Coriolis Pharma
Biopharmaceutical Research and Development Service

Orthogonal protein aggregation characterization (AUC, AF4/HF5 and HP-SEC)

www.coriolis-pharma.com

A breadth of services

Protein stability, efficacy, immunogenic potential and patient safety can be closely related to protein aggregation. Our expertise in in protein formulation development, including forced degradation and stability studies, as well as the related analytics, allows Coriolis Pharma to deliver outstanding service in protein aggregation characterization.

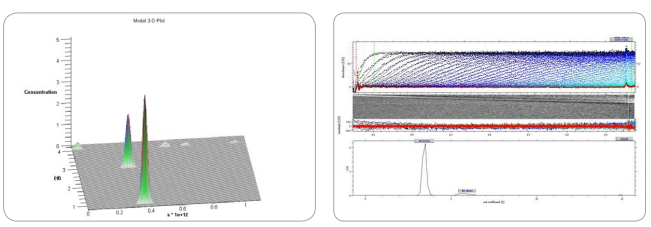
For a comprehensive characterization of protein aggregates, an orthogonal approach that includes methods which use different measurement principles is recommended. **HP-SEC** is often used as routine stability method for the quantification of soluble aggregates. In order to assess the suitability of HP-SEC, studies that involve the employment of orthogonal methods, for example **AUC** or **AF4/HF5** are required.

Putting results obtained from different measurement principles into context may not be straight forward. Coriolis Pharma offers all relevant analytical techniques for this purpose under one roof. This allows for a comprehensive data assessment for orthogonal studies. Our scientists are experts in the latest particle/aggregate characterization instrumentation and have released several high-ranking publications in this area.

Orthogonal protein aggregation characterization

Analytical ultracentrifugation (AUC)

- **Recognized as "gold standard" method for aggregation testing**
  - In many cases sample is analyzed in its original formulation
  - No risk of dissolution of aggregates by a mobile phase during analysis (as in HP-SEC and AF4)
  - No shear stress applied during analysis
- 2 AUC systems available for high sample throughput
- 8 hole (50 krpm) and 4 hole rotor (60 krpm) capabilities
- Absorbance and/or interference optics for detection
- **Unique service: Optionally, in-depth data processing using UltraScan-III on dedicated in-house supercomputing**
  - Superior peak resolution and higher reproducibility than standard data processing software (SEDFIT)
  - Evaluation of multiple species in one sample (e.g. different density, shape)
  - Monte-Carlo analysis based data processing reducing artefacts and noise
- **SV-AUC (Sedimentation Velocity AUC)**
  - Detection and quantification of aggregates
  - Assessment of native state of a protein within its formulation
  - Size distribution at a much higher resolution than HP-SEC
  - Sedimentation coefficient and shape determination
  - Analysis of peptides starting from ca. 3 kDa possible
  - Up to 100 nm aggregates can be characterized
- **SE-AUC (Sedimentation Equilibrium AUC)**
  - Assessment of the equilibrium constant Kd
  - Determination of the stoichiometry of complexes between different proteins
  - Measurement of highly concentrated samples (up to 80mg/ml) without dilution
  - Low-speed equilibrium experiments to increase accuracy in molecular weight determination



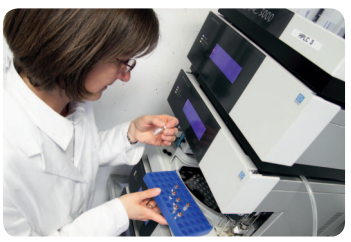
Data processing of a BSA sample using UltraScan (left) and SEDFIT (right).

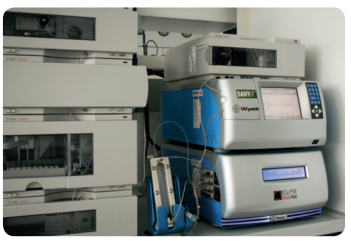
Asymmetric flow field flow fractionation (AF4), hollow fiber flow field flow fractionation (HF5)

- Quantification of monomer content, aggregates and fragments
- Molecular weight determination for monomer, aggregates and fragments
- Extremely wide separation range (small peptides (2 kDa) up to subvisible particle (up to ~ 50 µm)
- High flexibility with respect to mobile phase
- Available detectors: UV, fluorescence, MALLS, RI
- Column-free separation principle. No potential interaction with stationary phase material.

Size exclusion chromatography (HP-SEC)

- Quantification of monomer content, small soluble aggregates and fragments
- Molecular weight determination for monomer, aggregates and fragments
- Highly reproducible and robust
- Limitations with respect to mobile phase
- Available detectors: UV, fluorescence, MALLS, RI





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Increase the confidence in your data