Applications of native MS in biopharmaceutical industry:

- For determination of drug-to-antibody (DAR) ratio for conventional Cys-conjugated ADC;
- For characterization of protein/protein and protein-small molecule complexes:
  - Protein small molecule interactions are studied to evaluate binding affinity of small molecule drugs to protein. For this study titration curves are used. This approach is used instead of SPR technique.
  - Orphan ligand identification;
  - FcRN receptor binding;
- For characterization of antibody aggregates, size variants, and bispecific size variants. Native SEC MS is an orthogonal technique to the SEC/MALS.
- For charge reduction of highly heterogeneous mixtures. Native MS shifts charge envelop of biotherapeutic molecules to lower charges (higher m/z), thus giving a better separation in m/z values for complex mixtures allowing a better intact mass deconvolution.
- For improved m/z separation of close in mass species that are due to ion coalescence in the Orbitrap mass spectrometers might not be separated under denaturing conditions. Under native MS conditions mAb has charge states from 24+ to 27+, whereas under denaturing conditions, the charge state envelope is form 35+ to 57+.
- For characterization of molecules (intact mAbs, glycans, peptides, etc.) using ion-mobility MS (IM-MS):
  - Characterization of various conformers of the molecules and determination of their collisional cross-sections (CCS).

Instruments used for native MS:

- ThermoScientific Orbitrap EMR
- Agilent 6560 QTOF IM-MS
- Bruker 12T FTMS
- Waters Synapt G2 IM-MS
- Waters QTOF Ultima upgraded to high mass range
Sample introduction:

- Samples are buffer exchanged into ammonium acetate and are directly infused into mass spectrometer.
- Native size-exclusion chromatography coupled to MS. Ammonium acetate is used as a mobile phase. The concentration of ammonium acetate ranges from 20 to 200 mM.

Challenges with native MS:

- For ADC DAR determination, lower values of DAR are observed under native MS vs denatured MS:
  - Potential solutions – need to improve recovery of highly loaded species from the SEC column by either increasing ammonium acetate concentration or addition of low level of organic solvents. Waters SEC BEH column that is used for native SEC/MS contains C2 carbon that results in hydrophobic interaction with analyte.
- For HMMS characterization by SEC/MS, sensitivity and MS resolution are not adequate. Often need orthogonal methods to further characterize the HMMS species.
- For IM-MS characterization of conformers, only 1 or 2 charge states might show the difference between conformers.
- For protein-small molecule binding study, binding affinity need to be in low micromolar or higher, otherwise, complexes do not survive ionization in the ion source.