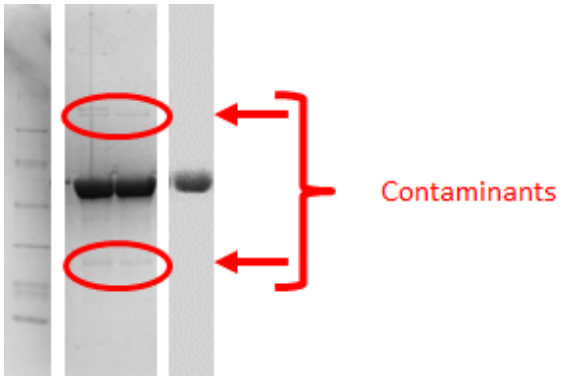
(ii) Minimal quality control parameters that should be tested on protein sample

- **Purity:** checked by SDS-PAGE, Capillary Electrophoresis (CE) or Reversed-Phase-HPLC (RP-HPLC). The objective here is to assess the presence (and level) of contaminants using techniques that are available to most laboratories. The assessment of sample purity by SDS-PAGE is illustrated below.

Example: SDS-PAGE



If SDS-PAGE is the sole method of assessment of sample purity then ideally no other band other than the expected one for your protein of interest should be detected, as illustrated in the example figure. The staining should be chosen according to the amount loaded on the gel in order to be able to detect contaminants of 1% or less of the total protein load. N.B.: the detection limit of Coomassie blue staining is approximately 100ng per band, for reverse zinc staining approximately 10ng per band, while fluorescent or silver stains have a detection limit of approximately 1ng of protein per band. In other words, if you load 10µL of a solution at 1mg/ml, you will load in total 10µg of protein meaning. In order to detect contaminants you will need sensitivity lower than 100ng per band and you should therefore use reverse zinc, fluorescent or silver stain to be able to assess contamination. It is also possible to perform TCA or DOC/TCA or acetone precipitation to concentrate ten or more times the sample.

Common staining protocols can be found in:

- *Quality assessment and optimization of purified protein samples: why and how? Bertrand Raynal, Pascal Lenormand, Bruno Baron, Sylviane Hoos and Patrick England Microbial Cell Factories* ***2014 ; 13:180****.*

More detail about other techniques can be found in:

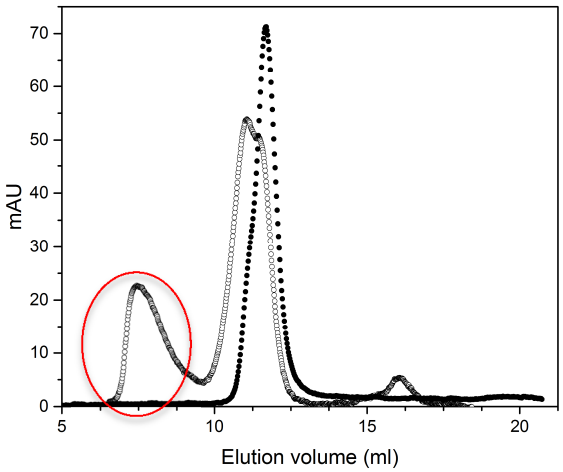
- *Applications of capillary electrophoresis in characterizing recombinant protein therapeutics. Zhao SS, Chen DDY* Electrophoresis ***2014; 35:96–108.***
- *Hydrophobic interaction chromatography for the characterization of monoclonal antibodies and related products. Fekete S, Veuthey JL, Beck A, Guillarme D. J Pharm Biomed Anal.* ***2016; 130:3-18.***

- **Homogeneity (aggregation state):** checked preferably by Size Exclusion Chromatography (SEC) and/or Dynamic Light Scattering (DLS) or by Size Exclusion Chromatography in combination with Multi Angle Light Scattering (SEC-MALS), Field Flow Fractionation (FFF) or Field Flow Fractionation in combination with Multi Angle Light

Scattering (FFF-MALS) or Analytical Ultracentrifugation (AUC). The objective is to assess if the sample has a tendency to form aggregates in the condition used to purify it, and to assess the potential for oligomerization of the protein sample.

Example: SEC

If you are using analytical size exclusion chromatography to assess homogeneity, only regular peaks corresponding to the prevalent monomeric or oligomeric species specific for that protein should be detected, and no aggregates should be detected in the void volume of the column situated in a position corresponding to 1/3 of the total volume of the size exclusion column used (e.g. for column with a total volume of 21ml the void volume is situated at an elution volume of 7ml). The figure below shows a good sample (filled dots) with a single, well-defined, symmetric peak, representing a homogeneous species, and a heterogeneous sample containing aggregates (open dots), note the 'aggregate' peak at the void volume of the column (circled) and the asymmetric main peak. SEC can, of course, also be used preparatively at larger scales to select specific oligomeric states e.g. if a dimer is functional in an experiment or assay but a monomer is not the peak corresponding to the size of the dimer may be selected. Please note that after pooling a specifically 'sized' peak any further processes e.g. concentration or buffer exchange may alter the oligomeric state. Pooled fractions from a single peak should be preferentially checked again by a further SEC round.

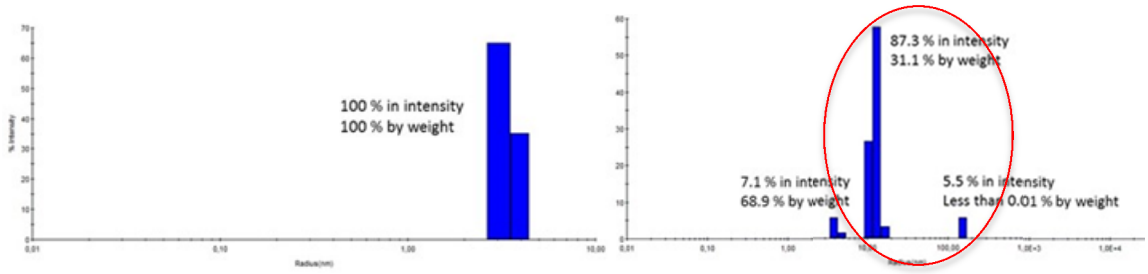


More detail about Size exclusion Chromatography can be found in:

- *Theory and practice of size exclusion chromatography for the analysis of protein aggregates. Fekete S, Beck A, Veuthey J-L, Guillarme D: J Pharm Biomed Anal* ***2014; 101:161–173.***
- *Useful practical information can be accessed in:* https://www.gelifesciences.com/en/de/solutions/protein-research/knowledge-center/protein-handbooks and http://wolfson.huji.ac.il/purification/

Example: DLS

If DLS is used to assess the homogeneity of your sample ideally only one species with a low polydispersity (with less than 20% dispersity of the peak) should be detected, and no aggregates should be detectable (as greater than 1-2 percent of your sample). The figure



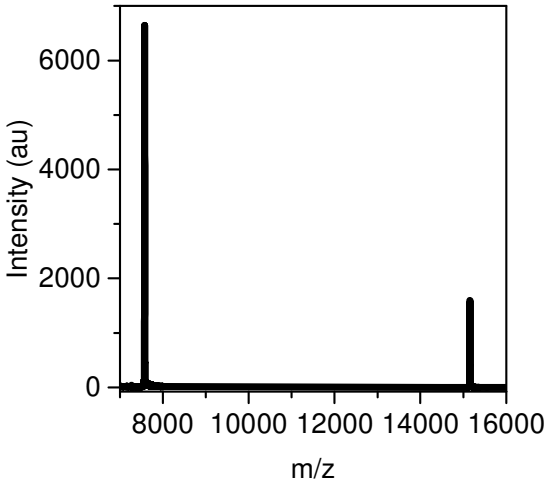
below shows a good sample (Left Figure) with a single peak and a heterogeneous sample containing aggregates (Right Figure).
One should remember that the signal is dependent on the size of the detected particle. For example in the right figure the aggregate of 150nm (right circle) represents more than 5% of signal, however less than 1% in weight, it is therefore negligible. On the contrary, the aggregates with an approximate size of 15nm (central peak) represent nearly 90% of the intensity signal but only 31% of the sample weight. This sample can clearly be defined as non-homogeneous.

More detail about DLS can be found in:

- *Dynamic light scattering: a practical guide and applications in biomedical sciences. Stetefeld J, McKenna SA, Patel TR. Biophys Rev.* ***2016; 8:409-427.***
- *Nobbmann U, Connah M, Fish B, Varley P, Gee C, Mulot S, Chen J, Zhou L, Lu Y, Shen F, Yi J, Harding SE: Dynamic light scattering as a relative tool for assessing the molecular integrity and stability of monoclonal antibodies. Biotechnol Genet Eng Rev* ***2007; 24:117–128.***
- *Philo JS: Is any measurement method optimal for all aggregate sizes and types? AAPS J* ***2006; 8:E564–571.***

- **Identity:** checked preferably by intact protein mass, peptide mass fingerprinting or Edman sequencing.

Example: Intact mass by MALDI-TOF



For confirmation of sample identity mass spectrometry is a technique that is now widely available, both in academic institutions and commercially. Mass spectrometry will identify species according to their mass over charge ratio. In the intact mass (also known as 'top-down' MS) example presented here (see figure), the purified protein has an expected mass of 15154.8 Da. Two peaks can be detected: one with a double charge and a ratio of 7578.34 m/z and one with a single charge and a ratio of 15155.56 m/z. Since these two peaks are in good agreement with the expected molecular mass, the produced protein is the expected one and no trace of degradation or contamination can be detected.

Example: tryptic digest/MS

You may also use 'bottom-up' mass spectrometry (e.g. tryptic digest/MS) to confirm the identity of our protein. This method will also detect (and identify) any contaminating proteins but will

not provide information on the integrity of your protein. It can be conveniently performed on samples excised from SDS-PAGE gels.

More detail about mass spectrometry can be found in:

- Analysis of intact protein isoforms by mass spectrometry. Tipton JD, Tran JC, Catherman AD, Ahlf DR, Durbin KR, Kelleher NL: J Biol Chem **2011, 286:25451–25458.**
- *Overview of peptide and protein analysis by mass spectrometry. Zhang G, Annan RS, Carr S, Neubert T Curr Protoc Protein Sci **2010, 62:16.1.1–16.1.30***