## Table 3: Spectroscopic Characterisation of Biopharmaceuticals

### SCOPE:

The biological activity of proteins- like immunoglobulins- being deployed to battle diseases like cancer, rely on the integrity of their higher order structure (HOS). Consequently, structural characterization of protein drugs is expected by regulatory agencies to be conducted throughout their development trajectory. Thus, a variety of analytical techniques can be used. The resulting data provides insight into the HOS of biopharmaceutical proteins, and into structural alterations induced by storage or certain types of stress. The set of spectroscopic characterization techniques commonly used in the pharmaceutical industry to address the integrity of secondary, tertiary and quaternary structure, comprises of far-UV/near-UV circular dichroism (CD), intrinsic/extrinsic fluorescence (FLU), Fourier transform-infrared (FT-IR), UV spectroscopy, differential scanning calorimetry (DSC) and dynamic light scattering (DLS). Despite the fact that the aforementioned characterization techniques have been used to characterize (pharmaceutical) proteins for quite a while already, efforts to improve data quality, analysis and interpretation continue to be undertaken, for example by using statistics to assess the significance of changes observed in spectral data. Characterization assays usually are not run under GMP and are therefore not required to be validated. Evidence of the fact that they are scientifically sound should be available however, in case data are being used in support of pharmaceutical product development.

### QUESTIONS FOR DISCUSSION:

1. What spectroscopic assays are available in your situation and which are being used for what purposes?
2. How is scientific soundness of your spectroscopic methods ensured (i.e. system suitability criteria, etc.)?
3. To what extent are statistical methods used to assess the quality of spectroscopic data and potential differences observed between sample spectra?
4. What (new) developments are seen in the arena of spectroscopic methods for protein characterization?
5. What are the main (dis)advantages of spectroscopic methods, for example compared to mass spectrometry-based methods for structural interrogation?

### DISCUSSION NOTES:

**Recent developments**

- New applications of spectroscopic techniques were presented during the AT Europe 2020:
- Laurence Barron, University of Glasgow: Raman Optical Activity (ROA),
    - application areas: biosimilar assessment, glycoproteins (distinct signals for glycosylation and peptide backbone), folding status

- John Hales, University College London, Virus Lasers and Decay-associated Chromatography :
    - application areas: ligand binding assays, mAb coformulations, online bioprocess monitoring (e.g. for continues manufacturing), early development, Identification + quantification from chromatographic techniques
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**Current status of spectroscopic techniques in biopharmaceutical industry**

- Spectroscopic assays are mostly developed in academia, transition to industry is hampered as regulatory authorities need to be convinced by the new techniques
- Spectroscopic assays can add structural information on all stages of development
- Vibrational circular dichroism (VCD) was mentioned as example for an accepted spectroscopic assay by regulators in USA
- Potency assays are the gold standard for release testing (from regulatory point of view), disadvantage: bioassays have high variations and poor robustness
- HOS analysis alone could increase variability and precision (routine methods needed)
- Structural attributes are underrepresented in analytical characterisation
- Questions: What is the added need/benefit of spectroscopic methods?, What cannot be done?
- New therapeutic modalities (e.g. bsAbs or coformulations) bring new challenges for the analytical characterization

**Requirements and challenges for implementation in biopharmaceutical industry:**

- Justification of spectroscopic methods in the biopharmaceutical industry is important
- Can different folding structures be resolved?
- How to capture spectra fingerprints? (e.g. assessment based on induced structural changes by induced denaturation, heat, light etc.)
- Challenge: Spectroscopic data are rich in information, how to make decisions about the data
- Differences in spectroscopic data: statistical difference vs. biological relevance
- Databases for identification with acceptable variance needed
- Spectroscopic data complexity is conflicting with requirements for release testing
- Moving from expert interpretation to numerical/binary outputs to define specifications, implementation of statistical tools needed
- Appearance/presentation of the data: visualization as something more familiar like a chromatogram would be helpful for moving towards release testing
- Note: Not a single spectroscopic technique covers all structural information
- Aim: Spectroscopic assays should be widespread + applicable for routine testing