

MS03 Crystallization and biophysical characterization

MS3-02

Protein quality control for improved data reproducibility and reliability

K. Remans ¹, A. De Marco ², N. Berrow ³, M. Lebendiker ⁴, M. Garcia-Alai ⁵, S. Knauer ⁶, B. Lopez-Mendez ⁷, A. Matagne ⁸, A. Parret ⁹, S. Uebel ¹⁰, B. Raynal ¹¹

¹EMBL - Heidelberg (Germany), ²University of Nova Gorica - Vipava (Slovenia), ³IRB - Barcelona (Spain), ⁴Hebrew University of Jerusalem - Jerusalem (Israel), ⁵EMBL - Hamburg (Germany), ⁶University of Bayreuth - Bayreuth (Germany), ⁷Novo Nordisk Foundation Center for Protein Research - Copenhagen (Denmark), ⁸University of Liège - Liège (Belgium), ⁹EMBL (present address: Charles River Laboratories, Beerse) - Hamburg (Germany), ¹⁰Max Planck Institute of Biochemistry - Martinsried (Germany), ¹¹Institut Pasteur - Paris (France)

Abstract

Purified proteins are used in various types of scientific experiments and fields. For example, in structural biology insights into the functional mechanisms can be obtained by elucidating the 3D molecular structure of proteins and protein complexes using technologies such as X-ray crystallography, cryo-EM and NMR. In biochemistry and biophysics, interactions with other proteins, nucleic acids and small molecules can be studied by determining affinities and specificities. Proteins can function as antigens to generate specific antibodies or as reagents in cell biology experiments. Furthermore, recombinant proteins can be used as tool molecules in genomics, chemical biology and microscopy assays. In order for these experiments to produce reliable and biologically relevant results, they must be performed using high-quality proteins that are active, properly folded, in the right oligomeric state and contain correctly inserted co-factors. Ensuring that these parameters are fulfilled requires quality control. As researchers inexperienced in handling proteins are not always aware of how to validate the quality of protein samples used in downstream experiments, a working group comprised of members of professional European biophysics (<https://arbre-mobieu.eu>) and protein production networks (<https://p4eu.org>) put together a number of guidelines addressing this problem. These guidelines comprise the minimal information that needs to be present to reliably reproduce the expression and purification of the protein of interest, minimal quality control checks for assessing purity, homogeneity/dispersity and identity and more extended quality control tests that need to be performed depending on the specific downstream application of the protein sample. An evaluation by the network members over a one-year period indicated that implementing these quality control guidelines facilitates the optimisation of the protein purification process and improves the reliability of downstream experiments. Therefore, investing in protein quality control benefits all life science stakeholders (researchers, editors and funding agencies alike) by increasing data veracity and minimising loss of valuable time and resources.

References

Berrow N., de Marco A., Lebendiker M., Garcia-Alai M., Knauer S.H., Lopez-Mendez B., Matagne A., Parret A., Remans K., Uebel S. and Raynal B. (2021) Quality control of purified proteins to improve data quality and reproducibility: results from a large-scale survey. *European Biophysics Journal* 50(3-4): 453-460

de Marco A., Berrow N., Lebendiker M., Garcia-Alai M., Knauer S.H., Lopez-Mendez B., Matagne A., Parret A., Remans K., Uebel S. and Raynal B. (2021) Quality control of protein reagents for the improvement of research data reproducibility. *Nature Communications* 12(1): 2795

Remans K., Lebendiker M., Abreu C., Maffei M., Sellathurai S., May M.M., Varčok O. and de Marco A. (2022) Protein purification strategies must consider downstream applications and individual biological characteristics. *Microbial Cell Factories* 21(1): 52