
Lucia Di Vagno
Eva-Illes Tothes
Chris Mussell
Chris Hopley
Government Chemist Team



Organic MS Team



Our close collaborators....

Paul Dalby (UCL)
Oliver Durran (UCB)
Perdita Barran (Uni Manchester)
Sebastien Boulo (JRC)
Lorna Ashton (Lancaster Uni)
Alison Ashcroft (Uni Leeds)
Frank Sobott (Uni Leeds)
Sarah Cianferani (Uni Strasbourg)
Kostas Thalassinou (UCL)
Jeremy Melanson (NRC)
Gavin O'Connor (PTB)
Vincent Delatour (LNE)
Chiara Portesi (INRIM)
Chris Burns (NIBSC)
Alan Reid (UKNEQAS)
Ralf Josef (BIPM)
Anja Kessler (RfB/RELA)
Claire Mills (Uni Manchester)
Zoja Soloviev (GSK, now Sanofi)

**24
June**

Session 3: Science for improved health outcomes

Session Chair: Helen Munday, Institute of Food Science And Technology

Time (BST)	Title	Speaker
10:00	Chair's introduction	Helen Munday, IFST <i>President</i>
10:05	The importance of standardization of biomarker measurements in nutrition	Ian Young, Queen's University Belfast <i>Clinical Professor</i>
10:30	Current perspectives in food toxicology	Stella Cochrane, Unilever <i>Science Leader, Allergy and Immunology</i>
10:55	Challenges in measuring clinical proteins and allergens	Milena Quaglia, LGC <i>Principal Scientist, Proteins</i>
11:20	CBD and controlled cannabinoids in consumer products	Selvarani Elahi, LGC <i>Deputy Government Chemist</i> Christopher Hopley, LGC <i>Principal Scientist, Organic Chemistry</i>
11:45	Microbiome for protected status	Mark Kennedy, Fera <i>Senior Risk Analyst</i>
12.10	Chair's final remarks	







